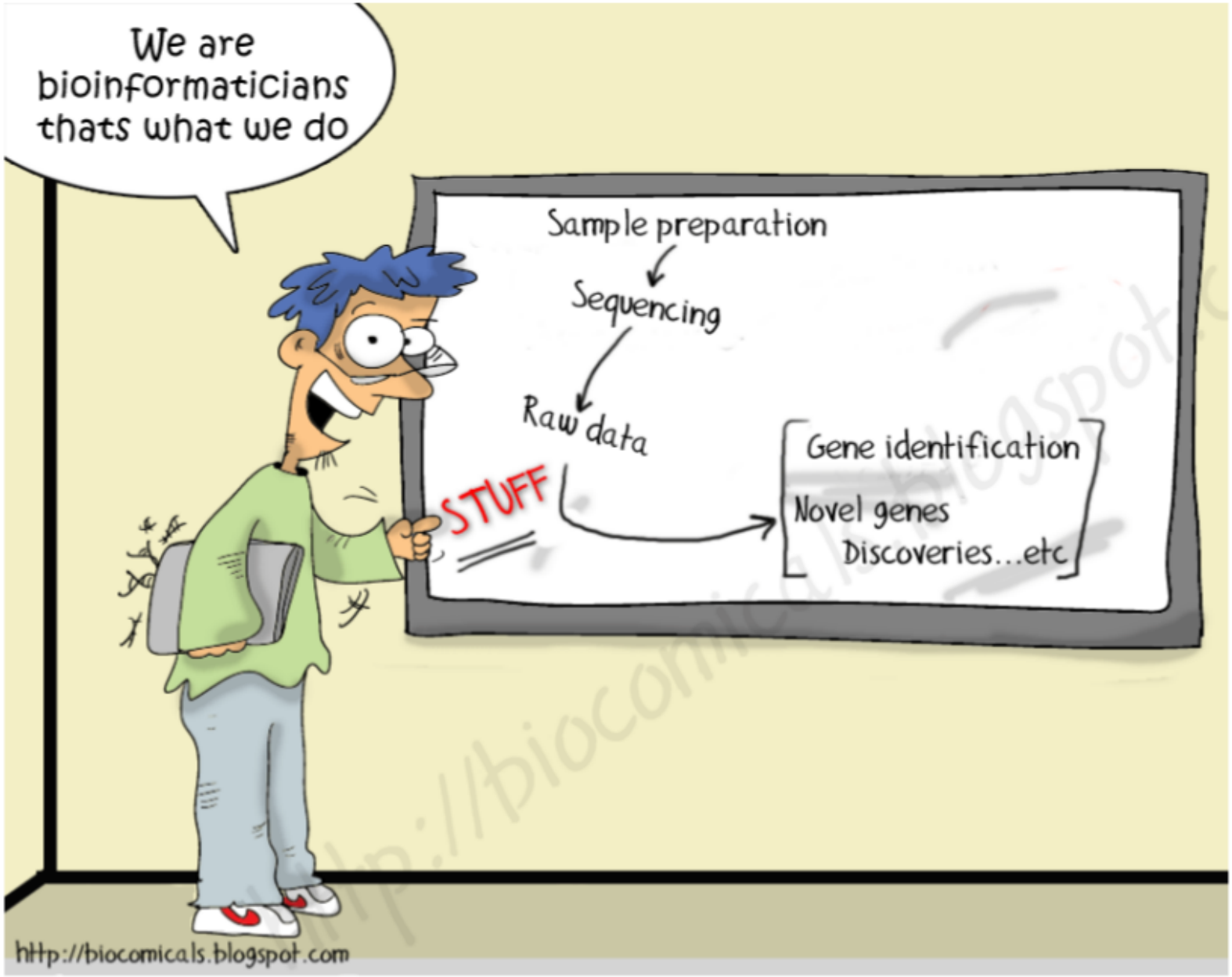
Imperial College London

Introduction to NGS data

Principles of genotype and SNP calling and estimation of allele frequencies

Matteo Fumagalli

22^{nd} August 2017

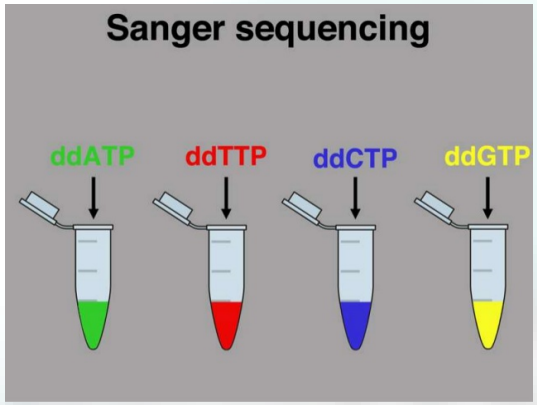

We are bioinformaticians thats what we do
Sample preparation
Sequencing
Raw data
STUFF
Gene identification
Novel genes
Discoveries...etc
http://biocomicals.blogspot.com

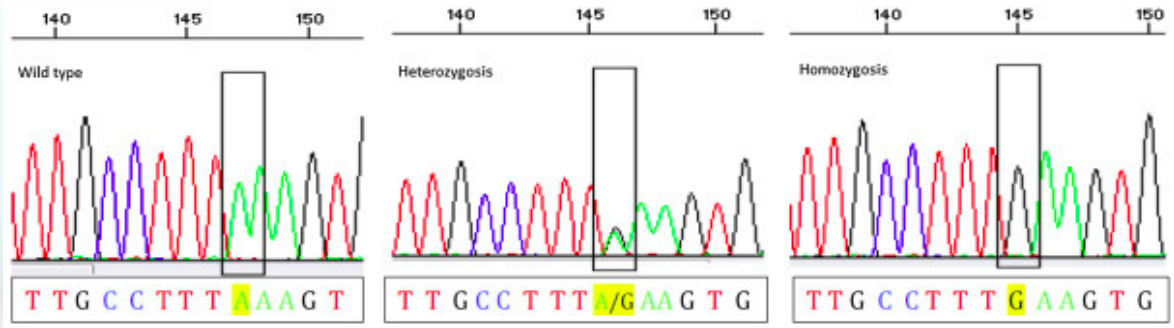
Presentation outline

1. Introduction
2. Genotype likelihoods
3. Genotype calling
4. SNP calling
5. Imputation

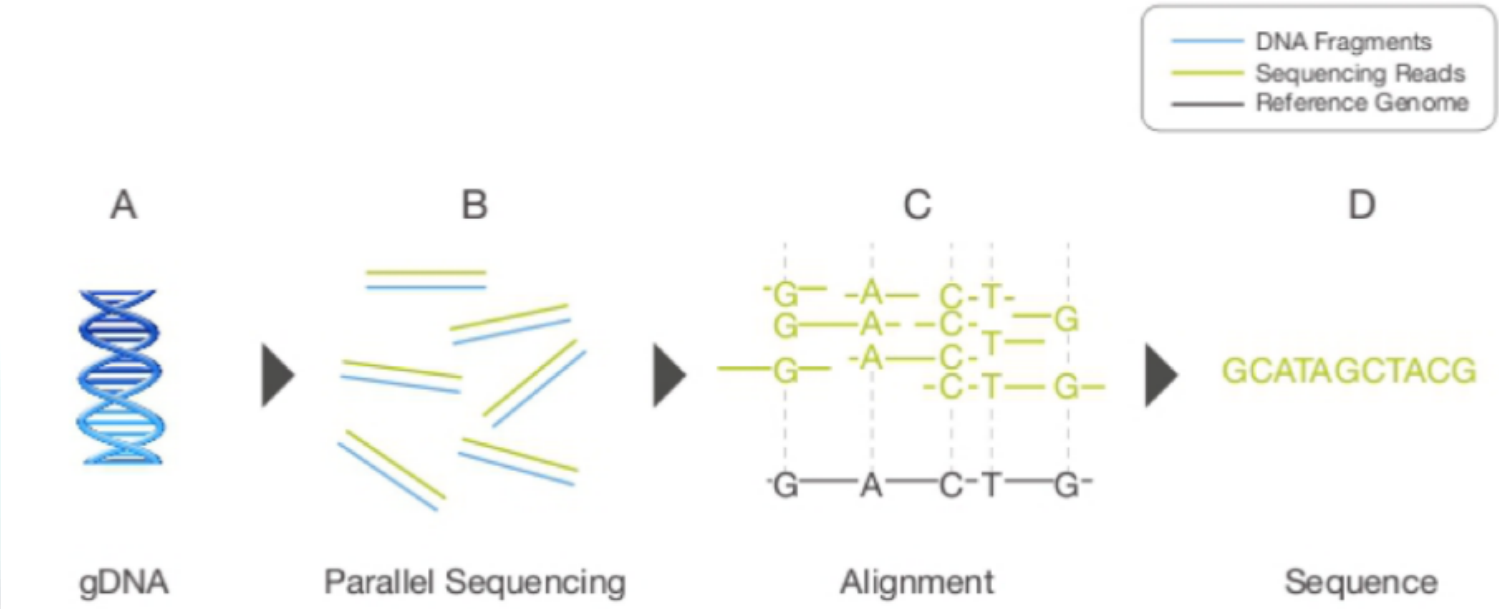
Sanger sequencing

aka first/former generation sequencing





Next Generation Sequencing



A. Extracted gDNA
B. gDNA is fragmented into a library of small segments that are each sequenced in parallel.
C. Individual sequence reads are reassembled by aligning to a reference genome
D. The whole-genome sequence is derived from the consensus of aligned reads.

From genomes to variants

Genome (FASTA)

```
>ARPM2ref|NC_000001.10|:2938046-2939467 Homo sapiens chromosome 1, GRCh37 primary
reference assembly
TGGAAGAGGCCTCAGCAGGCCCAGGCCACCTGGAGGGAGAGCAGACCTGCGGCTGAGGATGCAGGGCTCC
CGGGCACGGTGCTAGCCCTGCCTTGAGACACCCCGAGAGCTGTGGGAAGAGCTGTGGGATCCCCTATTGC
ATCACAAAGCGGCCCTGGAGGGCTGGTCTTTATTTTGATGAGGCTGAGAAGGGAAGGCTGCGGGCATGTT
TAATCCGCACGCTTTAGACTCCCCGGCTGTGATTTTTGACAATGGCTCGGGGTTCTGCAAAGCGGGCCTG
TCTGGGGAGTTTGGACCCCGGCACATGGTCAGCTCCATCGTGGGGCACCTGAAATTCCAGGCTCCCTCAG
```

Reads (FASTQ)

```
CCAATGATTTTTTTCCGTGTTTCAGAATACGGTTAA
+SRR038845.41 HWI-EAS038:6:1:0:1474 length=36
BCCBA@BB@BBBBAB@B9B@=BABA@A:@693:@B=
@SRR038845.53 HWI-EAS038:6:1:1:360 length=36
GTTCAAAAAGAACTAAATTGTGTCAATAGAAAACTC
+SRR038845.53 HWI-EAS038:6:1:1:360 length=36
```

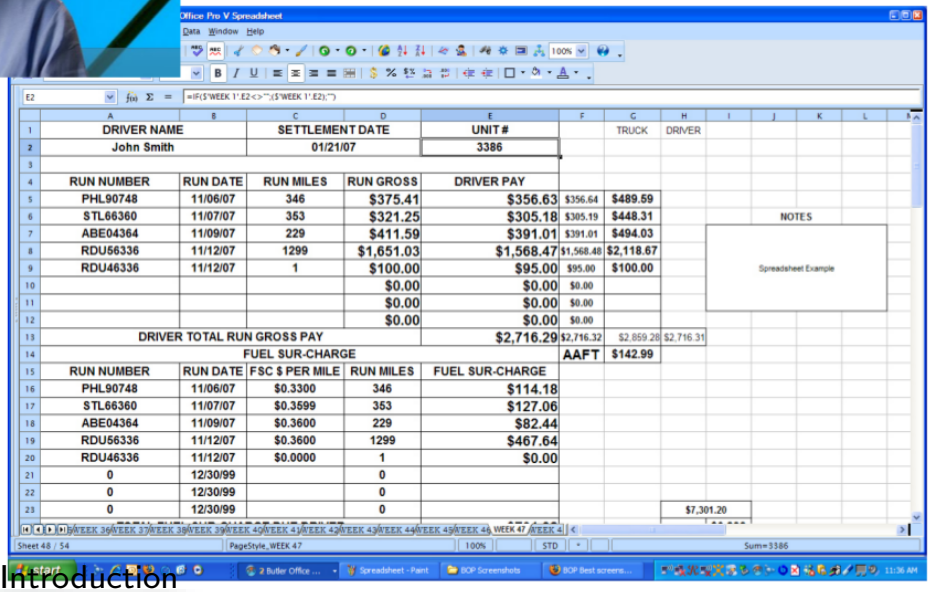
Mapped Reads (mpileup, BAM)

```
seq1 272 T 24  ,.$.....,.,.,...,,,,,,,,,^+. <<<+;<<<<<<<<<<=<;<;7<&
seq1 273 T 23  ,.....,.,.,...,,,,,,,,,A <<<;<<<<<<<<<3<=<<<;<<+
seq1 274 T 23  ,.$.....,.,.,...,,,,,,,,,    7<7;<;<<<<<<<<<=<;<;<<6
seq1 275 A 23  ,$.....,.,.,...,,,,,,,,,^l.  <+;9*<<<<<<<<<=<<:;<<<<
seq1 276 G 22  ...T,,.,.,.,...,,,,,,,,,  33;+<<7=7<<7<&<<1;<<6<
seq1 277 T 22  ..........,C.,,,,,,,G.  +7<;<<<<<<<&<=<<:;<<&<
seq1 278 G 23  .....,.,.,...,,,,,,,,,^k.   %38*<<;<7<<7<=<<<;<<<<<
seq1 279 C 23  A..T,,.,.,...,,,,,,,,,, ;75&<<<<<<<<<=<<<9<<:<<
```

Variants (VCF)

```
##fileformat=VCFv4.1
##fileDate=20140930
##source=23andme2vcf.pl https://github.com/arrogantrobot/23andme2vcf
##reference=file://23andme_v3_hg19_ref.txt.gz
##FORMAT=<ID=GT,Number=1,Type=String,Description="Genotype">
#CHROM  POS     ID      REF     ALT     QUAL    FILTER  INFO    FORMAT  GENOTYPE
chr1    82154   rs4477212       a       .       .       .       GT      0
/0
chr1    752566  rs3094315       g       A       .       .       .       GT      1
/1
chr1    752721  rs3131972       A       G       .       .       .       GT      1
/1
chr1    798959  rs11240777      g       .       .       .       .       GT      0
/0
chr1    800007  rs6681049       T       C       .       .       .       GT      1
/1
```

Forget about



DRIVER NAME	SETTLEMENT DATE	UNIT #
John Smith	01/21/07	3386

RUN NUMBER	RUN DATE	RUN MILES	RUN GROSS	DRIVER PAY
PHL90748	11/06/07	346	$375.41	$356.63
STL66360	11/07/07	353	$321.25	$305.18
ABE04364	11/09/07	229	$411.59	$391.01
RDU56336	11/12/07	1299	$1,651.03	$1,568.47
RDU46336	11/12/07	1	$100.00	$95.00
			$0.00	$0.00
			$0.00	$0.00
			$0.00	$0.00
DRIVER TOTAL RUN GROSS PAY				$2,716.29

FUEL SURCHARGE				
RUN NUMBER	RUN DATE	FSC $ PER MILE	RUN MILES	FUEL SURCHARGE
PHL90748	11/06/07	$0.3300	346	$114.18
STL66360	11/07/07	$0.3599	353	$127.06
ABE04364	11/09/07	$0.3600	229	$82.44
RDU56336	11/12/07	$0.3600	1299	$467.64
RDU46336	11/12/07	$0.0000	1	$0.00
0	12/30/99		0	
0	12/30/99		0	
0	12/30/99		0	

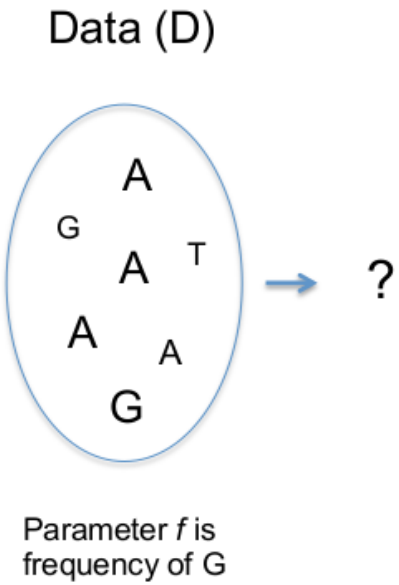
	TRUCK	DRIVER
$356.64	$489.59	
$305.19	$448.31	
$391.01	$494.03	
$1,568.48	$2,118.67	
$95.00	$100.00	
$0.00		
$0.00		
$0.00		
$2,716.32	$2,859.28	$2,716.31
AAFT	$142.99	

NOTES

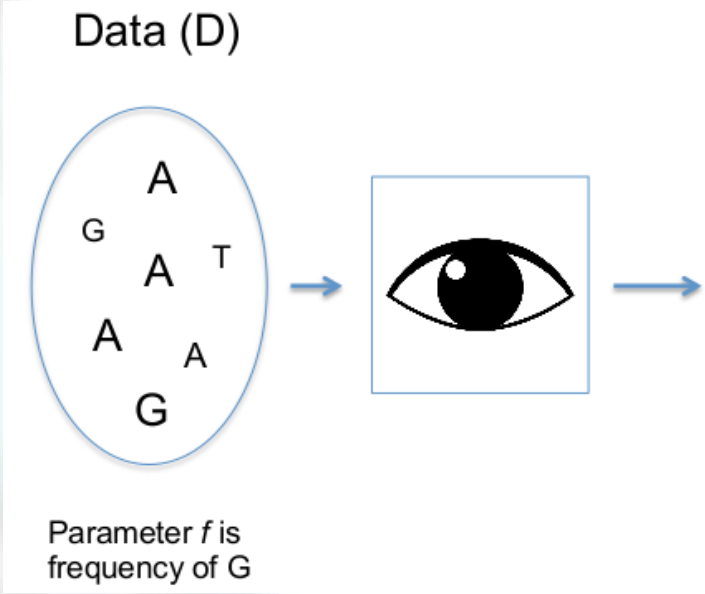
Spreadsheet Example

$7,301.20

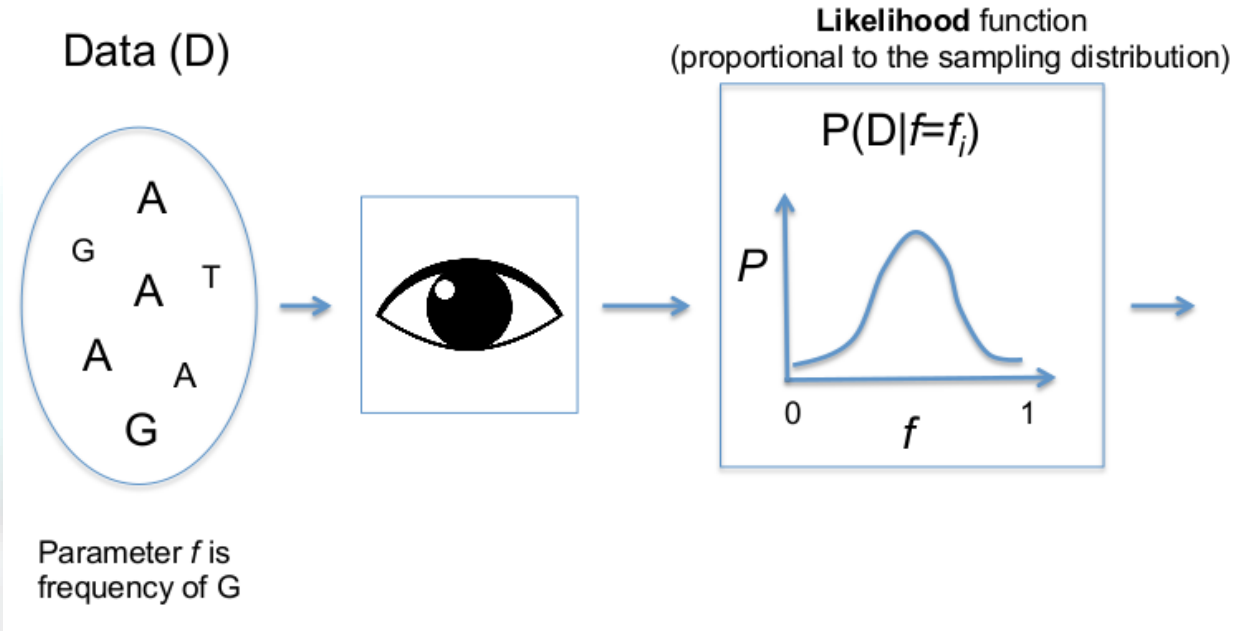
Statistical inference



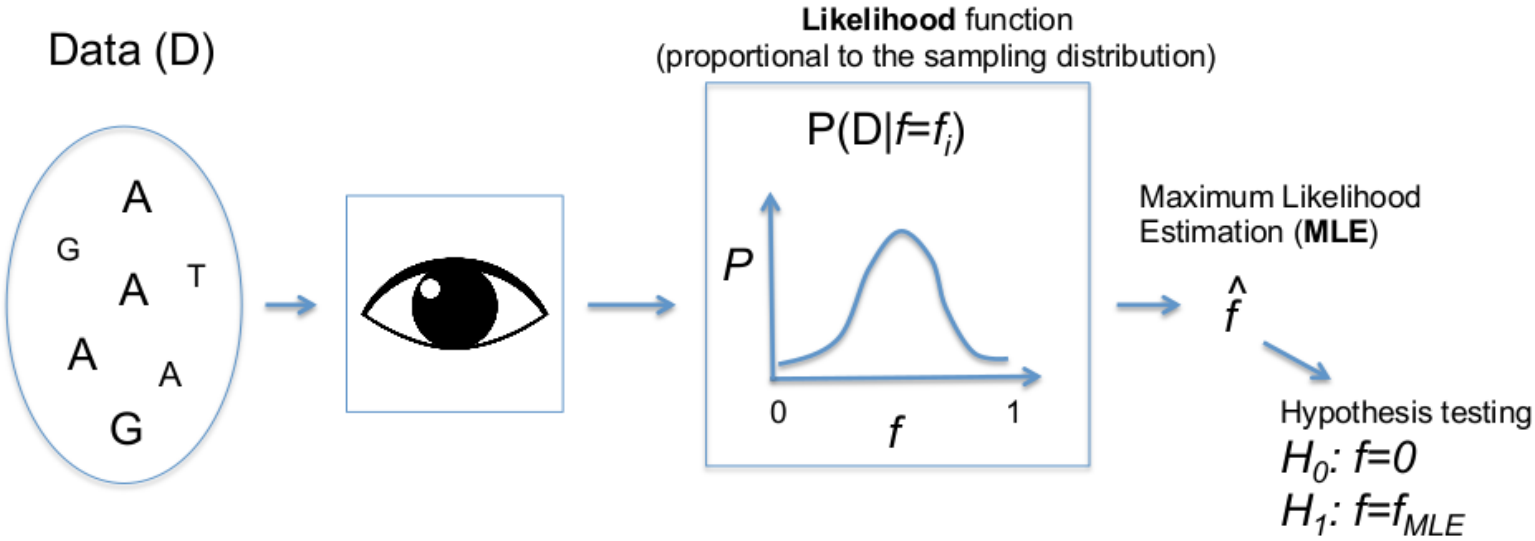
Statistical inference



Statistical inference



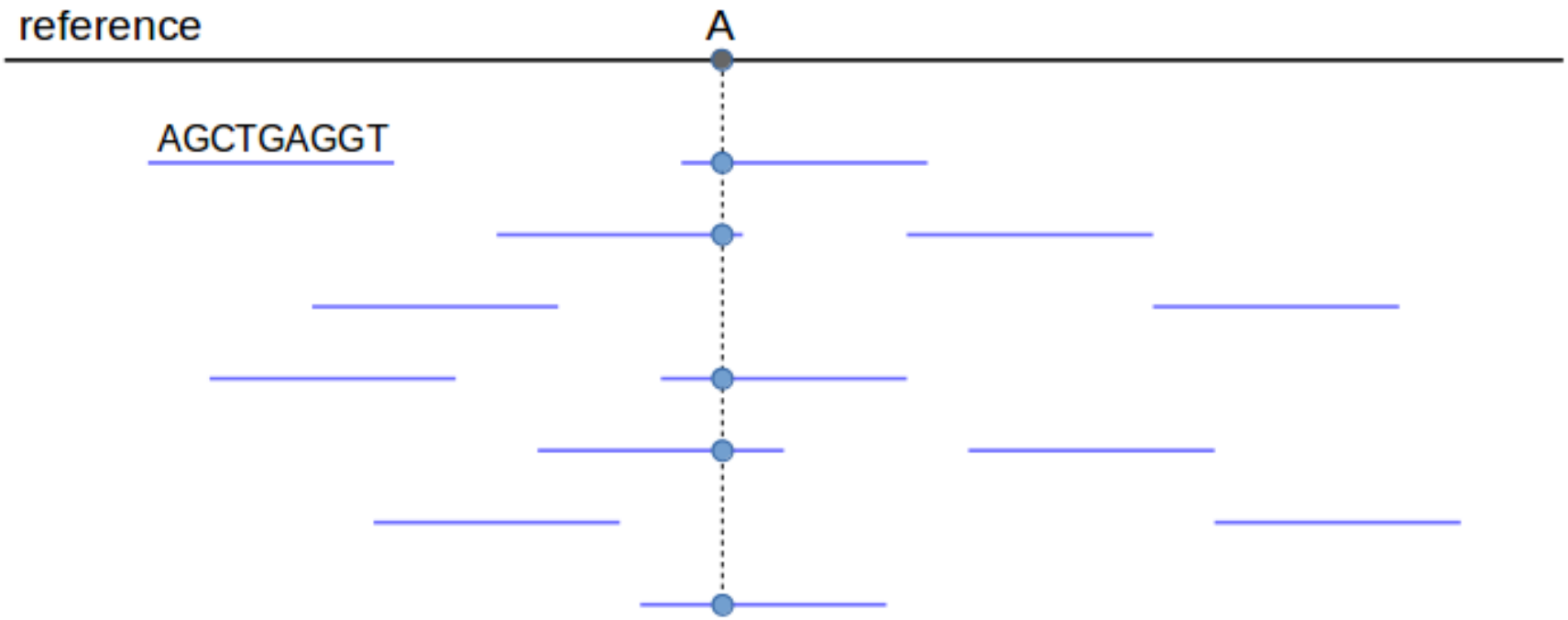
Statistical inference



Likelihood approach:

- All the information on the parameter is in the likelihood function (we use all the data!).
- More data leads to less bias and less variance.
- Suitable for hypothesis testing.

The data



- is a **nucleotide**/base/allele with a certain **quality** score

Genotype likelihoods

Likelihood

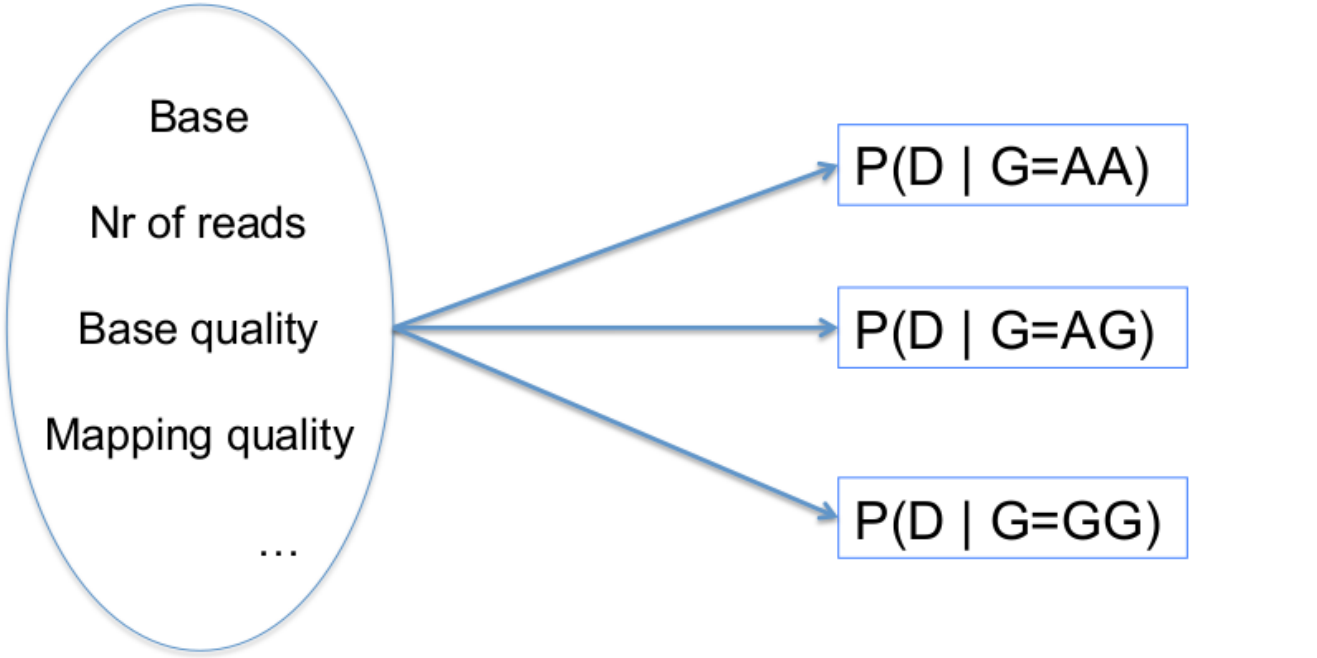
$P(D|G = \{A_1, A_2, ..., A_n\})$
with
$A_i \in \{A, C, G, T\}$ and n being the ploidy

How many genotypes likelihoods do we need to calculate for each each individual at each site?

Genotype likelihoods

Chrom1 272 A 24 AAAAAGGAGAGGTAAG <<<+;<<<<<<<<<<=<;<;7<&

Base quality in Phred scale



Calculating genotype likelihoods

Likelihood function

$$P(D|G = \{A_1, A_2, ..., A_N\}) = \prod_{i=1}^{R} \sum_{j=1}^{N} \frac{L_{A_j,i}}{N}$$

- $L_{A_j,i} = P(D|A_G = A_j)$
- $A_i \in \{A, C, G, T\}$
- R is the depth (nr. of reads)
- N is the ploidy (nr. of chromosomes)

Example:
AAAG with all quality scores equal to 20 (in phred score)
$P(D|G = AC) =?$

Calculating genotype likelihoods

Likelihood function

$$P(D|G = \{A_1, A_2, ..., A_N\}) = \prod_{i=1}^{R} \sum_{j=1}^{N} \frac{L_{A_j,i}}{N}$$

AAAG & Q=20

$$P(D|G = \{A, C\}) = ...$$

Calculating genotype likelihoods

Likelihood function

$$P(D|G = \{A_1, A_2, ..., A_N\}) = \prod_{i=1}^{R} \sum_{j=1}^{N} \frac{L_{A_j,i}}{N}$$

AAAG & Q=20

$N = 2; i = 1; A_1 = A; A_2 = C$

$$P(D|G = \{A, C\}) = (\frac{L_{A,1}}{2} + \frac{L_{C,1}}{2}) \times ...$$

What are $L_{A,1}$ and $L_{C,1}$?

Calculating genotype likelihoods

Likelihood function

$$P(D|G = \{A_1, A_2, ..., A_N\}) = \prod_{i=1}^{R} \sum_{j=1}^{N} \frac{L_{A_j,i}}{N}$$

AAAG & Q=20

$$L_{C,1} = \frac{\epsilon}{3}$$

$$L_{A,1} = 1 - \epsilon$$

$$P(D|G = \{A, C\}) = (\frac{1-\epsilon}{2} + \frac{\epsilon}{6}) \times ...$$

Calculating genotype likelihoods

Likelihood function

$$P(D|G = \{A_1, A_2, ..., A_N\}) = \prod_{i=1}^{R} \sum_{j=1}^{N} \frac{L_{A_j,i}}{N}$$

AAAG & Q=20

$$L_{C,1} = \frac{\epsilon}{3}$$

$$L_{A,1} = 1 - \epsilon$$

$$P(D|G = \{A, C\}) = (\frac{1-\epsilon}{2} + \frac{\epsilon}{6}) \times \frac{\epsilon}{3} \times \frac{\epsilon}{3} \times \frac{\epsilon}{3}$$

What is ϵ?

Calculating genotype likelihoods

Genotype	Likelihood (log10)
AA	-2.49
AC	**-3.38**
AG	-1.22
AT	-3.38
CC	-9.91
CG	-7.74
CT	-9.91
GG	-7.44
GT	-7.74
TT	-9.91

AAAG & $\epsilon = 0.01$

Genotype calling

Genotype	Likelihood (log10)
AA	-2.49
AC	-3.38
AG	-1.22
AT	-3.38
CC	-9.91
CG	-7.74
CT	-9.91
GG	-7.44
GT	-7.74
TT	-9.91

AAAG & $\epsilon = 0.01$

What is the genotype here?

Genotype calling

Genotype	Likelihood (log10)
AA	-2.49
AC	-3.38
AG	**-1.22**
AT	-3.38
CC	-9.91
CG	-7.74
CT	-9.91
GG	-7.44
GT	-7.74
TT	-9.91

AAAG & $\epsilon = 0.01$
What is the genotype?
AG.

Maximum Likelihood

The simplest genotype caller: choose the genotype with the highest likelihood.

Major and minor alleles

Likelihood function

$$\log P(D|G = A) = \sum_{i=1}^{R} \log L_{A_j,i}$$

AAAG & $\epsilon = 0.01$

Allele	Likelihood
A	**-2.49**
C	-3.38
G	**-1.22**
T	-3.38

We can reduce the genotype space to 3 entries (from 10).

Genotype calling

AAAG & $\epsilon = 0.01$ & A,G alleles

Genotype	Likelihood
AA	-5.73
AG	-2.80
GG	-17.12

Examples varying qualities and reads...

Genotype likelihood ratio

$$\log_{10} \frac{L_{G(1)}}{L_{G(2)}} > t$$

i.e. $t = 1$ meaning that the most likely genotype is 10 times more likely than the second most likely one

Pros and cons?

- Yes:
- No:

Genotype likelihood ratio

$$\log_{10} \frac{L_{G(1)}}{L_{G(2)}} > t$$

i.e. $t = 1$ meaning that the most likely genotype is 10 times more likely than the second most likely one

Pros and cons?

- Yes: genotype are called with higher **confidence**
- No: more **missing** data

Statistical thinking

Figure: Nessie, the Loch Ness Monster. True or fake?

Statistical thinking

- $T = \{0, 1\}$, whether I tell you I saw Nessie or not.
- $N = \{0, 1\}$, whether Nessie exists (I saw it) or not.

Questions

- What are $p(T = 1|N = 1)$ and $p(T = 1|N = 0)$?
- What is a Maximum Likelihood Estimate of N?

Statistical thinking

Our inference on N is driven solely by our observations, given by our likelihood function.

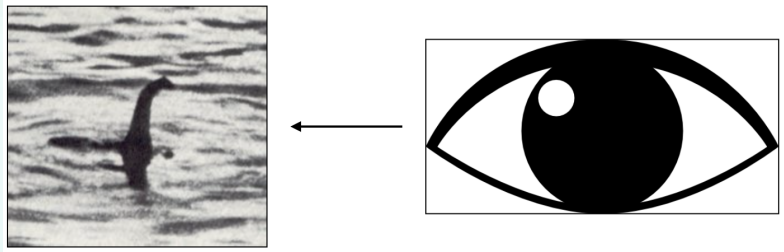


Figure: The eye: a "likelihood" organ.

Statistical thinking

In real life we take many decisions based not only on what we observe but also on some believes of ours.

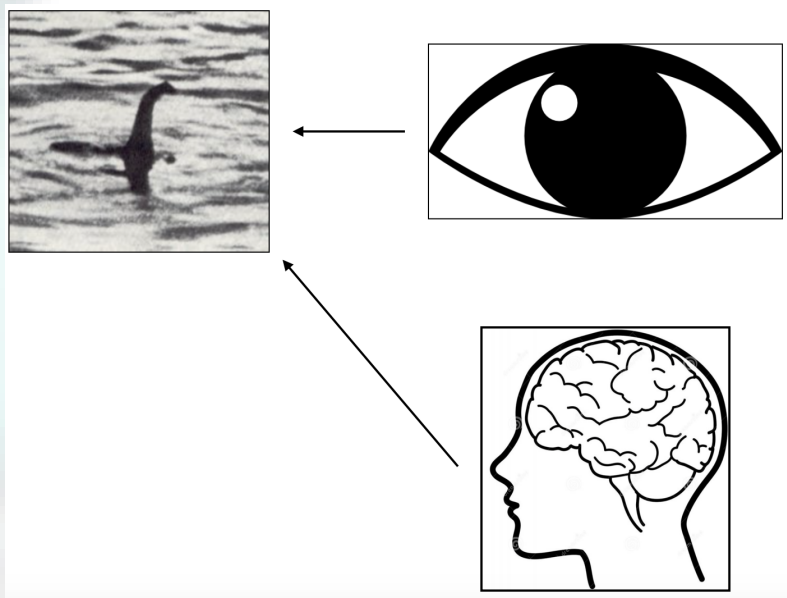


Figure: The brain: a "non-likelihood" organ.

Bayesian thinking

- with "eyes only" our intuition is that $p(N|T) \approx p(T|N)$
- with "the brain" our intuition is that $p(N|T) \approx p(T|N)p(N)$

Our "belief" expresses the probability $p(N)$ **unconditional** of the data.

Question

How can we define $p(N)$?

Bayesian thinking

The "belief" function $p(N)$ is called **prior probability** and the joint product of the likelihood $p(T|N)$ and the prior is proportional to the **posterior probability** $p(N|T)$.

The use of posterior probabilities for inferences is called Bayesian statistics.

Statistical inference

If D is the data and θ is your unknown parameter, then

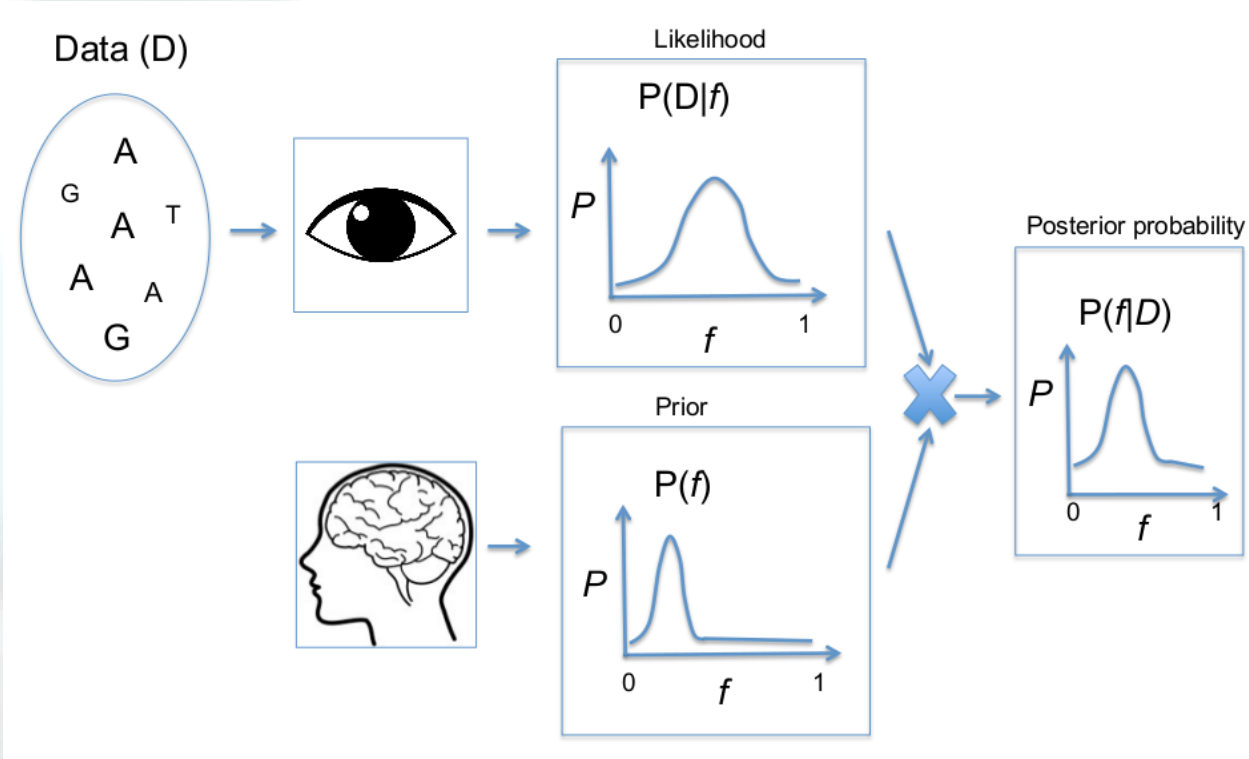
- the frequentist conditions on parameters and integrates over the data, $p(D|\theta)$,
- the Bayesian conditions on the data and integrates over the parameters, $p(\theta|D)$.

Statistical inference

Bayesian *vs.* Likelihoodist

- we derive "proper" probability distributions of our parameters rather than deriving a point estimate;
- a probability is assigned to a hypothesis rather than a hypothesis is tested;
- we can "accept" the null hypothesis rather than "fail to reject" it;
- parsimony imposed in model choice rather than correcting for multiple tests.

Bayesian inference



Bayesian concepts

Bayes' Theorem

$$p(\vec{\theta}|\vec{y}) = \frac{f(\vec{y}|\vec{\theta})\pi(\vec{\theta})}{m(\vec{y})} = \frac{f(\vec{y}|\vec{\theta})\pi(\vec{\theta})}{\int f(\vec{y}|\vec{\theta})d\vec{\theta}} \quad (1)$$

- $\vec{\theta}$ is not a fixed parameter but a random quantity with prior distribution $\pi(\vec{\theta})$
- $p(\vec{\theta}|\vec{y})$ is the posterior probability distribution of $\vec{\theta}$
- $\int p(\vec{\theta}|\vec{y})d\vec{\theta} = 1$

Genotype posterior probability

AAAG & $\epsilon = 0.01$ & A,G alleles

Genotype	Likelihood (log)	Prior	Posterior
AA	-5.73		
AG	-2.80		
GG	-17.12		

Genotype posterior probability

AAAG & $\epsilon = 0.01$ & A,G alleles

Genotype	Likelihood (log)	Prior	Posterior
AA	-5.73	1/3	0.05
AG	-2.80	1/3	0.95
GG	-17.12	1/3	0

Only call genotypes if the largest probability is above a certain threshold (e.g. 0.95).

Genotype posterior probability

AAAG & $\epsilon = 0.01$ & A,G alleles & **A is the reference allele**
$P(AA) > P(AG) > P(GG)$

Genotype	Likelihood (log)	Prior	Posterior
AA	-5.73	0.80	0.22
AG	-2.80	0.15	0.78
GG	-17.12	0.05	0

The reference allele is just one of the possible alleles, often chosen arbitrarily: why give it so much weight?

Genotype posterior probability

AAAG & $\epsilon = 0.01$ & A,G alleles & $f(A) = 0.7$ from a reference panel
$P(AA) =?$; $P(AG) =?$; $P(GG) =?$

Genotype	Likelihood (log)	Prior	Posterior
AA	-5.73		
AG	-2.80		
GG	-17.12		

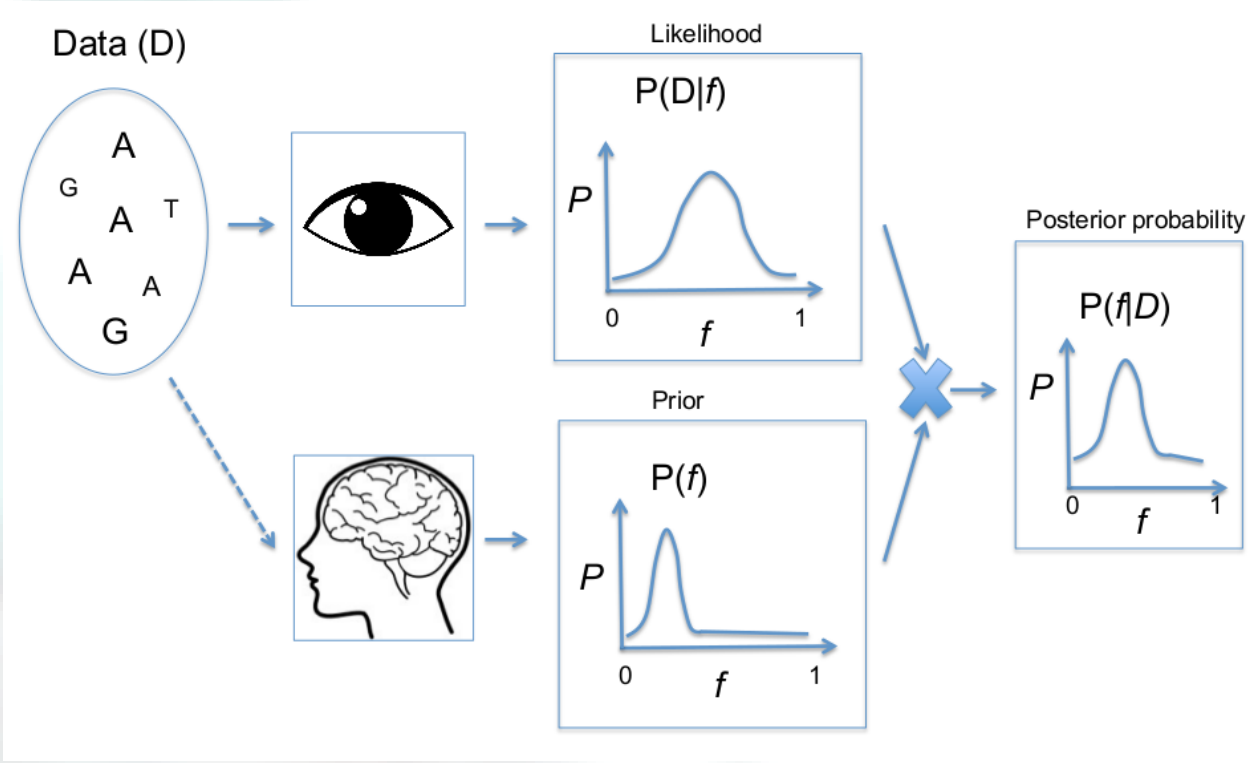
Genotype posterior probability

AAAG & $\epsilon = 0.01$ & A,G alleles & $f(A) = 0.7$ from a reference panel
$P(AA) =?$; $P(AG) =?$; $P(GG) =?$

Genotype	Likelihood (log)	Prior	Posterior
AA	-5.73	0.49	0.06
AG	-2.80	0.42	0.94
GG	-17.12	0.09	0

If the assumption of HWE can be reasonably met.

Empirical Bayesian inference



Genotype posterior probability

AAAG & $\epsilon = 0.01$ & A,G alleles & $f(A) = 0.6$ from the data itself

$P(AA) = ?$; $P(AG) = ?$; $P(GG) = ?$

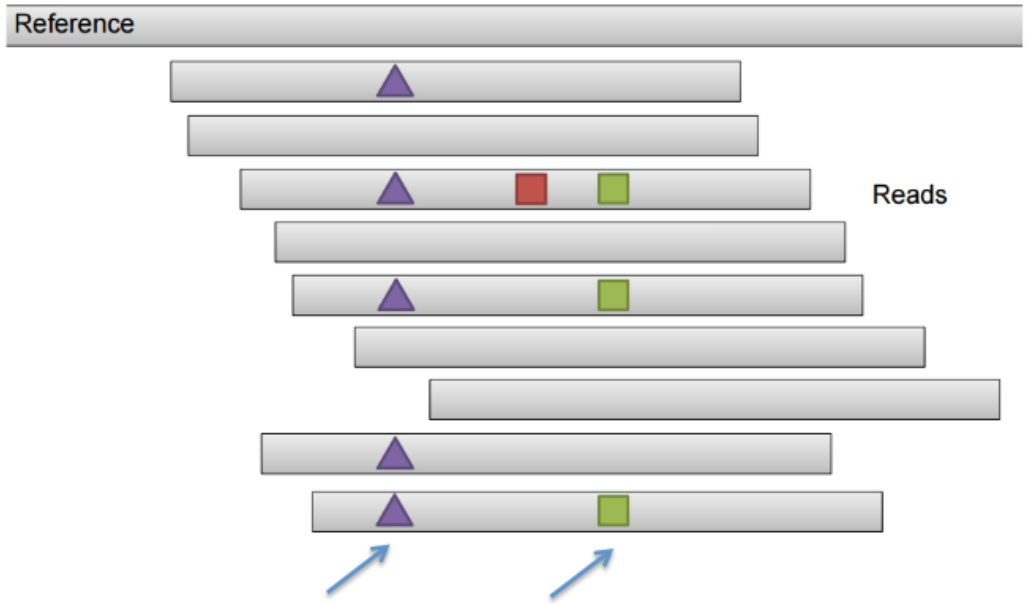
Genotype	Likelihood (log)	Prior	Posterior
AA	-5.73	0.49	0.04
AG	-2.80	0.42	0.96
GG	-17.12	0.09	0

- if the assumption of HWE can be reasonably met
- if you have enough samples to have a robust estimate of the allele frequencies

How can we estimate allele frequencies?

SNP calling procedures

- Alignment-based caller



We completely rely on how reads have been mapped

Figure from Erik Garrison

SNP calling procedures

- Assembly-based caller (as in GATK)

Local re-alignment around putative variants; better resolution for INDELs detection.

- Haplotype-based caller (as in freebayes)

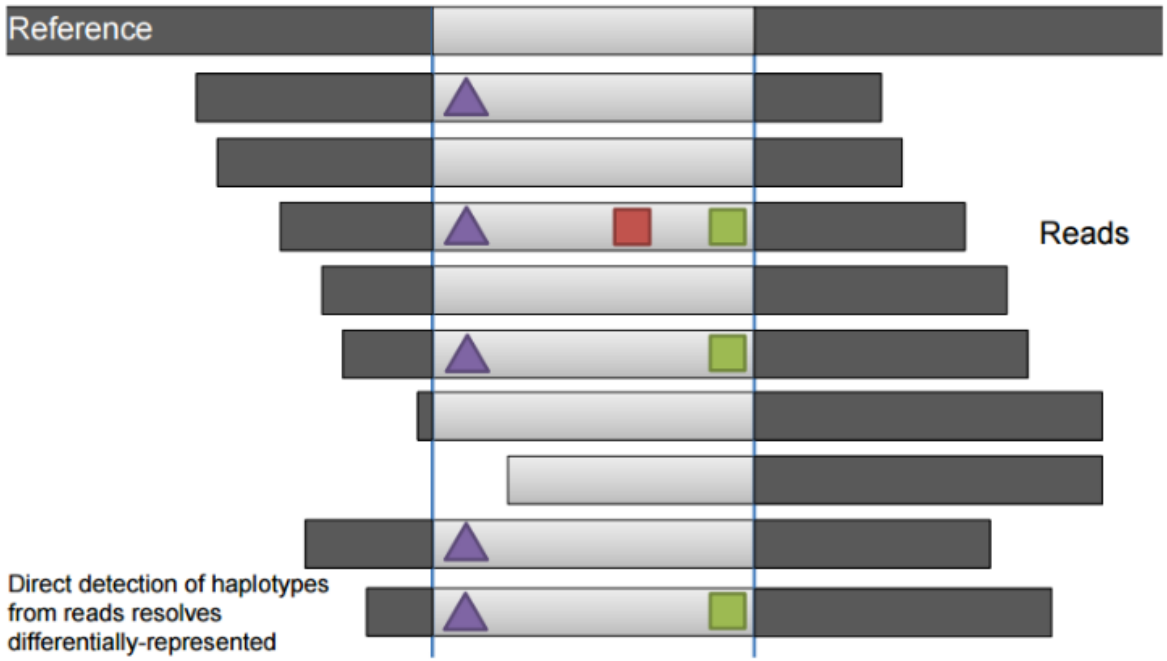


Figure from Erik Garrison

Estimating allele frequencies

Assuming 2 alleles (A,G) with true allele frequency of 0.50

Sample	True genotype	Reads allele A	Read allele G
1	AA	7	0
2	AA	25	1
3	AG	5	3
4	AG	4	4
5	GG	0	2
6	GG	0	4

What is the simplest estimator of allele frequencies?

Estimating allele frequencies

Assuming 2 alleles (A,G) with true allele frequency of 0.50

Sample	True genotype	Reads allele A	Read allele G
1	AA	7	0
2	AA	25	1
3	AG	5	3
4	AG	4	4
5	GG	0	2
6	GG	0	4
Total		41	14

$$\hat{f} = \frac{\sum_{i=1}^{N} n_{A,i}}{\sum_{i=1}^{N} (n_{A,i} + n_{G,i})}$$

$\hat{f} = 0.75$

What is wrong with this estimator?

Estimating allele frequencies

Assuming 2 alleles (A,G) with true allele frequency of 0.50

Sample	True genotype	Reads allele A	Read allele G
1	AA	7	0
2	AA	25	1
3	AG	5	3
4	AG	4	4
5	GG	0	2
6	GG	0	4
Total		41	14

$$\hat{n_A} = \sum_{i=1}^{N} (1-\epsilon) n_{A,i} + \epsilon n_{G,i} - \epsilon n_{A,i} - (1-\epsilon) n_{G,i}$$

$\hat{f} = 0.77$

Estimating allele frequencies

Maximum Likelihood estimator

$$P(D|f) = \prod_{i=1}^{N} \sum_{g \in \{0,1,2\}} P(D|G = g)P(G = g|f)$$

Estimating allele frequencies

Maximum Likelihood estimator

$$P(D|f) = \prod_{i=1}^{N} \sum_{g \in \{0,1,2\}} P(D|G=g)P(G=g|f)$$

$P(D|G=g)$ is the genotype likelihood and $P(G=g|f)$ is given by HWE (for instance).

In our previous example, $\hat{f} = 0.46$ which is much closer to the true value than previous estimators.

SNP calling

Challenges

- If high levels of missing data, then genotypes can be lost.
- Rare variants are hard to detect.
- Trade off between false positive and false negative rates.

How to call SNPs?

- If at least one heterozygous genotype has been called.
- If the estimated allele frequency is above a certain threshold.

SNP calling

Call a SNP if

$$\hat{f} \geq t$$

where t can be the minimum sample allele frequency detectable (e.g. $t = 1/2N$ with N diploids).

Likelihood Ratio Test

A Likelihood Ratio Test (LRT) compares the goodness of fit between the null and the alternative model:

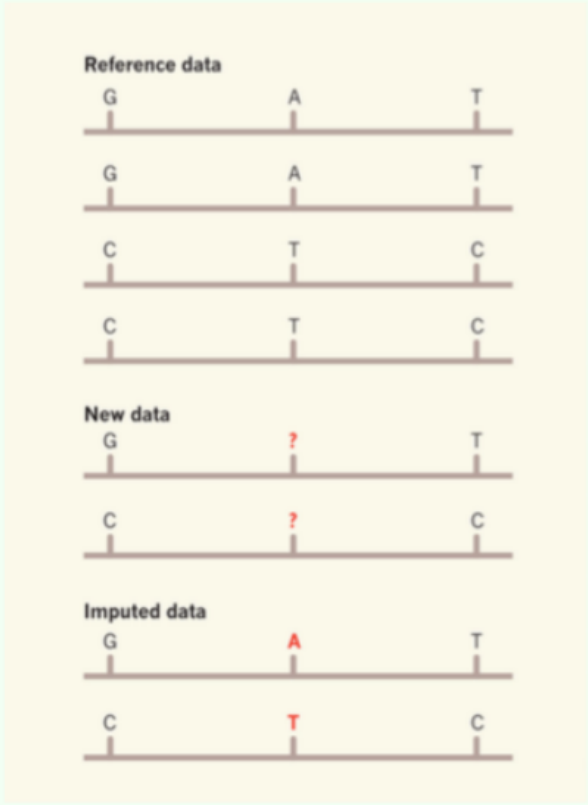
- Null model: $f = 0$
- Alternative model: $f \neq 0$

$$T = -2 \log \frac{L(f = 0)}{L(f = \hat{f}_{MLE})}$$

where T is χ^2 distributed with 1 degree of freedom.

Haplotype imputation

Haplotype imputation - simplified



Reference

- 1000 Genomes
- Phased using family structures

new data

- partial information

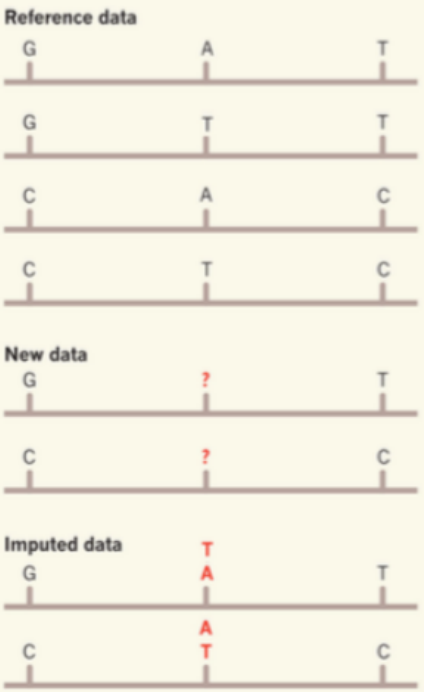
Imputed data

- Probabilistic approach
- The results retains the uncertainty of both the genotype and the haplotypes

Anders Albrechtsen

Haplotype imputation

Haplotype imputation - simplified



Reference

- 1000 Genomes
- Phased using family structures

new data

- Data with known and unknown genotypes

Imputed data

$p(? = T) =$
$p(? = A) =$

Anders Albrechtsen

Haplotype imputation

Haplotype imputation - simplified

Reference

- haplotype frequencies

new data

- Data with known and unknown genotypes

first haplotype

$$p(? = T) = \frac{0.56}{0.56+0.03} = 0.95$$
$$p(? = A) = \frac{0.03}{0.56+0.03} = 0.05$$

second haplotype

$$p(? = T) = \frac{0.21}{0.21+0.2} = 0.51$$
$$p(? = A) = \frac{0.2}{0.21+0.2} = 0.49$$

Anders Albrechtsen

Haplotype imputation

Haplotype imputation - simplified

Bayes formula

$$p(H = h|f, G) = \frac{P(G|H=h)P(H=h|f)}{\sum_{h'} P(G|H=h')P(H=h'|f)}$$

$P(G|H = h)$

1 if consistent

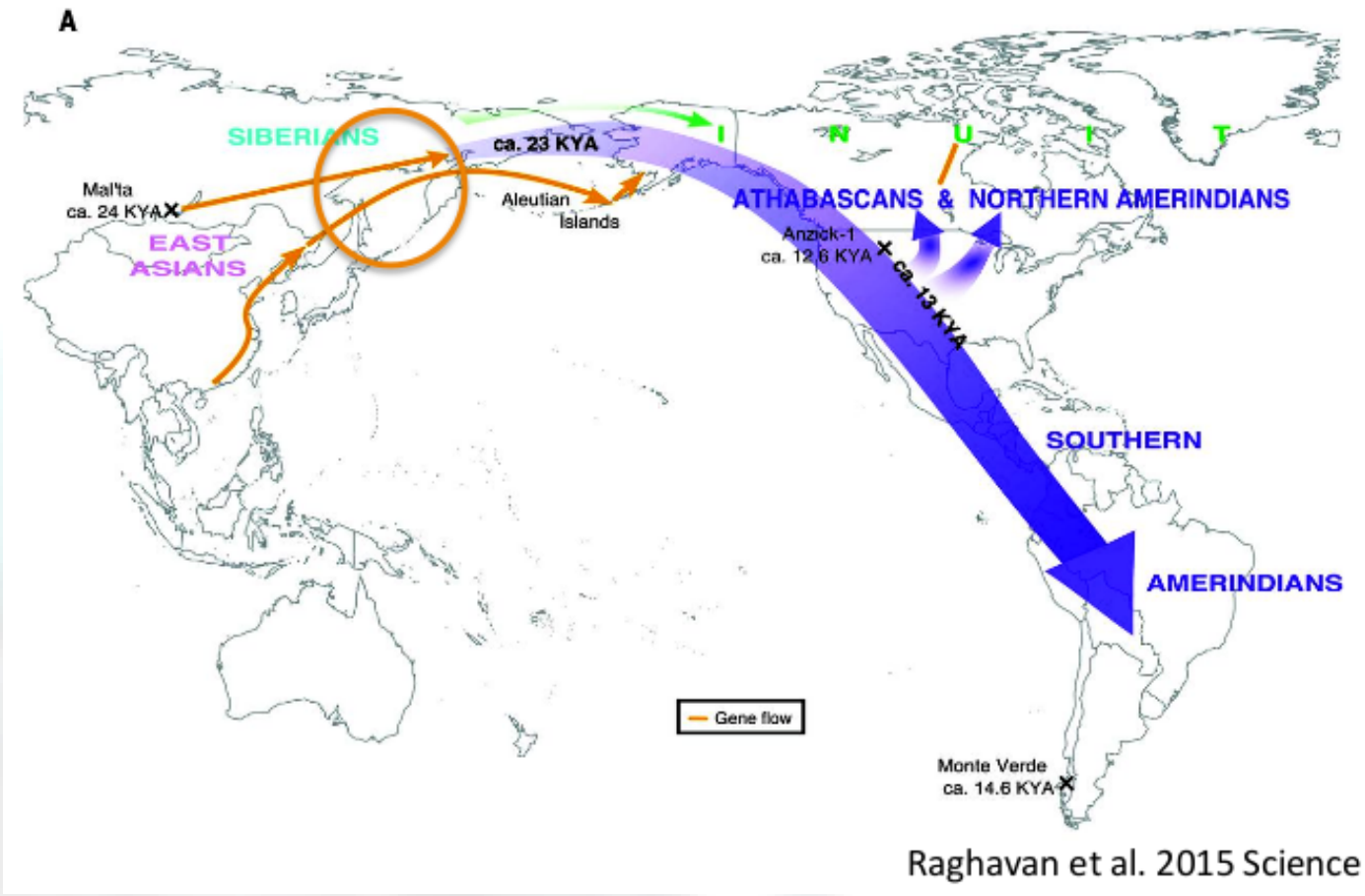
0 otherwise

first haplotype

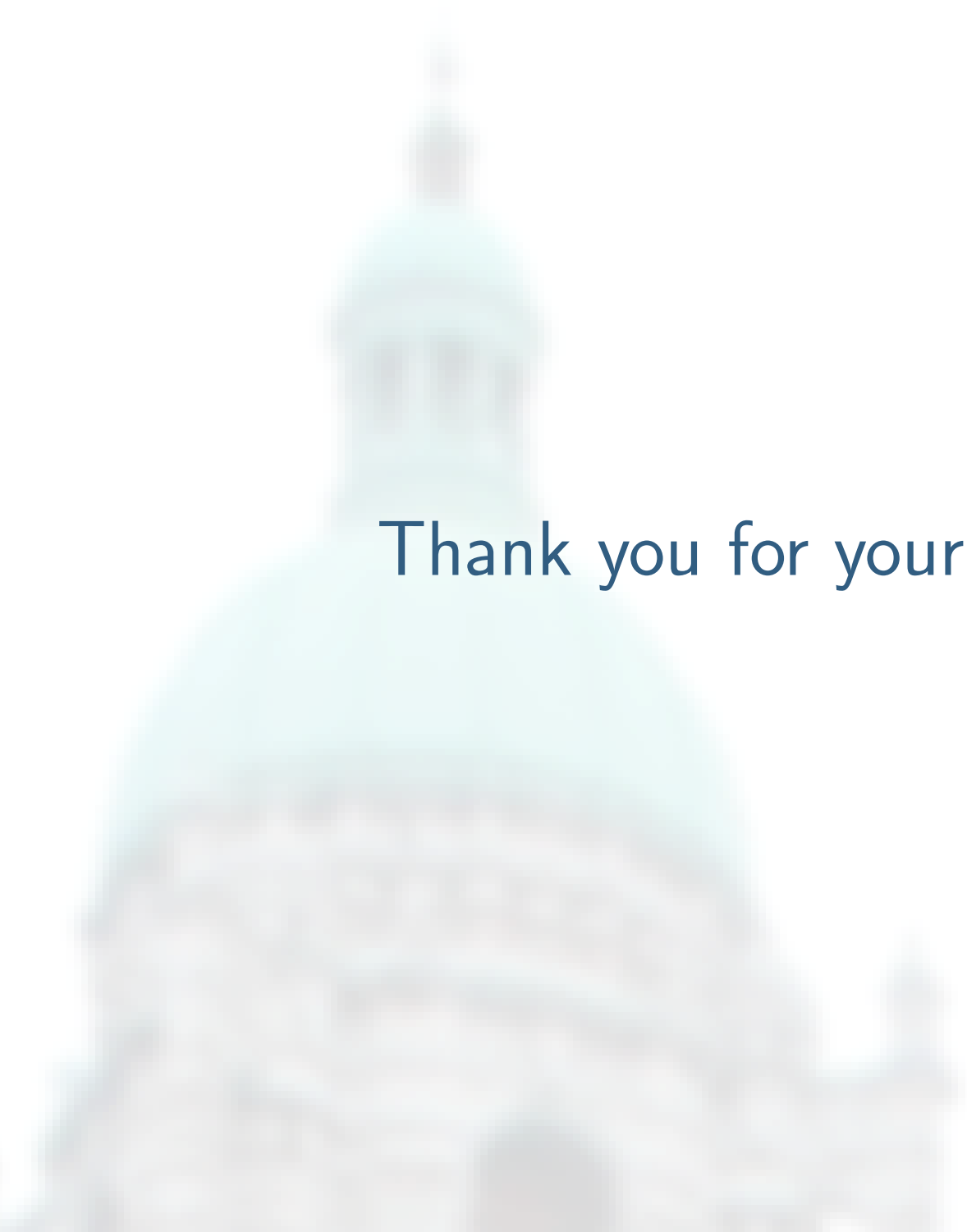
$$p(? = T) = \frac{0.56}{0.56+0.03} = 0.95$$
$$p(? = A) = \frac{0.03}{0.56+0.03} = 0.05$$

Anders Albrechtsen

Practical



Raghavan et al. 2015 Science



Imperial College London

Thank you for your attention