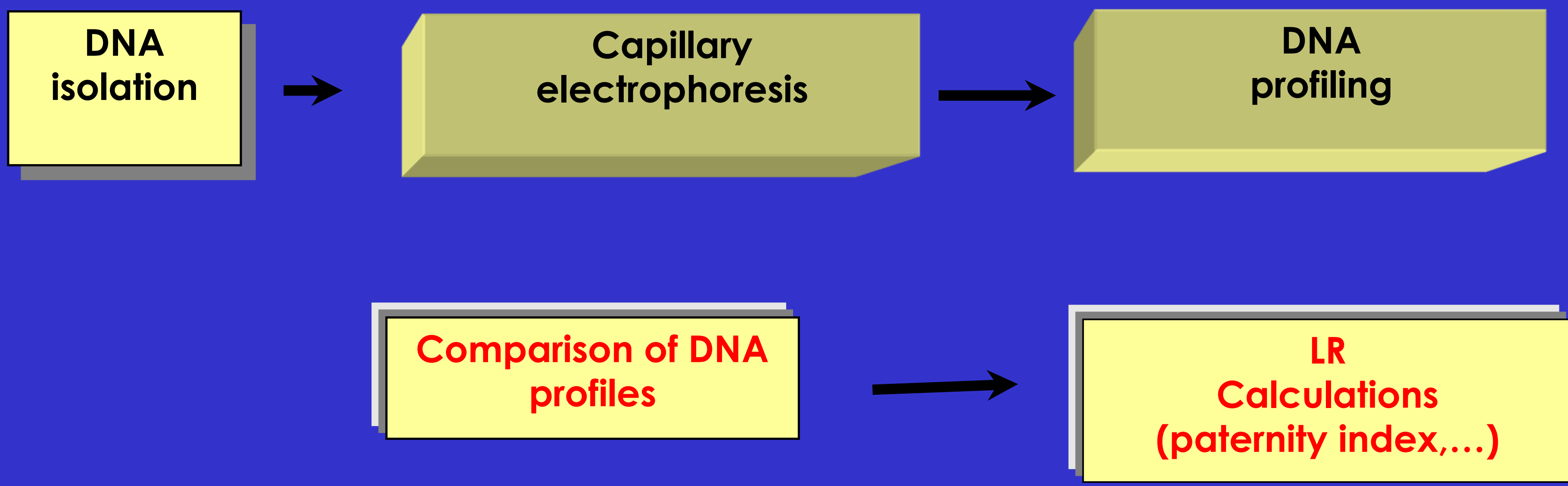
Forensic Genetics and Legal Medicine
2019-2020

4th May 2020

Kinship testing
(standard paternity testing)


DNA isolation
Capillary electrophoresis
DNA profiling
Comparison of DNA profiles
LR
Calculations
(paternity index,…)

Probability of mutually exclusive events

$$Pr(X \text{ or } Y) = Pr(X) + Pr(Y)$$

$Pr(1) + Pr(3) = 2/6$

Probability of independent events

Pr(X and Y) = Pr (X) * Pr (Y)

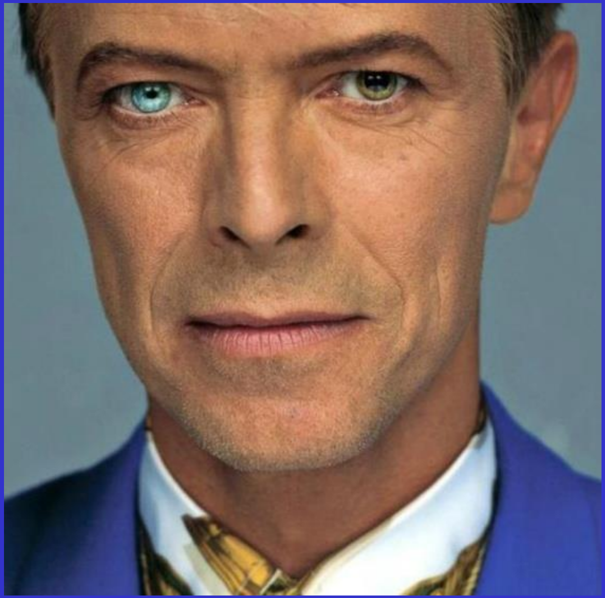
Pr(5) * Pr(6) = 1/36

Conditional probabilty: events are dependent

$$Pr(B/A) = Pr(A \text{ and } B) / Pr(A)$$

or

$$Pr(A \text{ and } B) = Pr(B/A) * Pr(A)$$



The frequency of individuals with a blue right eye is 25% Pr(dx)=0.25

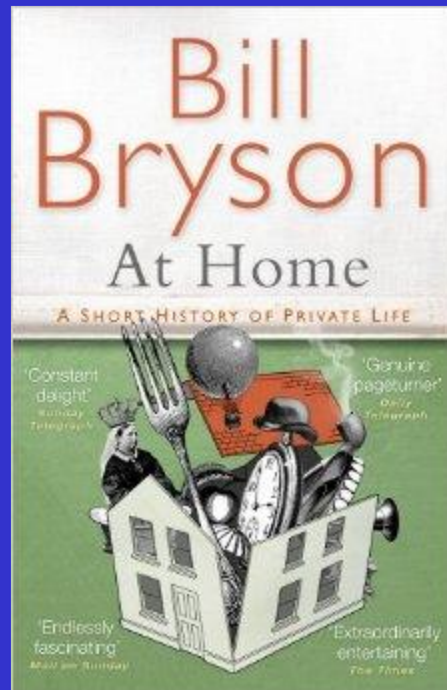
The frequency of individuals with a blue left eye is 25% Pr(sx)=0.25

Probability of having two blue eyes?

- ~~0.25 X 0.25 = 0.06~~

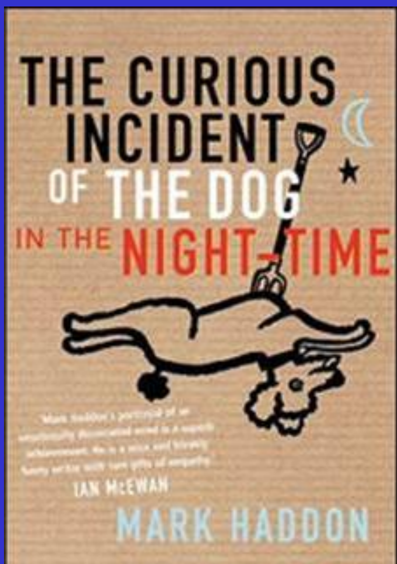
Pr(left eye is blue / right eye is blue) ~ 1

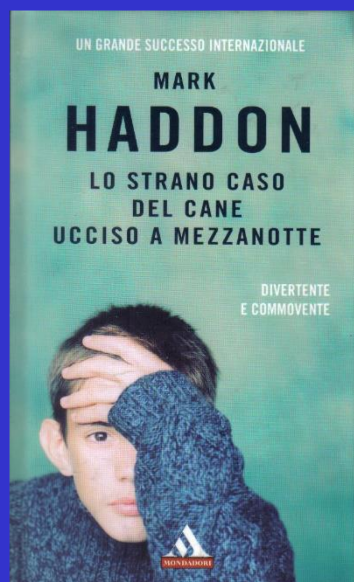
- Pr (both left and right eye are blue) = 1 x 0.25



Thomas Bayes

*And now ladies and gentlemen, the Monty Hall problem**





**Go to last two slides for solution*

BAYES THEOREM: revising a probability value based on additional information that is later obtained.

- ✓ B1 and B2 are two mutually exclusive and exhaustive events
- ✓ A is the conditioning element

$$Pr(B1/A) = \frac{Pr(B1_{and}A)}{Pr(A)^*} = \frac{Pr(B1_{and}A)}{Pr(B1_{and}A) + Pr(B2_{and}A)} = \frac{Pr(A/B1) * Pr(B1)}{Pr(A/B1)^* Pr(B1) + Pr(A/B2)^* Pr(B2)}$$

Imagine you are blindfolded and then asked to pick a ball from a bag…

- ✓ **Pr(B1)** = p of picking a black ball from the bag **1/3**
- ✓ **Pr(B2)** = p of picking a white ball from the bag **2/3**

What would you bet, black or white?

- ✓ Each ball carries a number (1 or 2)
- • of black balls, ¾ carry number 1: **Pr(A/B1)** = **3/4**
- • of white balls, ¼ carry number 1: **Pr(A/B2)** = **1/4**
- ✓ **A** = the ball you picked carries number 1!

And after this additional information?

***Pr(A)** = p of picking a number 1 ball is the sum of p_s of picking a black number 1 ball or a white number 1 ball

Pr(B1/A) = 3/5

For B2 we'll have:

$$Pr(B2/A) = \frac{Pr(A/B2) * Pr(B2)}{Pr(A/B2) * Pr(B2) + Pr(A/B1) * Pr(B1)}$$

We can also calculate the ratio of Pr B1 and B2 given A:

$$\frac{Pr(B1/A)}{Pr(B2/A)} = \frac{Pr(A/B1) * Pr(B1)}{Pr(A/B1) * Pr(B1) + Pr(A/B2) * Pr(B2)} * \frac{Pr(A/B2) * Pr(B2) + Pr(A/B1) * Pr(B1)}{Pr(A/B2) * Pr(B2)}$$

$$\frac{Pr(B1/A)}{Pr(B2/A)} = \frac{Pr(A/B1)}{Pr(A/B2)} * \frac{Pr(B1)}{Pr(B2)}$$

In our "bag & balls" example, Pr(B1/A) / Pr (B2/A) = 3/2

In DNA identity testing the two mutually exclusive and exhaustive hypotheses are:

A: the tested subject is the donor of the stain;

D: the tested subject is not the donor of the stain.

Additional information comes from genetic data G.

$$\frac{Pr\,(A / G)}{Pr\,(D / G)} = \frac{\mathbf{Pr\,(G / A)}}{\mathbf{Pr\,(G / D)}} \times \frac{Pr\,(A)}{Pr\,(D)}$$

↓

$$\textbf{Likelihood Ratio (LR)} = \frac{\mathbf{1}}{\textbf{Random match probability}}$$

In paternity testing the two mutually exclusive and exhaustive hypotheses are :

P: the tested subject is the biological father;

N: the tested subject is not the biological father.

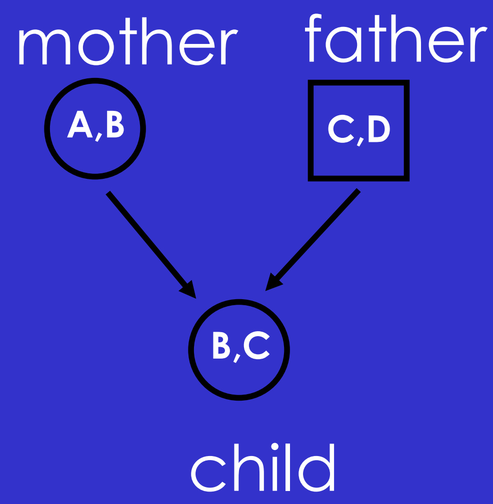
Additional information comes from genetic data G.

$$\frac{Pr\,(P\,/\,G)}{Pr\,(N\,/\,G)} = \frac{\mathbf{Pr\,(G\,/\,P)}}{\mathbf{Pr\,(G\,/\,N)}} \times \frac{Pr\,(P)}{Pr\,(N)}$$

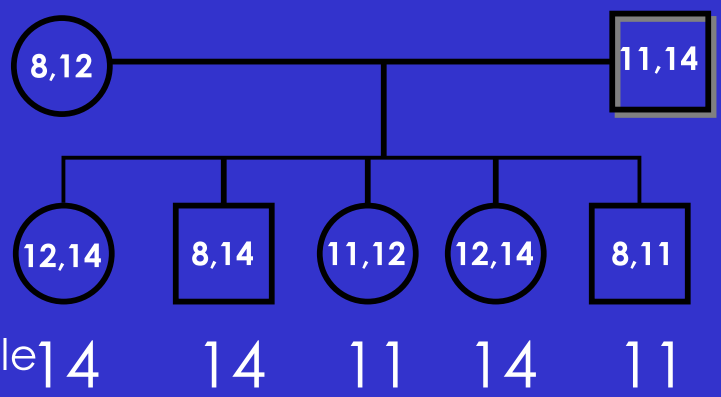
↓ Likelihood ratio **Paternity index (PI)**

↓ **Probability of paternity (W)** → from German Wahrscheinlichkeit (probability)

(A) Mendelian Inheritance



(B) Example



Obligate paternal allele 14 14 11 14 11

PATERNITY INDEX, PI

Consider a biallelic locus with alleles P and Q
Let's assume the child is "PQ", mother "QQ" and alleged father "PQ"

$$\frac{Pr(P / G)}{Pr(N / G)} = \frac{\mathbf{Pr(G / P)}}{\mathbf{Pr(G / N)}} \times \frac{Pr(P)}{Pr(N)}$$

Pr (G / P) = ???

Pr (F_{PQ}/M_{QQ} & P_{PQ}) = Pr (F_{PQ} and M_{QQ} & P_{PQ}) / Pr (M_{QQ} & P_{PQ})

Possibile couples	Freq. Possibile couples	Freq. of children PP	Freq. of children PQ	Freq. of children QQ
PPXPP	p^4	p^4	-	-
PPXPQ	2^2p^3q	$2p^3q$	$2p^3q$	-
PPXQQ	$2p^2q^2$	-	$2p^2q^2$	-
PQXPQ	$2^2 p^2q^2$	p^2q^2	$2p^2q^2$	p^2q^2
PQXQQ	$2^2 pq^3$	-	$2pq^3$	$2pq^3$
QQXQQ	q^4	-	-	q^4

$$Pr\ (F_{PQ}/M_{QQ}\ \&\ P_{PQ}) = Pr\ (F_{PQ} \text{ and } M_{QQ}\ \&\ P_{PQ})\ /\ Pr\ (M_{QQ}\ \&\ P_{PQ})$$

PATERNITY INDEX, PI

Consider a biallelic locus with alleles P and Q
Let's assume the child is "PQ", mother "QQ" and alleged father "PQ"

$$\frac{Pr(P / G)}{Pr(N / G)} = \frac{\mathbf{Pr(G / P)}}{\mathbf{Pr(G / N)}} \times \frac{Pr(P)}{Pr(N)}$$

$Pr(G / P) = ???$

$Pr(F_{PQ}/M_{QQ} \ \& \ P_{PQ}) = Pr(F_{PQ} \text{ and } M_{QQ} \ \& \ P_{PQ}) / Pr(M_{QQ} \ \& \ P_{PQ})$

$Pr(G / N) = ???$

$Pr(F_{PQ}/M_{QQ}) = Pr(F_{PQ} \text{ and } M_{QQ}) / Pr(M_{QQ})$

Possibile couples	Freq. Possibile couples	Freq. of children PP	Freq. of children PQ	Freq. of children QQ
PPXPP	p^4	p^4	-	-
PPXPQ	$4\,p^3q$	$2p^3q$	$2p^3q$	-
PPXQQ	$2p^2q^2$	-	$2p^2q^2$	-
PQXPQ	$4p^2q^2$	p^2q^2	$2p^2q^2$	p^2q^2
PQXQQ	$4pq^3$	-	$2pq^3$	$2pq^3$
QQXQQ	q^4	-	-	q^4

$$Pr\ (F_{PQ}/M_{QQ}) = Pr\ (F_{PQ} \text{ and } M_{QQ})\ /\ Pr\ (M_{QQ})$$

$$\frac{Pr(P/G)}{Pr(N/G)} = \frac{Pr(G/P)}{Pr(G/N)} \times \frac{Pr(P)}{Pr(N)} = \frac{1/2}{p} \times \frac{Pr(P)}{Pr(N)}$$

$Pr(G/P) = Pr(F_{PQ}/M_{QQ} \ \& \ P_{PQ}) = Pr(F_{PQ} \text{ and } M_{QQ} \ \& \ P_{PQ}) / Pr(M_{QQ} \ \& \ P_{PQ}) = pq^3 / 2pq^3 = 1/2$

$Pr(G/N) = Pr(F_{PQ}/M_{QQ}) = Pr(F_{PQ} \text{ and } M_{QQ}) / Pr(M_{QQ}) = (p^2q^2 + pq^3) / q^2 = pq^2 \times (p + q) / q^2 = p$

If p = 0.2 (1/5) then PI = 5/2 = 2.5. The observed genotypes are 2.5 times more likely according to the hypothesis of paternity.

PI values obtained with a standard set of 16 independent STRs can be freely multiplied, reaching, on average, PI combined values of ~ 5×10^{10}

PROBABILITY OF PATERNITY (W)

It is reasonable to assume that, a priori, the probability of paternity and non paternity are equal (Essen-Moeller transformation), consequently:

$$\frac{Pr(P / G)}{Pr(N / G)} = \frac{1/2}{p} * 1$$

If p = 0.2, PI = 5/2 given the observed genotypes: it means that in 5 cases out of 7 (71%) paternity is true, whereas in 2 cases out of 7 the observed genetic compatibility is adventitiuos. 5/7 = 5/2 / (5/2+1)

W is then calculated according to the general formula:

$$W = \frac{PI}{PI + 1}$$

What PI/W to enough paternity?

- Gendiagnostikgesetz (new German law regulating human genetics as well as paternity analyses, 2013): W > 99.9% (PI > 1000)
- Italian Society for Human Genetics (SIGU, 2013): PI > 10000
- Italian working group of the International Society for Forensic Genetics (GeFI, 2018): W > 99.99% (PI > 10000)

Gc = child
Gm = mother
Gtm = alleged father

#	G_C	G_M	G_{TM}	Numerator (X)	Denominator (Y)	Paternity Index (PI)
1	PP	PP	PP	1	p	1/p
2			PQ	1/2	p	1/2p
3			QR	0	p	0
4		PQ	PP	1/2	p/2	1/p
5			PQ	1/4	p/2	1/2p
6			PR	1/4	p/2	1/2p
7			QR	0	p/2	0
8	PQ	PP	QQ	1	q	1/q
9			PQ	1/2	q	1/2q
10			QR	1/2	q	1/2q
11			RS	0	q	0
12		PQ	PP	1/2	(p+q)/2	1/(p+q)
13			PQ	1/2	(p+q)/2	1/(p+q)
14			PR	1/4	(p+q)/2	1/[2(p+q)]
15			QR	1/4	(p+q)/2	1/[2(p+q)]
16			RS	0	(p+q)/2	0
17		QR	QQ	0	p/2	0
18			PQ	1/4	p/2	1/2p
19			QR	0	p/2	0
20			QS	0	p/2	0
21			RS	0	p/2	0

$$\Pr(PP|PP) = \frac{[p(1-\theta)+2\theta]\,[p(1-\theta)+3\theta]}{(1+\theta)(1+2\theta)}$$

$$\Pr(PQ|PQ) = \frac{2[p(1-\theta)+\theta]\,[q(1-\theta)+\theta]}{(1+\theta)(1+2\theta)}$$

Formulas can be modified in order to accomodate:

- Coancestry

θ / F_{ST} = the probability that two alleles, one taaken at random from each of two individuals are identical by descent (0.01-0.03)

Mutations

Mutation rate of standard forensic STRs (μ) is on average 2 out of 1000 meiosis (i.e. 3% chance of mutation whit a 15 STRs panel).

μ varies according to:

- parent's sex (higher in males than females depending on gametogeneis)
- Father's age (higher in older fathers)
- STR molecular structure (higher for more complex STRs)

Dedicated software treat mutation according to mutation models of different complexity. Easiest way:

PI_{μ} = PI at a locus showing a mismatch
μ = locus specific mutation rate
PE_x = locus specific average probability of exclusion

$$PI_{\mu} = \mu / PE_x$$

$$\downarrow$$

$$H^2(1-2H(1-H)^2)$$

H = locus heterozygosity
$H = 1 - \Sigma p^2$
p = frequency of each allele for that STR

Apparent Mutations Observed at STR Loci in the Course of Paternity Testing*

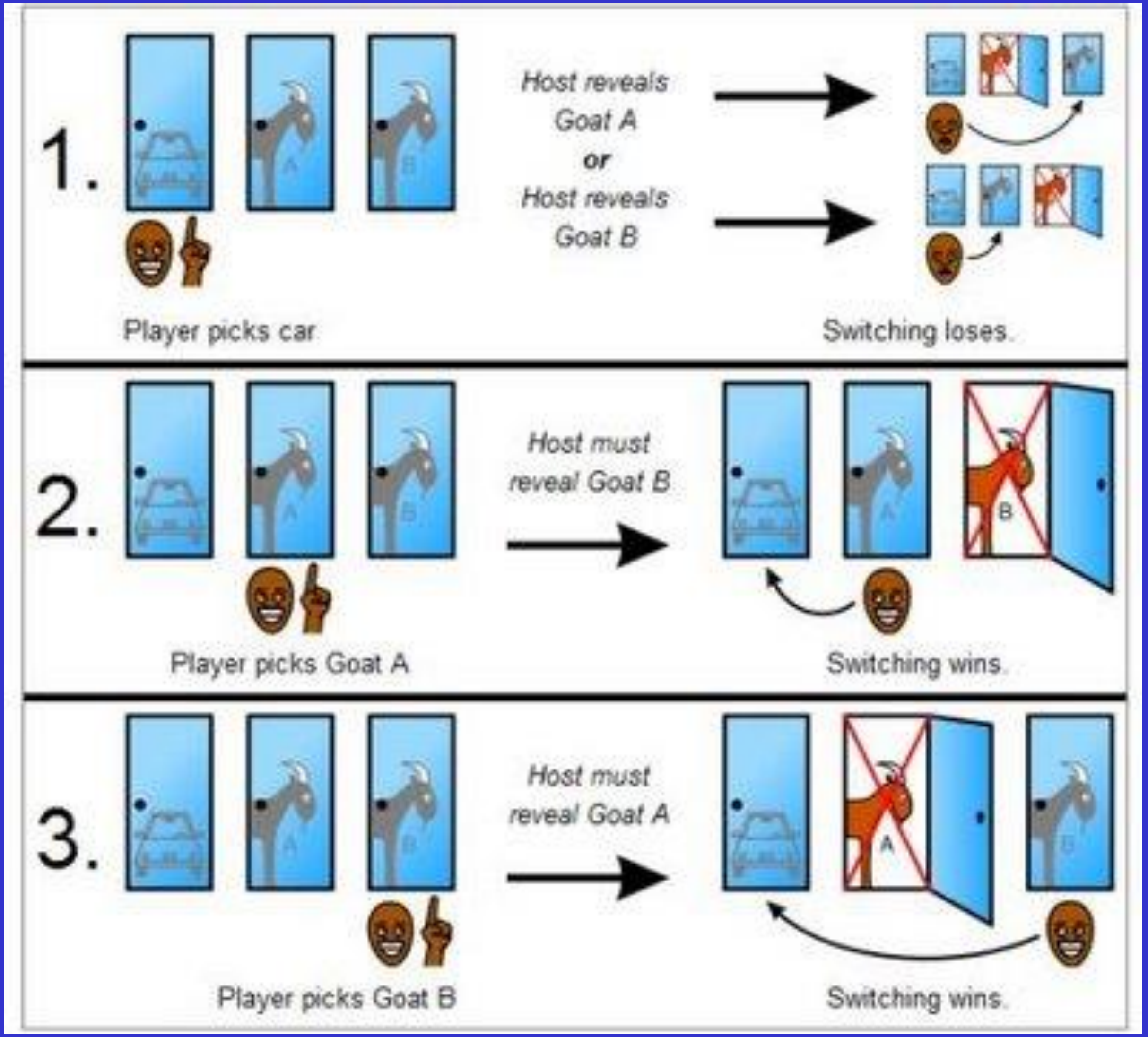
STR System	Maternal Meioses (%)	Paternal Meioses (%)	Number from either	Total Number of Mutations	Mutation Rate
CSF1PO	95/304,307 (0.03)	982/643,118 (0.15)	410	1,487/947,425	0.16%
FGA	205/408,230 (0.05)	2,210/692,776 (0.32)	710	3,125/1,101,006	0.28%
TH01	31/327,172 (0.009)	41/452,382 (0.009)	28	100/779,554	0.01%
TPOX	18/400,061 (0.004)	54/457,420 (0.012)	28	100/857,481	0.01%
VWA	184/564,398 (0.03)	1,482/873,547 (0.17)	814	2,480/1,437,945	0.17%
D3S1358	60/405,452 (0.015)	713/558,836 (0.13)	379	1,152/964,288	0.12%
D5S818	111/451,736 (0.025)	763/655,603 (0.12)	385	1,259/1,107,339	0.11%
D7S820	59/440,562 (0.013)	745/644,743 (0.12)	285	1,089/1,085,305	0.10%
D8S1179	96/409,869 (0.02)	779/489,968 (0.16)	364	1,239/899,837	0.14%
D13S317	192/482,136 (0.04)	881/621,146 (0.14)	485	1,558/1,103,282	0.14%
D16S539	129/467,774 (0.03)	540/494,465 (0.11)	372	1,041/962,239	0.11%
D18S51	186/296,244 (0.06)	1,094/494,098 (0.22)	466	1,746/790,342	0.22%
D21S11	464/435,388 (0.11)	772/526,708 (0.15)	580	1,816/962,096	0.19%
Penta D	12/18,701 (0.06)	21/22,501 (0.09)	24	57/41,202	0.14%
Penta E	29/44,311 (0.065)	75/55,719 (0.135)	59	163/100,030	0.16%
D2S1338	15/72,830 (0.021)	157/152,310 (0.10)	90	262/225,140	0.12%
D19S433	38/70,001 (0.05)	78/103,489 (0.075)	71	187/173,490	0.11%
SE33 (ACTBP2)	0/330 (<0.30)	330/51,610 (0.64)	None reported	330/51,940	0.64%

*Data used with permission from American Association of Blood Banks (AABB) 2003 Annual Report.

How many mismatches are enough to exclude paternity?

- Gendiagnostikgesetz (new German law regulating human genetics as well as paternity analyses, 2013): at least 15 STR need to be typed and >3 mismatches need to be observed to declare paternity exclusion
- Italian Society for Human Genetics (SIGU, 2013): regardless of the number of observed mismatches, it is always necessary to perform LR (PI) calculations and paternity can be excluded when PI is < 0.0001 → Formally correct, but possibly unpractical for Courts
- Italian working group of the International Society for Forensic Genetics (GeFI, 2018): at least 15 STR need to be typed and >2 mismatches need to be observed to declare paternity exclusion (PI calculation optional)

And now ladies and gentlemen, the Monty Hall problem…solved



A priori

Pr(N) = Pr(car not changing door) = Pr(car) =1/3

Pr(C) = Pr(car changing door)= Pr(door chosen does not hide car) * Pr(new door picked doesn't hide goat) = 2/3 * ½ = 1/3

…to change or not to change is irrelevant

Additional information (A)

Monty (who knows where the car is) shows that, behind one of the doors which was not chosen, there's a goat

Conditional probability

Pr(A/N) = Pr(Monty shows that goat, given that the door initially chosen hides the car) =1/2

Pr(A/C) = Pr(Pr(Monty shows that goat, given that the door initially chosen hides a goat) = 1

A posteriori

$$\frac{Pr(N/A)}{Pr(C/A)} = \frac{Pr(A/N)}{Pr(A/C)} * \frac{(Pr(N)}{Pr(C)} = \frac{1/2}{1} * \frac{1/3}{1/3} = \mathbf{1/2}$$

…twice more likely to win the car if changing door!!!