ESWG - Proficiency test 2023
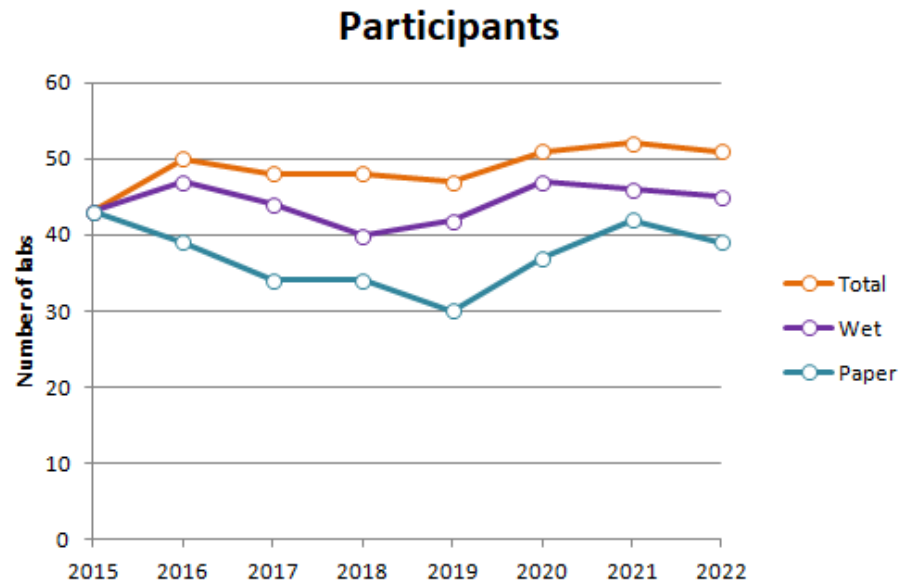
Daniel Kling – Head Organizer

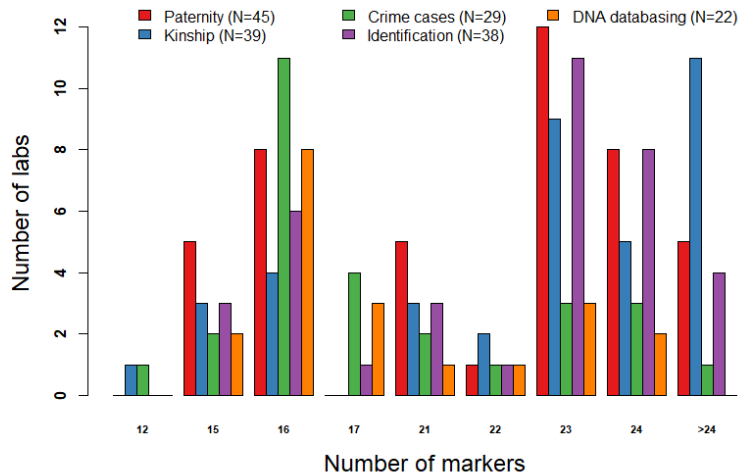daniel.kling@rmv.se

Summary

- Summarizing statistics for 2022
- <u>51</u> labs participated
- <u>37</u> completed paper challenge
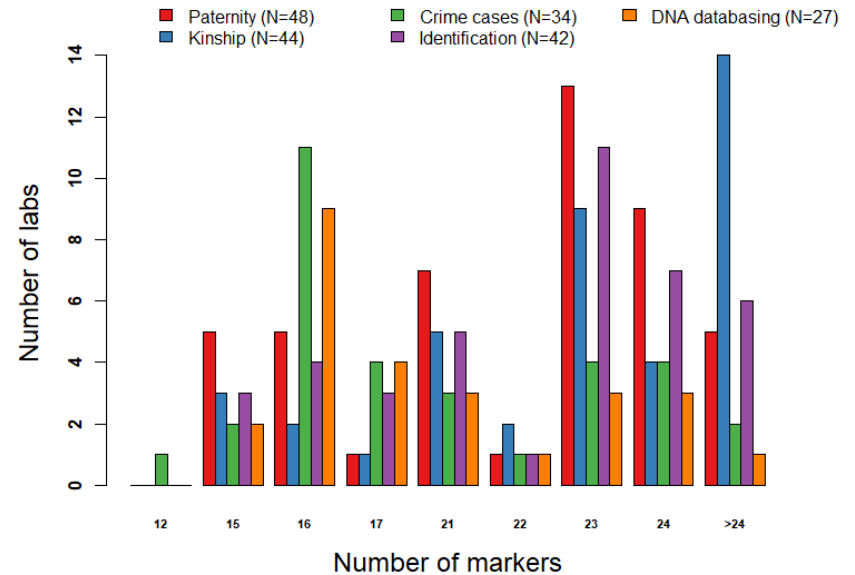- <u>47</u> completed wet exercise

Participants
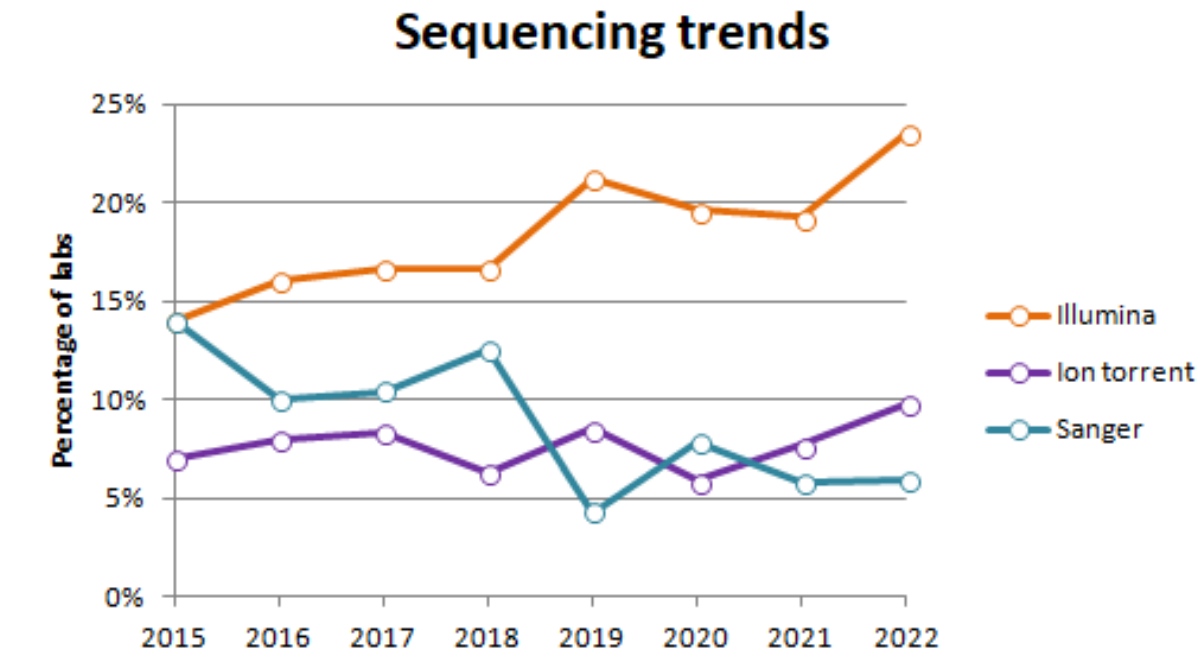
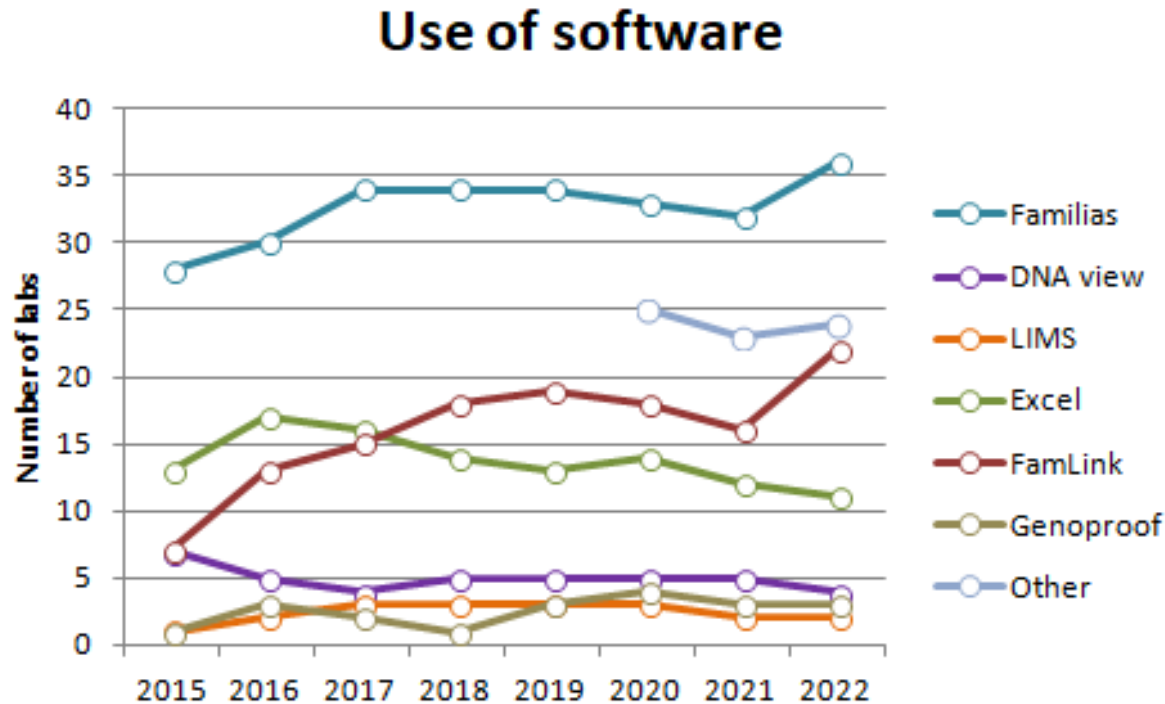# Questionnaire – Markers used

2021

2022

Questionnaire – Sequencing trends

- 19 labs (37%) performs sequencing

Sequencing trends

Questionnaire – Software trends

Other include DBLR. EuroForMix. STRmix and more

Questionnaire – Subpopulation effects

Never includes: 21

Always include: 15

When it is known: 16

Roughly 60% accounts for subpopulation effects

Questionnaire – Linked markers

<u>Not accounted for:</u> 10 -> 11

<u>Not used:</u> 14 -> 13

<u>Exclude one:</u> 16 -> 13

<u>Accounts for:</u> 9 -> 13

Slight increase in labs accouting for linkage

WET EXERCISE

Wet exercise - Background

ESWG WET EXERCISE 2023

This year's wet exercise includes a child (sample labeled Child) seeking his/her biological father. Conduct a paternity test for the putative father (sample labeled Alleged father).

Use a frequency database appropriate for a European population. Report the likelihood ratios (LR) for the individual genetic markers included in the tests as well as the combined LR. State which frequency database you have used for the calculations. Similar to previous years, all results should be reported in the electronic spreadsheet questionnaire.

Samples and procedure
The samples (two in total) consist of blood on FTA cards (diluted spots). We recommend direct amplification with buffers available from vendors (alternatively direct amplification with modern multiplexes). Other extraction procedures have not been tested.

Please perform the DNA tests according to your procedures for kinship analysis and report the data and conclusions in the questionnaire attached to the information email. If different kits are included in the analysis and any discrepancies between overlapping markers occur, please state the difference(s) in the comment field.

Due date
The due date is August 31th, results submitted after this deadline may be dismissed.

Wet exercise - Summary

➢ Overall very concordant results (despite the use of potentially different databases)

➢ 47 labs participated

➢ Consult the Excel summary for details

➢ For the wet exercise some labs' results have been highlighted (red or orange) which indicates a result that deviates. Certificates will still be issued.

| Combined LR (reported) | | 8,19E+06 | 5,60E+12 | 2,20E+10 | 4,93E+06 | | | | | 6,24E+14 | 1,62E+10 | | | | | 9,08E+09 | 1,5E+11 | | | 3,18E+11 | 3,46E+41 |
Combined LR (product)		3,58E+06	5,19E+12	9,53E+09	1,77E+06	5,33E+10	1,88E+09	8,10E+06	4,54E+09	2,26E+14	6,83E+09	0,00E+00	2,14E+10	0,00E+00	2,56E+09	4,04E+09	6,55E+10	0,00E+00	3,52E+11	1,35E+11	2,37E+20
	Number of markers -->	16	27	24	15	23	21	15	21	33	22	0	21	0	22	21	24	0	22	24	34
D3S1358	2,43	2,29	2,44	2,32	2,78	2,88	2,33	2,30	2,30	2,76	2,38		2,38	2,50		2,38	2,25	2,31		2,34	2,48
D19S433	3,18	3,59	2,71	2,55	3,13	2,88	2,33		3,80	3,88	3,54		3,54	3,08		2,55	3,08	3,73		3,08	3,28	2,47
D2S1338	2,35	2,47	3,26	2,99	2,27	1,77	2,94		2,50	4,86	2,38		2,37	2,25		2,35	2,24	2,66		2,25	3,65	2,20
D22S1045	0,72	0,64	0,72	0,72		0,80	0,76		0,70	0,86	0,65		0,65	0,81		0,82	0,75	0,67		0,81	0,74	0,72
D16S539	9,70	9,02	9,97	7,74	5,83	8,86	9,85	7,80	11,10	9,46	10,30		10,30	11,31		8,96	9,82	12,02		11,30	6,88	9,48
D18S51	2,03	2,00	1,62	1,60	2,08	1,81	2,00	2,20	1,80	1,88	2,03		2,03	2,03		2,19	1,80	1,81		2,03	2,32	2,46
D1S1656	4,73	4,89	4,26	3,80		6,60	6,38		3,40	4,73	5,01		5,01	6,93		3,43	4,60	4,65		6,94	6,27	7,00
D10S1248	8,45	8,22	9,09	6,88		7,02	7,23		11,10	8,68	9,03		9,03	8,92		6,68	6,24	12,24		8,93	7,54	120,25

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Wet exercise

- ➢ A single alleged father
- ➢ No labs reported SNP markers this year

Note: log scale on y-axis
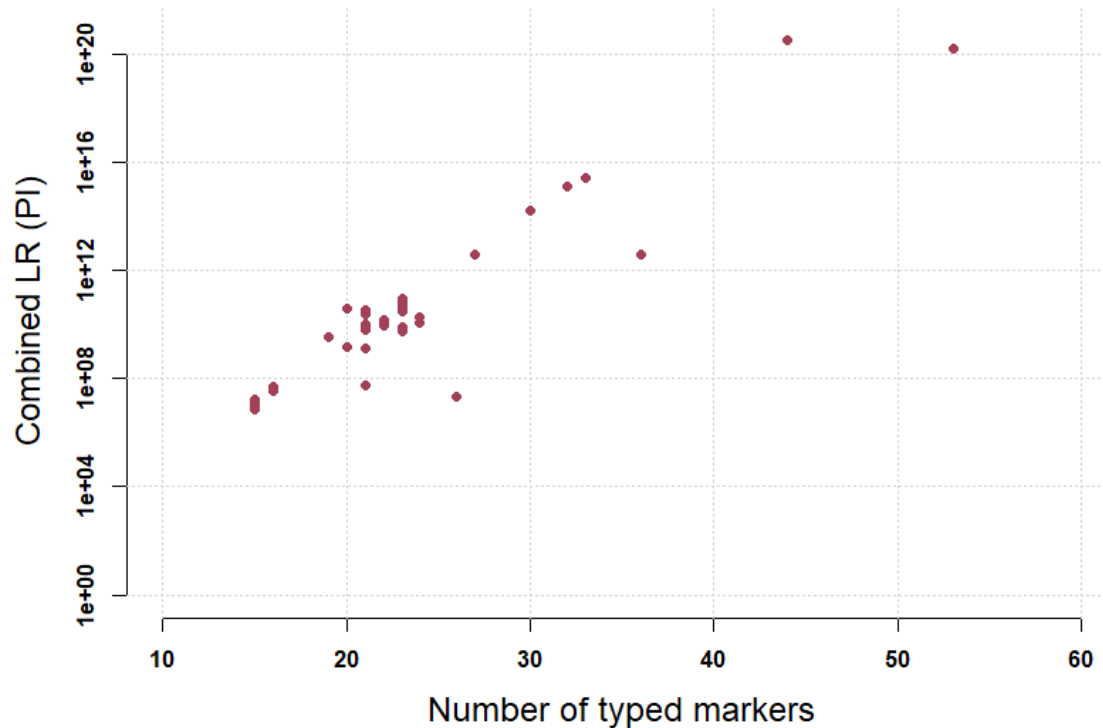
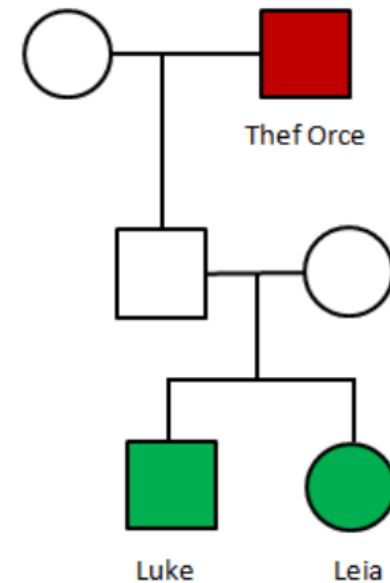# Wet exercise – per marker LR variation

Two markers with large variation
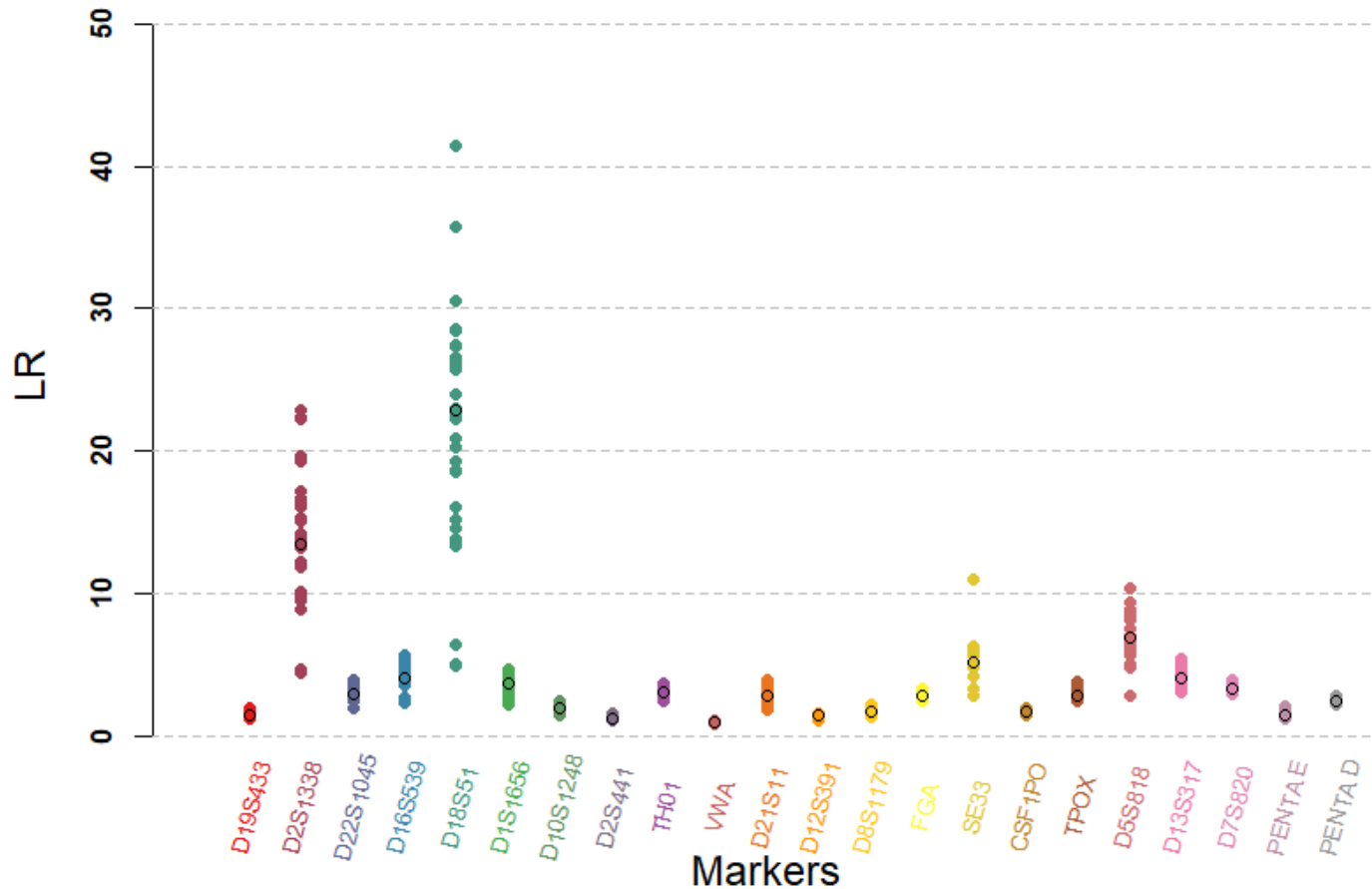
PAPER CHALLENGE

Paper challenge - Background

- Grandparent/grandchild case
- Autosomal data
- Y-data
- SNP data

Paper challenge – Setup

Paper challenge – Part a)

➢ Compute the pairwise LRs between the individuals. include half and full siblings. <u>Disregard pedigree!</u>
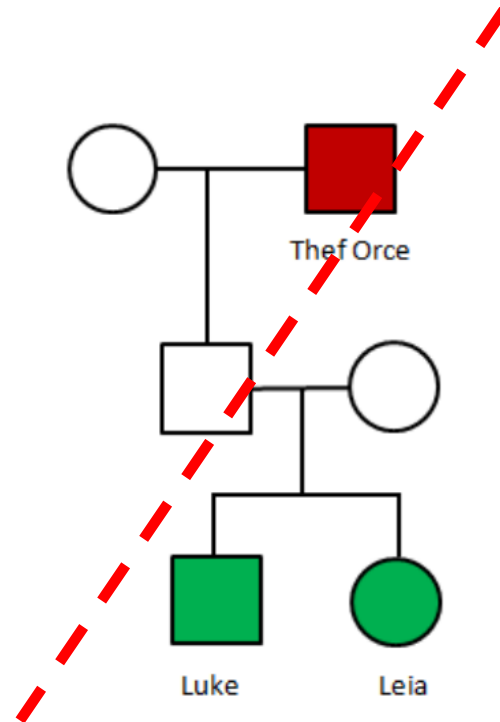
Paper challenge – Part a)

➢ Compute the pairwise LRs between the individuals. include half and

Individual 1	Individual 2	LR full sibs	LR half sibs
Luke	Leia	23	394
Thef Orce	Luke	0.96	118
Thef Orce	Leia	1734	608
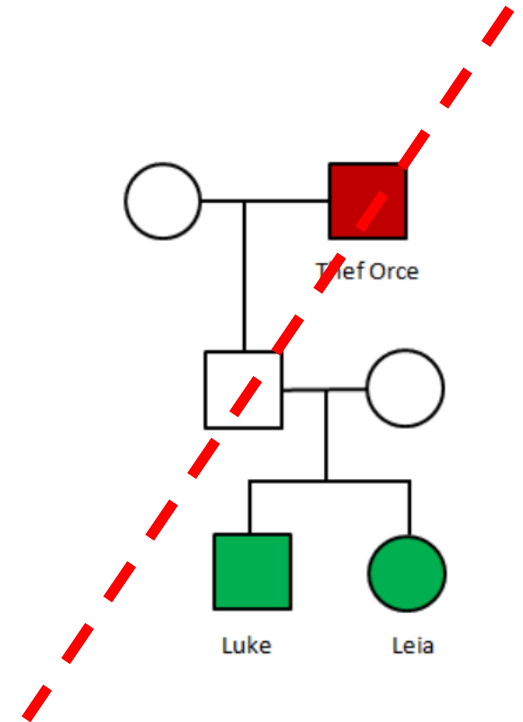
Paper challenge – Part b)

- Compute the LR comparing the pedigree in the illustration above with the alternative hypothesis where Luke and Leia are unrelated to Thef Orce [in the pedigree].
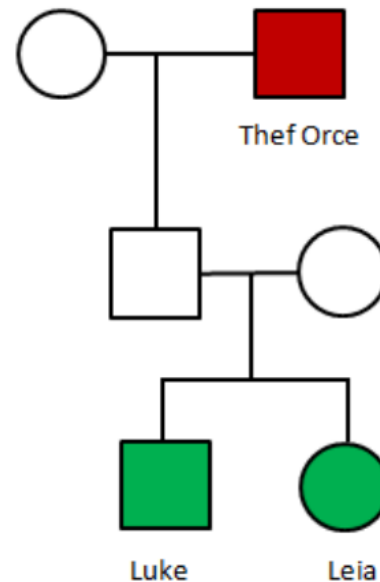
Demonstration in Familias

Paper challenge – Part b)

➢ Use a software or manual calculations to compute LR comparing full siblings and half siblings with unrelated. Two LRs per comparison

CSF1PO	1.12
D13S317	1.29
D16S539	1.74
D18S51	15.08
D19S433	5.98
D21S11	2.06
D2S1338	1.25
D3S1358	2.30
D5S818	1.75
D7S820	0.45
D8S1179	1.21
FGA	2.20
TH01	1.23
TPOX	1.43
D10S1248	0.63
D12S391	15.50
D1S1656	1.29
D22S1045	1.28
D2S441	1.81
SE33	2.09
Total autosomal LR	308623

i.   <u>All labs reached the same conclusion (in favor)</u>

ii.   Most reported LR=308623

iii.   Some reported other LRs

        a)   Inhouse databases

        b)   Exclude linked markers

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Paper challenge – Part c)

A more distant story (Optional)

In the ruins of an old temple, a lost archive is found. From the shelves we are able to obtain the Y-chromosomal profile of a paternal grandfather of T. Orce. See pedigree below.


Figure 2. Pedigree ilustrating the main hypothesis (H1). Data is available for the individuals with either green or red fill.
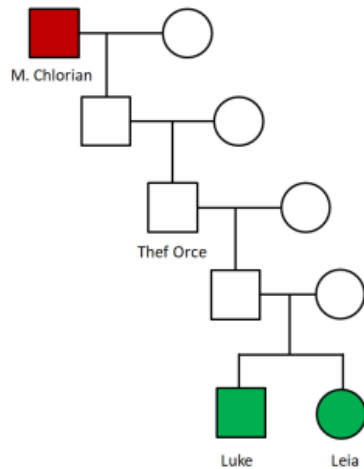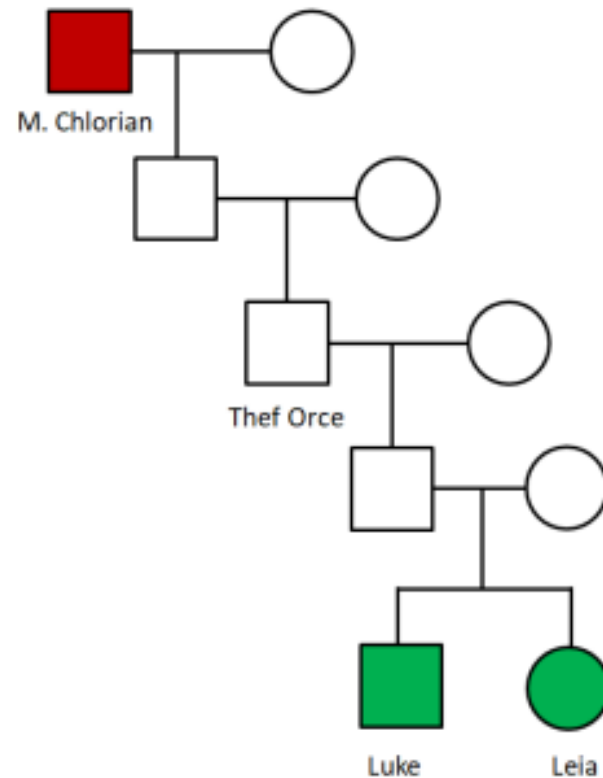
c) [Optional] The Y-profile of Luke is extracted from the walls of the cave. In total, data for 22 Y-STRs are available. Estimate the weight of evidence that Luke and his great great grandfather M. Chlorian are paternally related as depicted in the pedigree above. In the calculations you can assume that the haplotype of Luke has never been observed while the haplotype of M. Chlorian has been observed 10 times. The size of the Y-database is 100,000. For simplicity we can assume that the mutation rate is equal to 0.001 for all included markers and that there is an equal chance for a loss or gain of a tandem repeat in the mutation model. Further assume that there is a 90% chance for a mutation to be single step, 9% two step and so forth.

Demonstration YHRD

Paper challenge – Part c)

➢ Manual derivations

a) 4 generations (meiosis)
b) One marker (DYS389) with a single step mutation
c) 21 STR markers without mutations
d) Mutation rate (mu) = 0.001
e) No mutation = (1-mu) = 0.999

M. Chlorian

Thef Orce

Luke   Leia

Paper challenge – Part c)

> Manual derivations

a) 4 generations (meiosis)
b) One marker (DYS389) with a single step mutation
c) 21 STR markers without mutations
d) Mutation rate (mu) = 0.001
e) No mutation = (1-mu) = 0.999

H1: M. Chlorian is the paternal ancestor of Luke
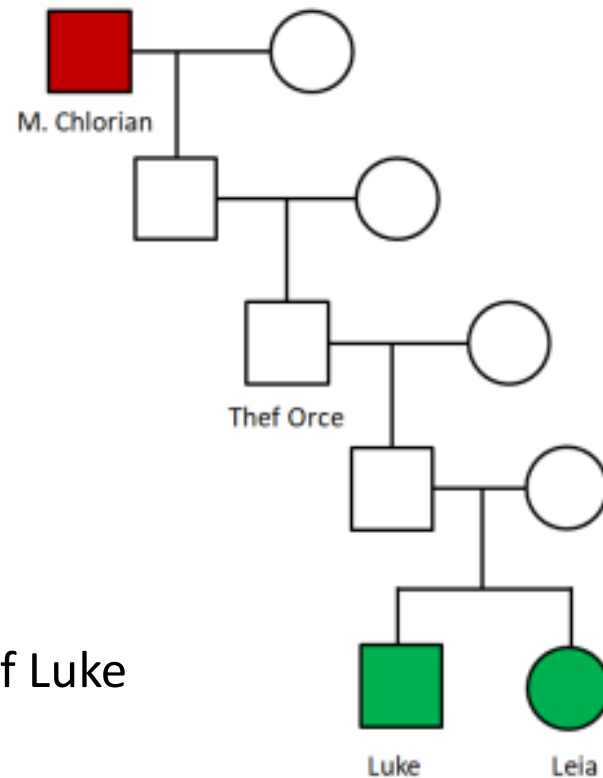H2: M. Chlorian and Luke are unrelated

M. Chlorian
Thef Orce
Luke   Leia

Paper challenge – Part c)

➢ Manual derivations

a) 4 generations (meiosis)
b) One marker (DYS389) with a single step mutation
c) 21 STR markers without mutations
d) Mutation rate (mu) = 0.001
e) No mutation = (1-mu) = 0.999

H1: M. Chlorian is the paternal ancestor of Luke
H2: M. Chlorian and Luke are unrelated

$L(H2) = H\_mc * H\_l$

M. Chlorian

Thef Orce

Luke   Leia

Paper challenge – Part c)

> Manual derivations

M. Chlorian

Thef Orce

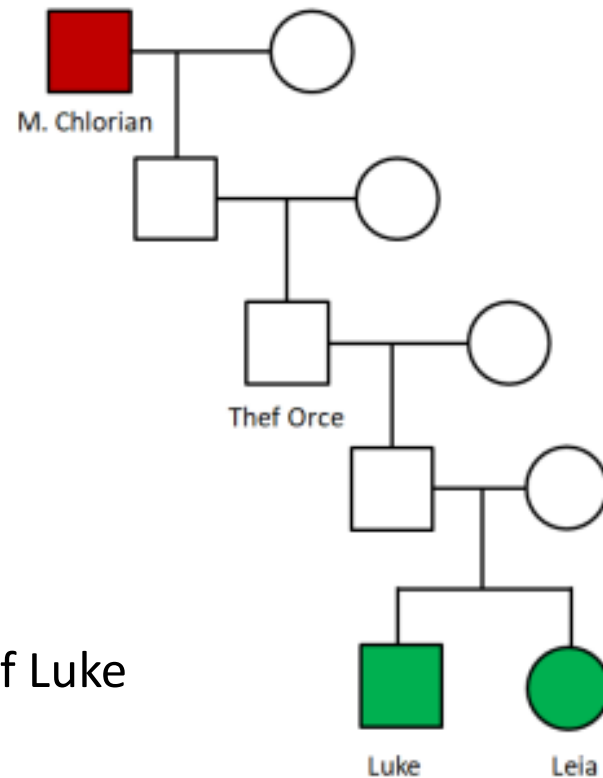Luke   Leia

a) 4 generations (meiosis)
b) One marker (DYS389) with a single step mutation
c) 21 STR markers without mutations
d) Mutation rate (mu) = 0.001
e) No mutation = (1-mu) = 0.999

H1: M. Chlorian is the paternal ancestor of Luke
H2: M. Chlorian and Luke are unrelated

L (H1) = H_mc * [no mutations in 21 markers over 4 generations…
a single mutation in 1 marker over 4 generations..]

Paper challenge – Part c)

➢ Manual derivations

M. Chlorian

Thef Orce

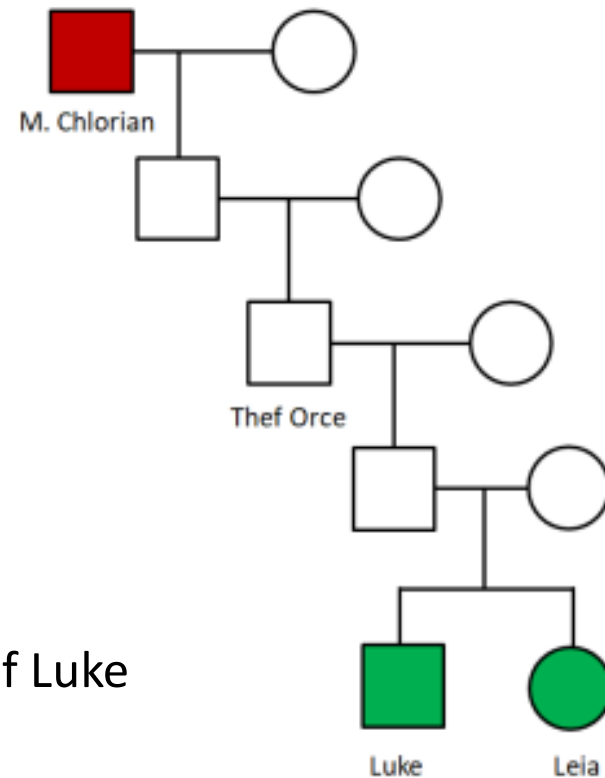Luke   Leia

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d) Mutation rate (mu) = 0.001
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H1: M. Chlorian is the paternal ancestor of Luke
H2: M. Chlorian and Luke are unrelated

$L(H1) = H\_mc * [(1-mu)^{4*21} *$
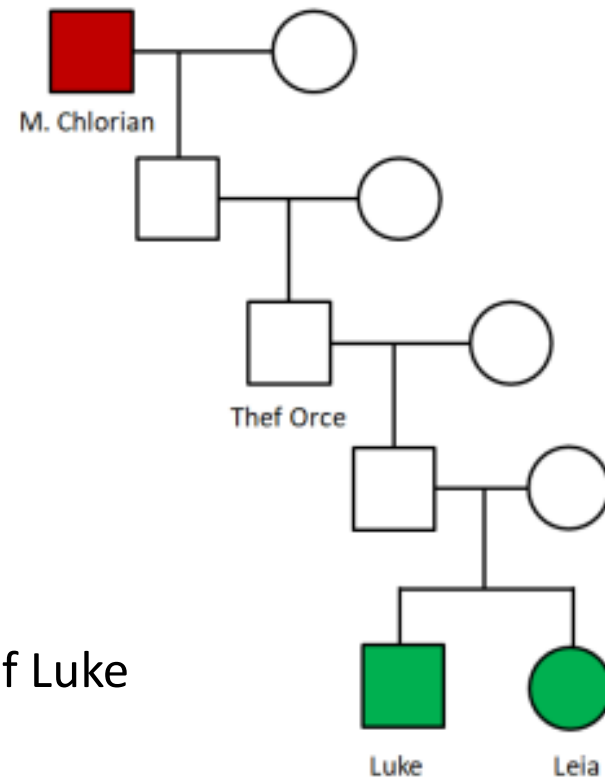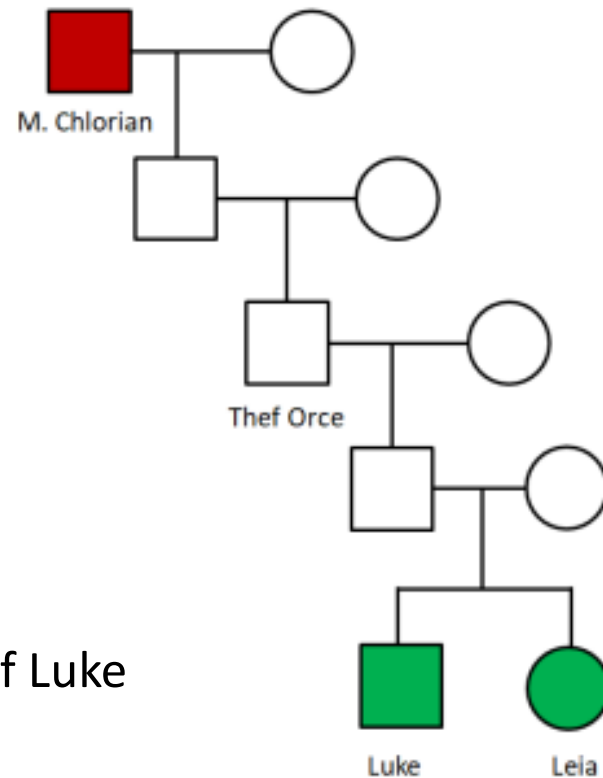
        $4*0.9*mu/2* (1-mu)^3]$

Paper challenge – Part c)

➢ Manual derivations

a) 4 generations (meiosis)
b) One marker (DYS389) with a single step mutation
c) 21 STR markers without mutations
d) Mutation rate (mu) = 0.001
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H1: M. Chlorian is the paternal ancestor of Luke
H2: M. Chlorian and Luke are unrelated

$$LR = L(H1)/L(H2) = [(1-mu)^{4*21} * 4*0.9*mu/2* (1-mu)^3] / H\_I$$

M. Chlorian

Thef Orce

Luke   Leia

Paper challenge – Part c)

➢ Manual derivations

M. Chlorian

Thef Orce

Luke   Leia

a) 4 generations (meiosis)
b) One marker (DYS389) with a single
    step mutation
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H1: M. Chlorian is the paternal ancestor of Luke
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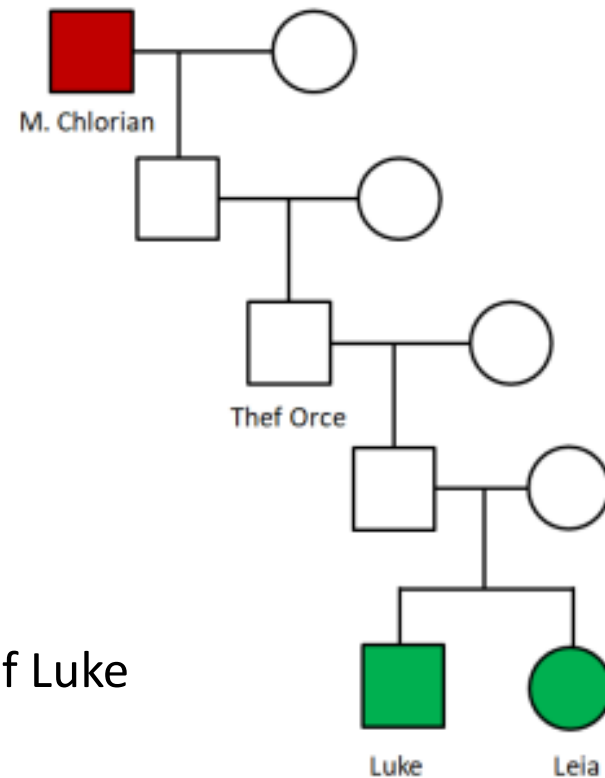LR = [$(1-mu)^{4*21}$ * 4*0.9*mu/2* $(1-mu)^3$] / H_I = [0.91 * 0.00179 / H_I

Paper challenge – Part c)

➤ Manual derivations

a) 4 generations (meiosis)
b) One marker (DYS389) with a single step mutation
c) 21 STR markers without mutations
d) Mutation rate (mu) = 0.001
e) No mutation = (1-mu) = 0.999

H1: M. Chlorian is the paternal ancestor of Luke
H2: M. Chlorian and Luke are unrelated

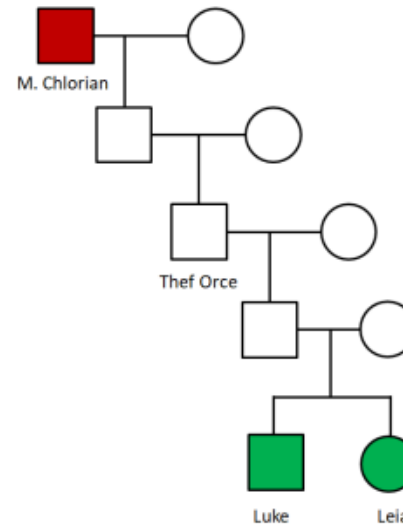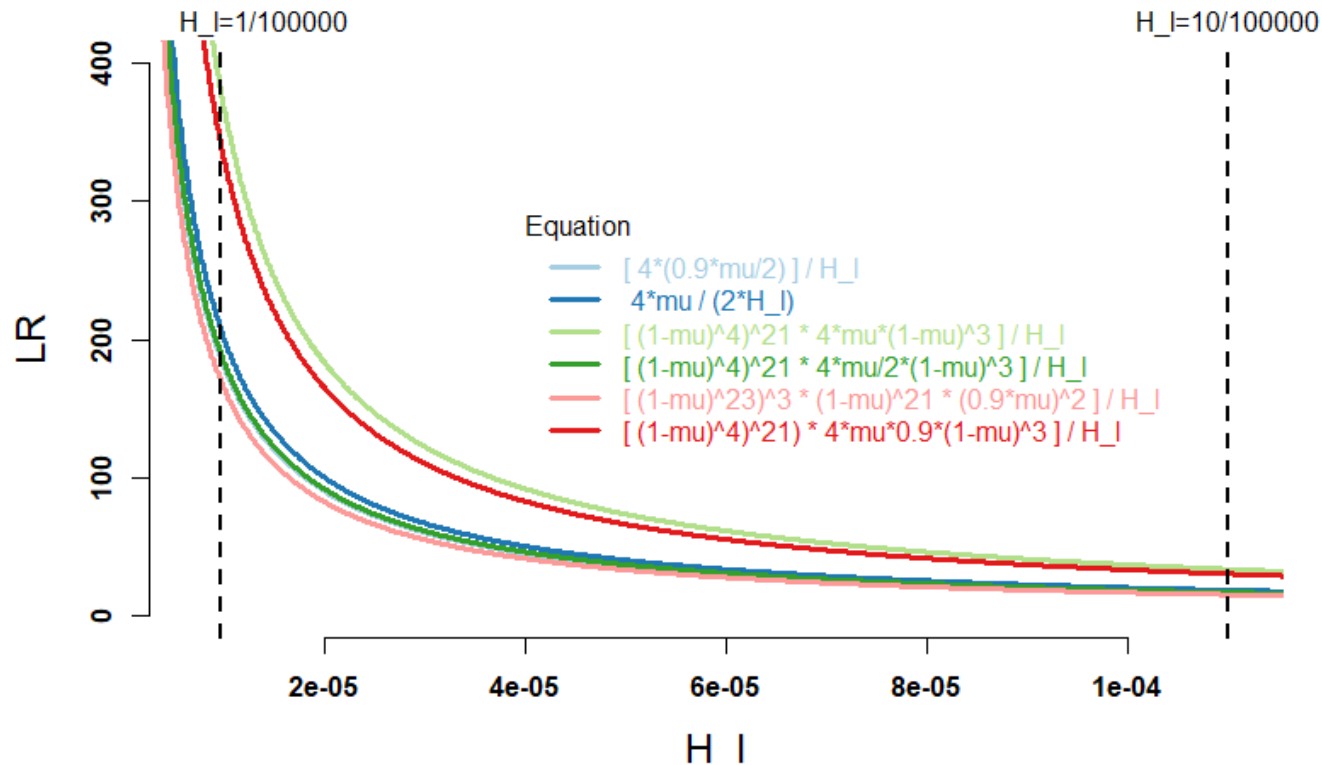$LR = [(1\text{-}mu)^{4*21} * 4*0.9*mu/2* (1\text{-}mu)^3] / H\_l = \underline{[0.91 * 0.00179] / H\_l}$

Simplified $LR = [4*0.9*mu/2] / H\_l = 0.0018 / H\_l$

Paper challenge – Part c)

➢ Results

Paper challenge – Part c)

➢ Reported LR

1. YHRD -> LR=375 (with Worldwide population haplotypes and H_l=1 / 103281)
2. Manual formulas -> A large range, some reporting two mutations (LR<1) while the majority reported a single mutation (LR ranging from 36 up to >1000)

Paper challenge – Part d)

d) [Optional] Ultimately, we are able to decipher the genetic code for an expanded SNP panel from both the caves and the temple with data for Luke and M. Chlorian. We are stunned to realize there is a 100% overlap with the recently published FORCE panel with data for 3,931 autosomal kinship markers. Compute the pairwise LR for the alleged relationship between the two individuals (depicted in the pedigree above). All necessary information is given in the files linked to at the top of this document. For brevity, genetic data is omitted from this document.
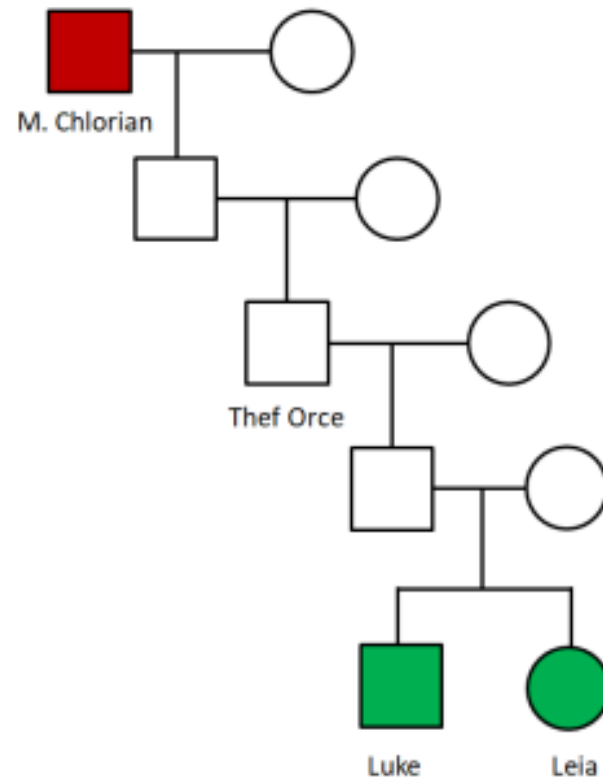
Demonstration FamLink2

Paper challenge – Part d)

➢ Results

LR (linkage) = 303 (or slightly greater)
LR (no linkage) = 1.3

Not accounting for linkage greatly
underestimate the LR!

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Paper challenge – Summary

➢ Video will be available through https://familias.name/ESWG/

➢ We will arrange the tests next year unless another lab(s) is willing to take the reins!?

➢ SNP test next year?

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ESWG - Proficiency test 2023

Daniel Kling – Head Organizer

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