That's a very strange boy

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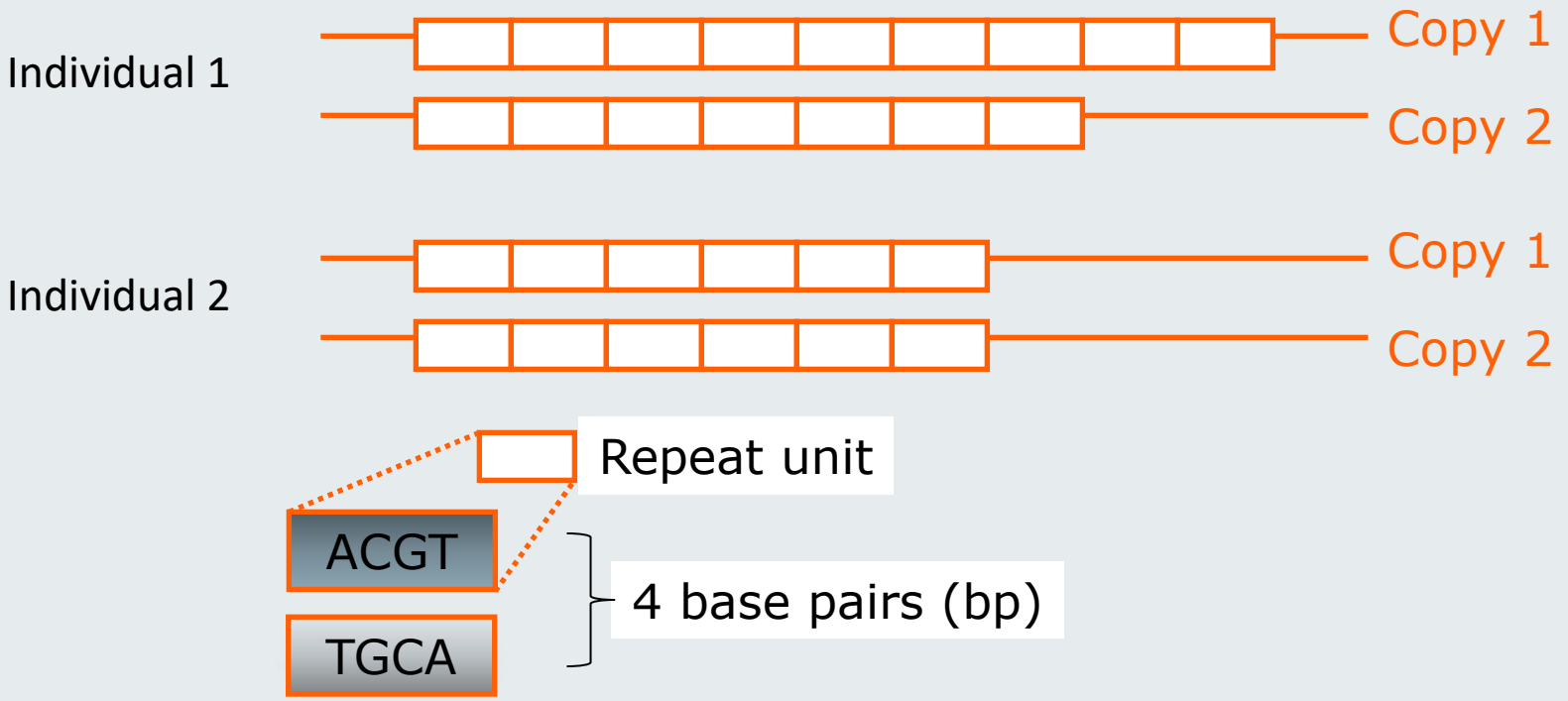
Outline

- Background
  - Paternity testing
    - Non-invasive assessment of paternity
  - Initial concerns
- Case 1
  - Short tandem repeat testing
- Case 2
  - Single nucleotide polymorphisms
- Case 3
  - Y phylogeny
- Case 4
  - The sting
- Outcome

The background

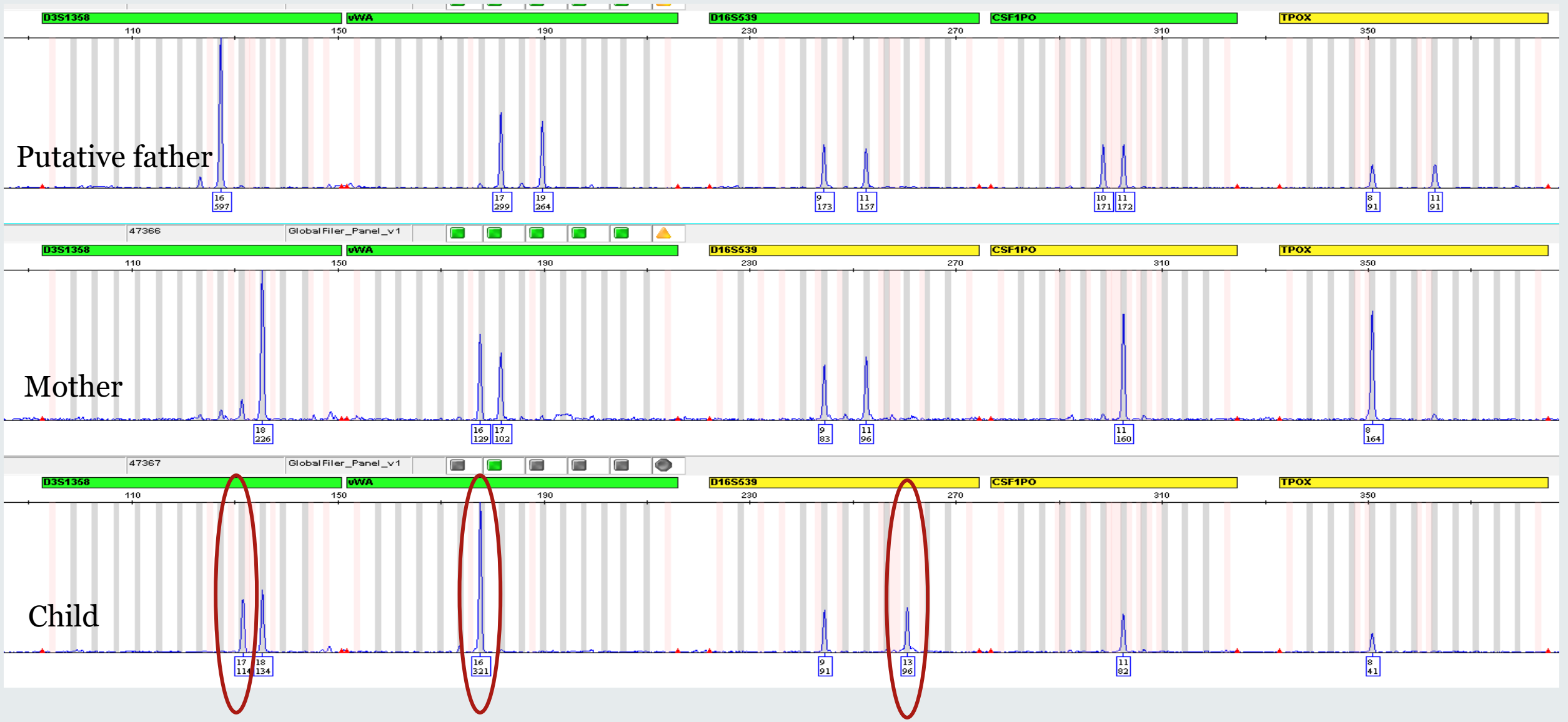
New Scientist Special Investigation

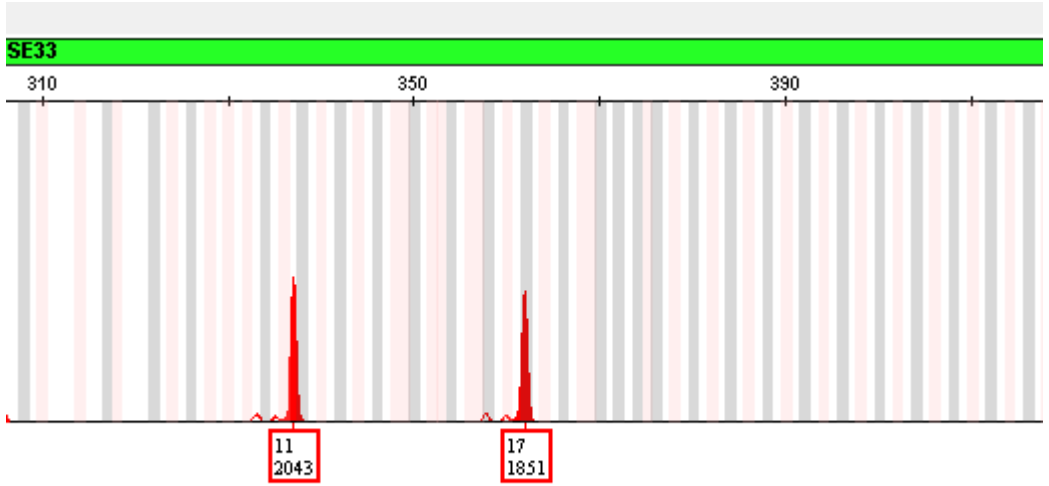
Short tandem repeat



Short sequences of 4 (generally) nucleotide bases, **repeat**ed in **tandem**

Paternity trio

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17
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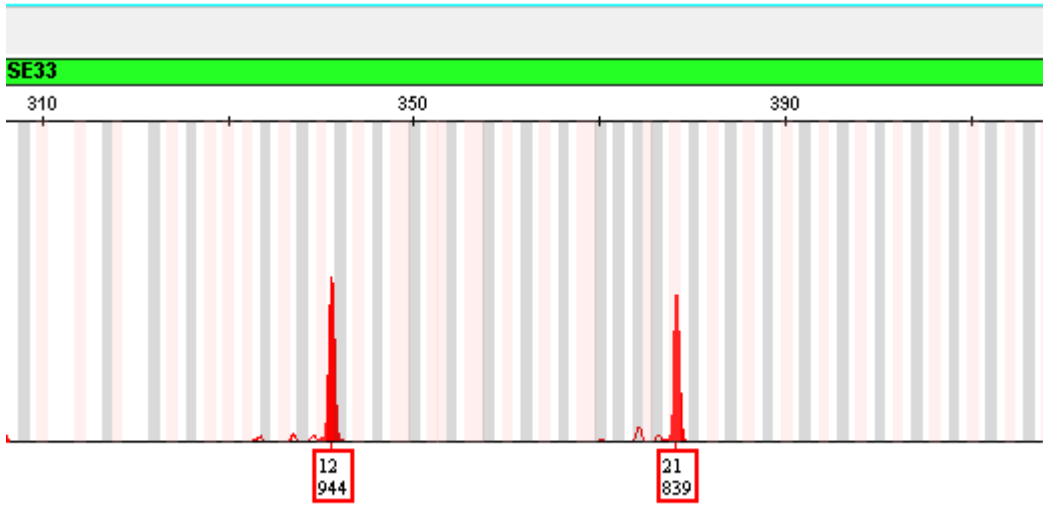
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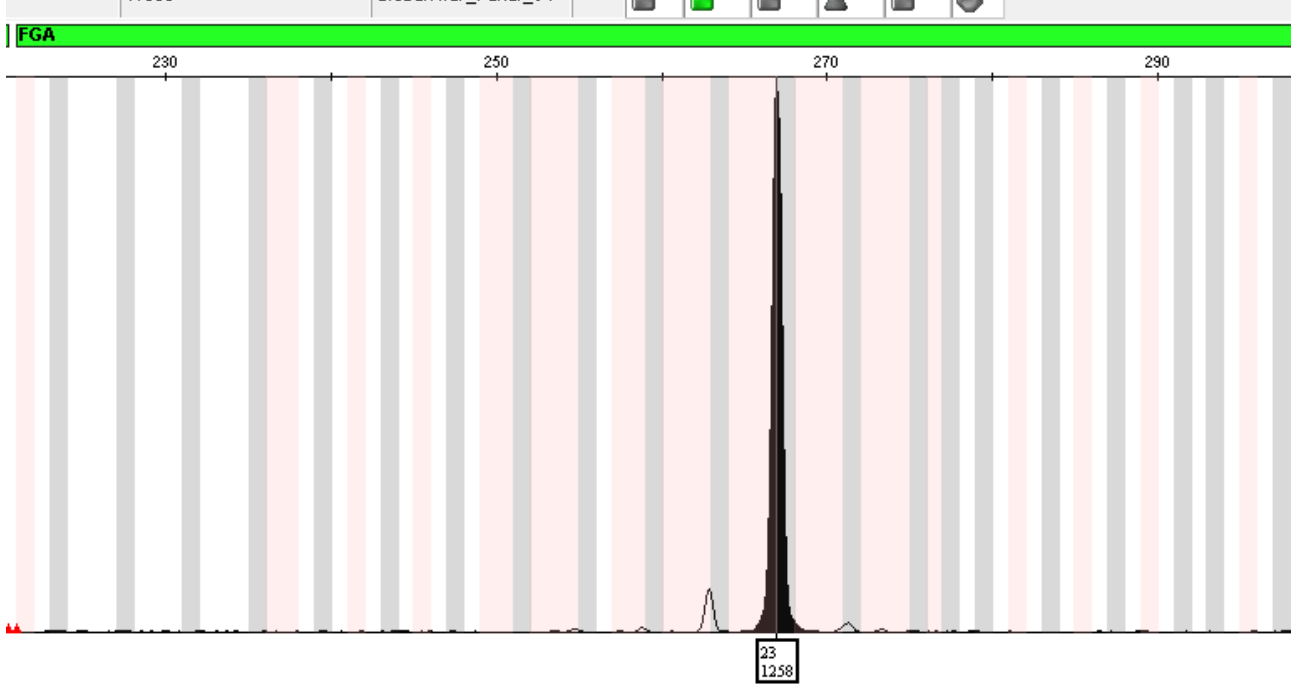

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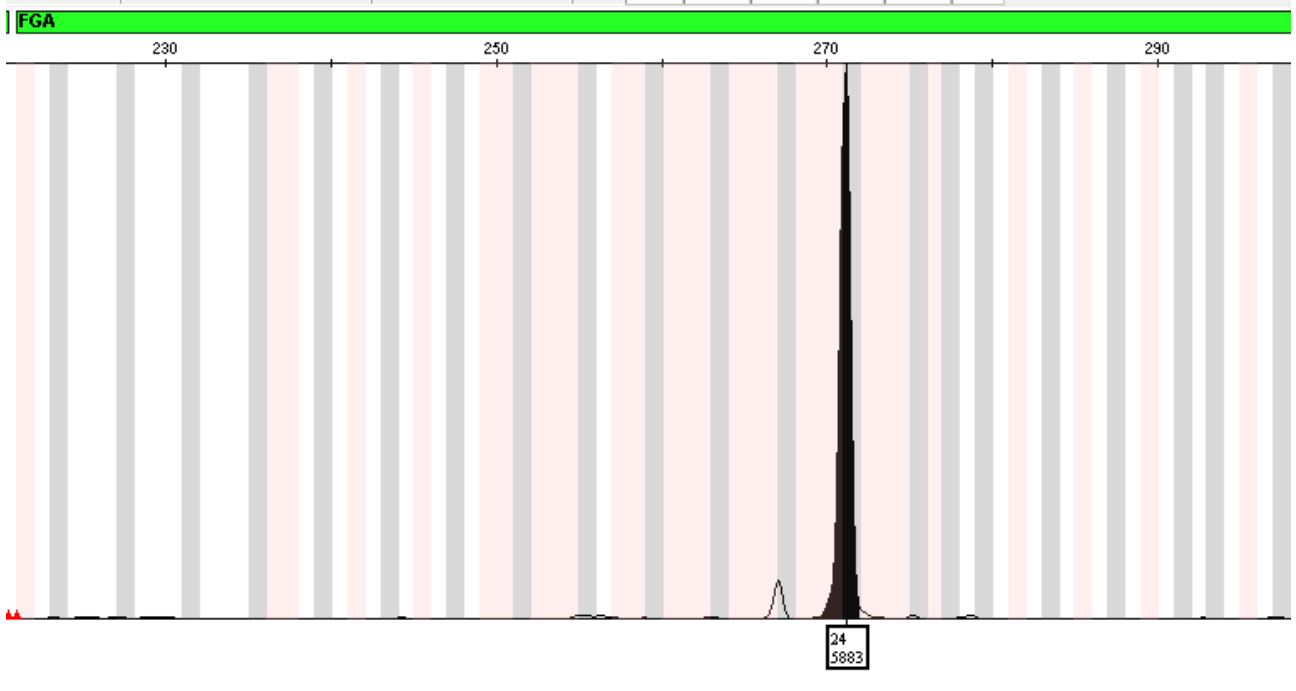


Paternity as a diagnostic test

A good test is one which will minimize the number of false positive and false negative results

False positive – telling someone that they have/may have a disease, when in fact they do not

Significant false positive rates for mammograms – but follow-up to investigate

False positive – telling someone that they are the father of a child, when they are not

Not tolerable – an easy mistake in some circumstances – to be avoided at all costs

Paternity as a diagnostic test

False negative – implying that the test result means that they do not have the disease, when in fact they do

Significant false negative rates for cervical cytology – but followed up with repeats over time

False negative – telling someone that they are not the father of a child, when they are

Not tolerable - but an easy mistake – to be avoided at all costs

Unlike diagnostic/screening tests – normally a 'one off'

Paternity

- Practitioners need to be ACCREDITED if the results are to be relied on
  - Few are accredited
  - Many offer internet based services
  - Value of service often unclear
    - Highly accurate
    - 99.99% accurate
- UK based practitioners are examined annually and need to undertake regular independent proficiency tests
- One UK based practitioner offers invasive(amniocentesis/CVS) prenatal accredited tests

Non-invasive paternity

- Practitioners need to be ACCREDITED if the results are to be relied on
  - No one is accredited
  - Several offer internet based services
    - 'No risk'
    - 99.99% accurate
  - What does 99.99% accurate mean? The results, or the conclusion?
  - All results should be presented as a likelihood ratio of the GENETIC ODDS, not a percentage as that involves knowing the PRIOR ODDS

Non-invasive paternity – detection of fetal material in maternal blood

- More fetal material in maternal circulation as gestation lengthens
- Need to be able to do something reliably between 10 and 14 weeks to reduce emotional and physical stress
- Problem of differential amplification of large amounts of maternal DNA
- VARIOUS SOLUTIONS SUGGESTED
  - Lo et al (1997) Lancet
    - More fetal material in serum than in plasma – clotting process releases DNA from cells
  - Gupta (2004) Clin Chim Acta
    - Cell free fetal DNA released from placental cells by apoptosis
  - Enrichment processes (2005) – columns, magnetic capture, size selection
  - Guo (2012) N Eng J Med
    - Formaldehyde addition to minimise release of maternal DNA from intact cells
  - Ou (2014) Transfusion
    - Differential methylation in *SERPINB5* and *RASSF1A* genes using methylation specific PCR or methylation sensitive restriction enzymes

2002 concerns

- Miss A pregnant at the time of splitting up with Mr A and getting together with Mr B
  - Prenatal test – 99.9% certain that Mr B was father
- Mr A devastated
- Mr B gave up the idea about going to law school to look after child
- Doubts about paternity within 6 months – two new tests shower Mr B could not be the father
- Mr B split with Miss A, but continues to share with child's care
- Miss A and Mr B sued the lab in Arizona and were awarded $1 million – they have seen one of this
- Laboratory ceased trading under trading name

Case 1

Short tandem repeats (STRs)

2005 – opinion requested

- Married female (white) – Catholic – living in a Mediterranean country – pregnant
  - Possible father is husband, or black American pilot (one incident)
- Prenatal test done in Canadian laboratory
  - Likelihood Ratio supporting paternity 500
  - Ethical dilemma
  - How good is the lab?
- LR 100 is required standard of proof in US, but we considered that too low, given the number of DNA tests done
- Report reviewed
- CVS done

Test results

	D3	Th01	D21	D18	D5	D13	D7	D16	CSF	VWA	D8	TPOX	FGA
PF	15	7	28	18	12	12	9	9	8	15	14	8	20
	16	8	30	19			12	11	10	17			23
Circ		7					9	9	8		14		
M	16	9	29	16	11	12	12	11	9	17	9	8	22
	17	9.3	31.2		12	13			11		12		24

Test results

	D3	Tho1	D21	D18	D5	D13	D7	D16	CSF	VWA	D8	TPOX	FGA
PF	15	7	28	18	12	12	9	9	8	15	14	8	20
	16	8	30	19			12	11	10	17			23
Circ		7					9	9	8		14		
M	16	9	29	16	11	12	12	11	9	17	9	8	22
	17	9.3	31.2		12	13			11		12		24

	D3	Tho1	D21	D18	D5	D13	D7	D16	CSF	VWA	D8	TPOX	FGA
PF	15	7	28	18	12	12	9	9	8	15	14	8	20
	16	8	30	19			12	11	10	17			23
CVS	17	7	29	15	10	12	8	9	11	17	12	8	21
	18	9.3		16	11		12	11	12		15	9	22
M	16	9	29	16	11	12	12	11	9	17	9	8	22
	17	9.3	31.2		12	13			11		12		24

Case 2

Single nucleotide polymorphisms (SNPs)

2008

- Couple approached us to have a blood sample taken to send to Canada for non-invasive paternity test
  - She wanted to get pregnant with her boyfriend but she was also a sex worker and she was worried about a split condom with this man
- She received a call with the results – the tested man is the father and you are going to have a boy
  - Termination booked
  - But scan the day before suggested it was a girl
- She received another call – 'we got it wrong – it's a girl'
- Dilemma – 'how can they get that wrong?'
- Cancelled her termination appointment and booked an amniocentisis instead

Test results

Reported results

- ‘They’ve done ten tests and there are numbers in all the boxes for the child’ – ‘Sounds as though it might be right but send it to me and I will have a look’.
- Not STRs but SNPs
  - 6 on the X chromosome – revealed fetus female
  - 4 on chromosome 1
  - The SNPs are unique to the fetus and are unlikely to have similar matches with random men in the population
- Very low power to try to prove paternity
  - 10 STRs equivalent to 50 SNPs
  - No proof that SNPs on these two chromosomes are independent

  If they were LR = 50

Test results

Follow up

- STR tests exclude man from paternity
- SNPS repeated

SNP	X1	X2	X3	X4	X5	X6	1-1	1-2	1-3	1-4
PF	T	G	A	T	T	G	GG	AA	CC	CT
Fetal	TT	GG	AG	AT	TT	GT	AG	AA	CC	CT
Maternal	GT	AG	AG	AA	CT	TT	AG	AA	CC	TT
Repeat	GT	nt	AG	AT	CT	GT	GG	AA	CG	CT

Case 3

Y chromosome phylogeny

2008

- Pregnant woman in US approached Canadian lab with a man
- Fetus was male – 11 Y chromosome SNPs used
- Putative father and fetus gave identical results
- ‘Man cannot be excluded from paternity – a random man is very unlikely to share the same SNP profile as the fetus’

SNP	Y1	Y2	-	Y4	Y5	Y6	Y7	Y8	Y9	Y10	Y11
PF	A	T	G	A	C	G	T	A	C	T	G
Fetal	A	T	G	A	C	G	T	A	C	T	G
Repeat	A	C	A	C	A	C	T	G	T	T	A

- A second case also gave different results from the Canadian lab in five of the SNPs and millions of European man would also match the correct result

Y haplogroups

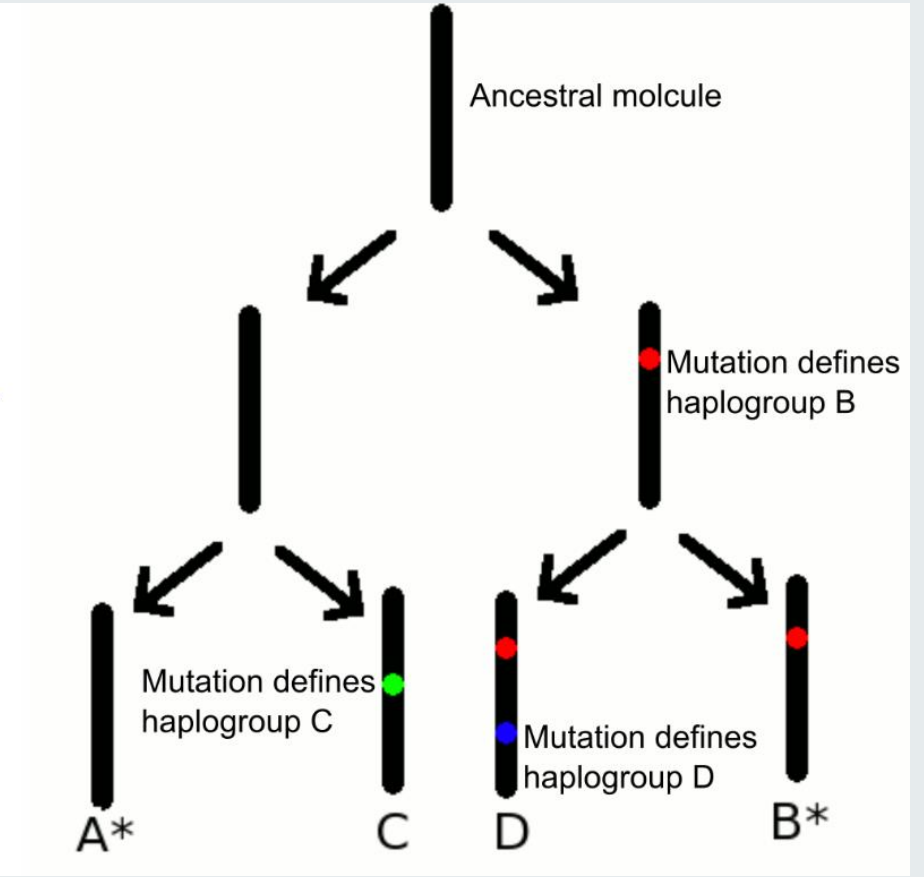
Inferring Human History: Clues from Y-Chromosome Haplotypes

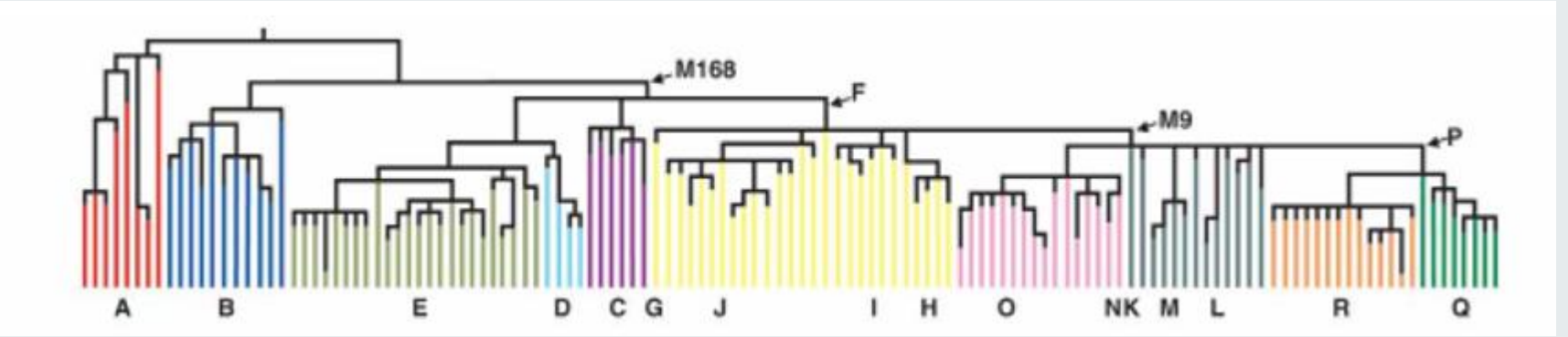
P.A. UNDERHILL
Department of Genetics, Stanford University School of Medicine, Stanford, California 94305-5120

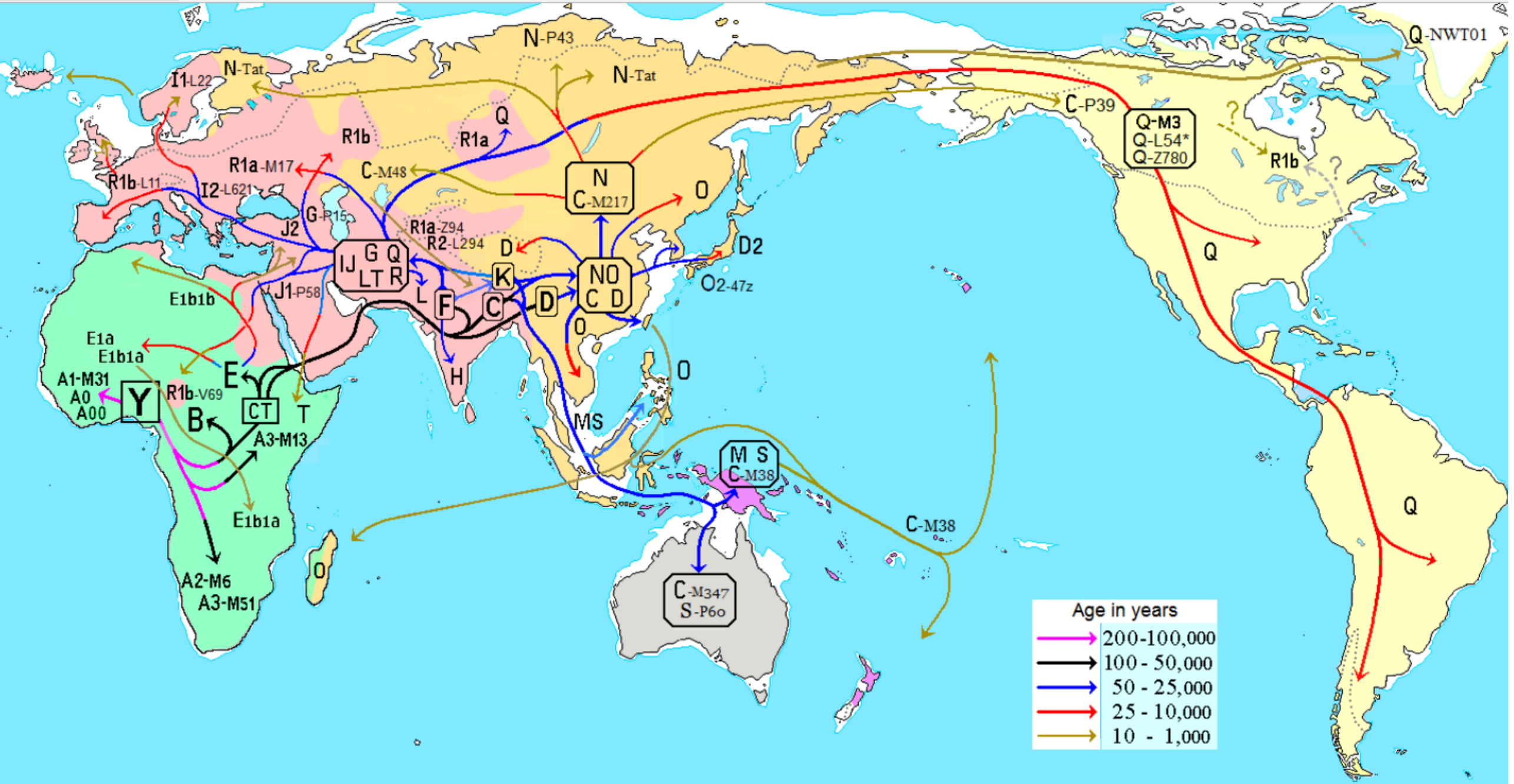
Fathers and sons: the Y chromosome and human evolution

MARK A. JOBLING AND CHRIS TYLER-SMITH

It should be possible to use Y chromosome DNA polymorphisms to trace paternal lineages for evolutionary and other studies, but progress in these areas has been slow because it has been difficult to find suitable markers. However, it is now possible to use selected, slowly evolving polymorphisms to draw a rudimentary Y chromosome tree, while more rapidly evolving polymorphisms allow most independent Y chromosomes to be distinguished. Different populations often have characteristically different Y chromosomes, and Y chromosome studies are soon likely to make a major contribution to our understanding of the origins of modern humans.



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I2-L621
G-P15
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R1a-Z94
R2-L294
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J1-P58
E1b1b
E1a
E1b1a
A1-M31
A0
A00
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R1b-V69
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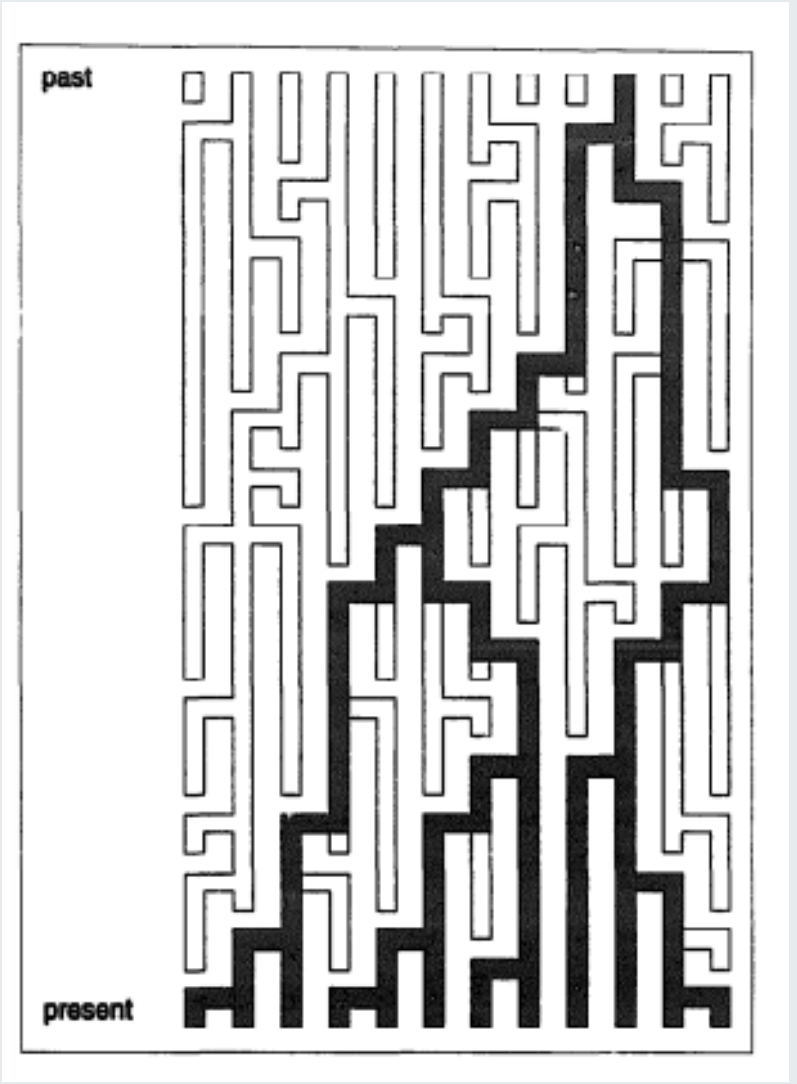
A3-M13
H
O
MS
M S
C-M38
C-M38
E1b1a
A2-M6
A3-M51
O
C-M347
S-P60
Q
Q
Age in years
200-100,000
100 - 50,000
50 - 25,000
25 - 10,000
10 - 1,000

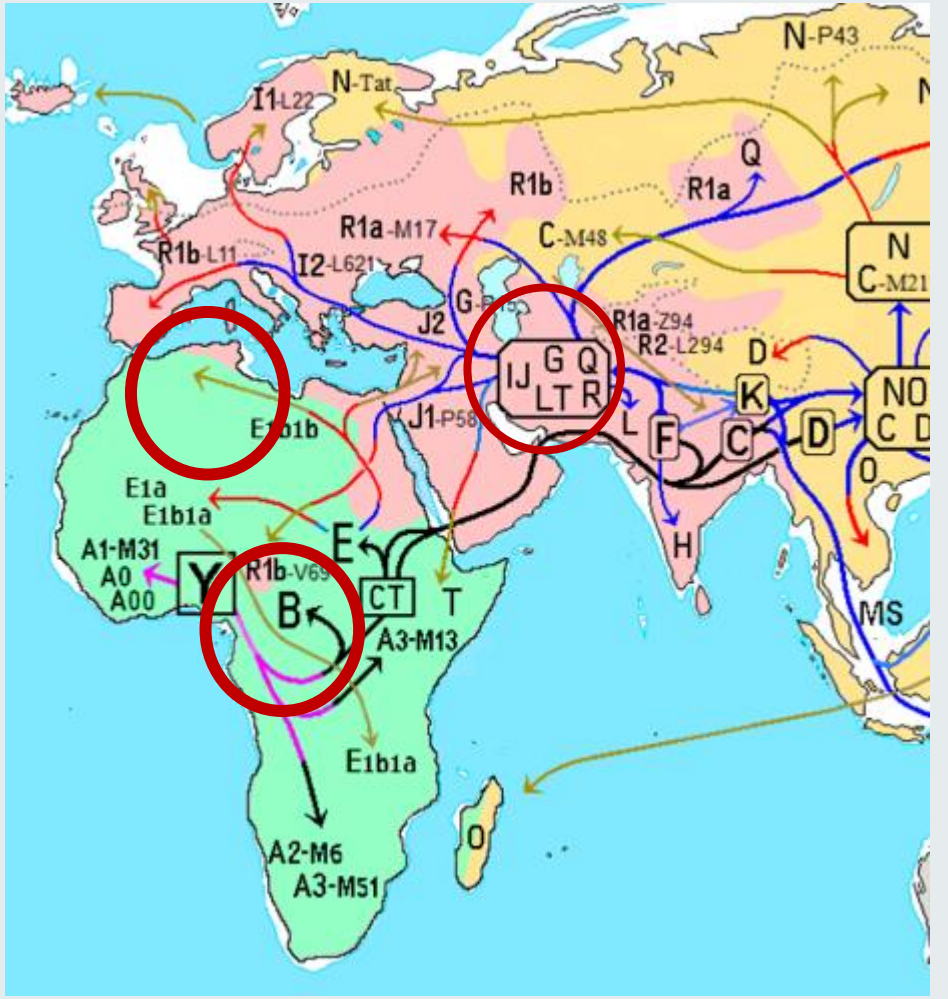
Canadian test haplogroups – this is a very strange boy

SNP	Y1	Y2	Y3	Y4	Y5	Y6	Y7	Y8	Y9	Y10	Y11
PF	A	T	G	A	C	G	T	A	C	T	G
Fetal	A	T	G	A	C	G	T	A	C	T	G
Haplo-group	NO	K-R	R	R	R1	K-R	F-R	P	B	NO	E1b1b1b1

E1b1b1b1 is a rare haplogroup

This really is a very strange boy





Case 4

The Sting

2009

- Significant number of other cases with false paternity attributions seen by an accredited laboratory in the US
- Concern that a number of women will have terminated their pregnancy based on the results
- The Canadian laboratory recommends testing all possible fathers
- Contamination due to poor practice? What about positive and negative controls?
- The case:
  - Female (UK based) – not pregnant – confirmed beta-hCG
  - Male A (US based)
  - Male B (UK based)
- The results
  - Male A cannot be excluded as the biological father. The prenatal markers are unique to the fetus and are unlikely to have similar matches with a random man
  - Male B is excluded as the biological father
  - It's a girl – no male contamination at least

Test results

Follow up

- 1 in 17 men would not be excluded as the father of the phantom fetus
- Man B was excluded at 3 sites
- Typing errors in Female and Man A

SNP	X1	X2	X3	X4	X5	X6	X7	1-1	1-2	1-3	1-4
Man A	C	C	T	A	T	T	G	G	GG	GG	CC
Man B	C	C	T	A	A	T	T	AG	AG	CG	TT
Phantom fetus	TC	CC	TT	AG	TT	TT	GG	AG	AG	CC	CC
Female	TC	CC	GT	AG	TT	CT	GG	AA	AG	CC	CT

The Outcome

New Scientist

19

SPECIAL INVESTIGATION 1 December 2010

The danger of unreliable paternity tests

By Peter Aldhous

Editorial: *No room for genetic errors*

The Outcome

- 2008 Y chromosome case shown to Canadians for comment – this would be a new human as its genetics are inconsistent with the Y chromosome known ancestry tree
  - 'We don't perform ancestry testing'
- Other cases
  - 'They've only tested on father – we recommend to test all possible fathers'
  - 'We don't use those Y SNPs any more'
  - 'We haven't made errors – it is your laboratory that has made the errors'
  - 'Our protocols are trade secrets, but are based on excellent research data'
- 2011 after publication the laboratory filed a multimillion dollar defamation suit against the New Scientist
  - Mediation and offer of sub one million compensation to Canadian not accepted
  - Insurers now determined to take it further on principle
  - Court case 2018
    - No records or samples of any tests are retained
    - Plaintiffs – 2 experts
    - Defendants – 6 world-renowned experts plus testimony from two affected families
  - Judgement ?

Thank you


King's College London
Franklin-Wilkins Building
Franklin
Biophysicist
Photo 51
An historic photograph