HIV-1 subtype C in Karonga District, Malawi
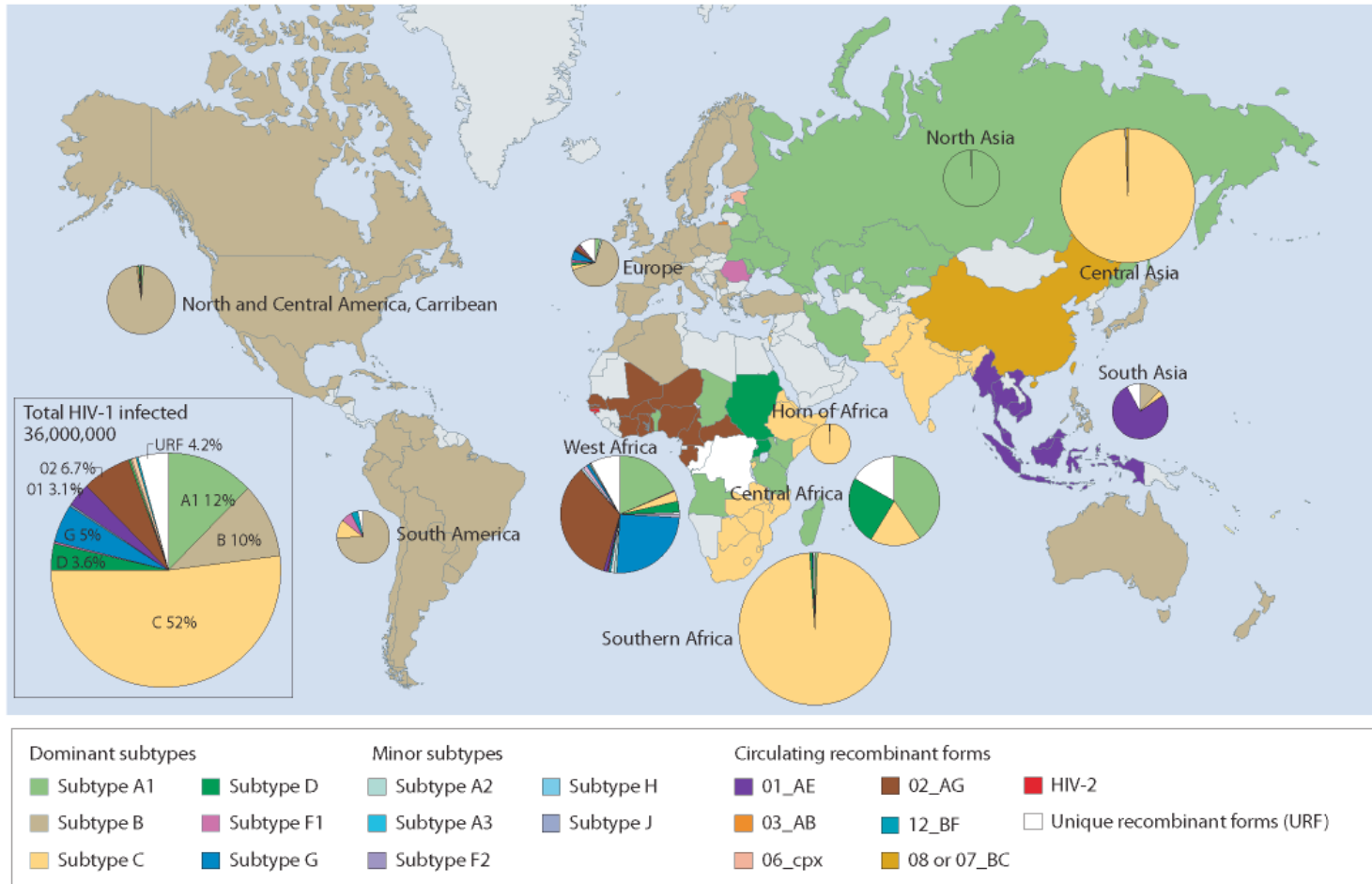
Simon Travers

HIV-1; closely related to HIV found in chimps
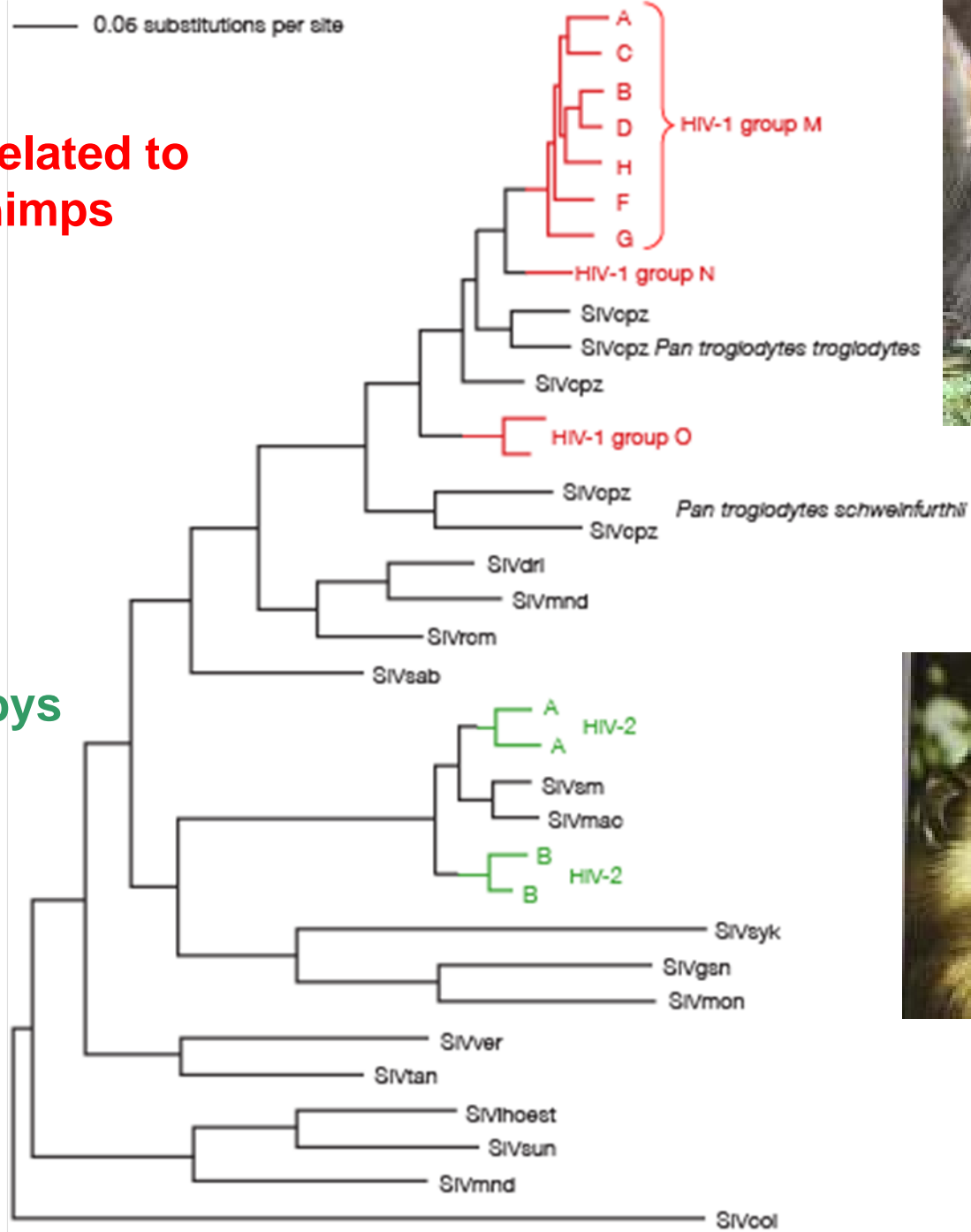
HIV-2; closely related to HIV found in mangabys

Worldwide Distribution of HIV-1 group M subtypes

HIV-1 in Africa

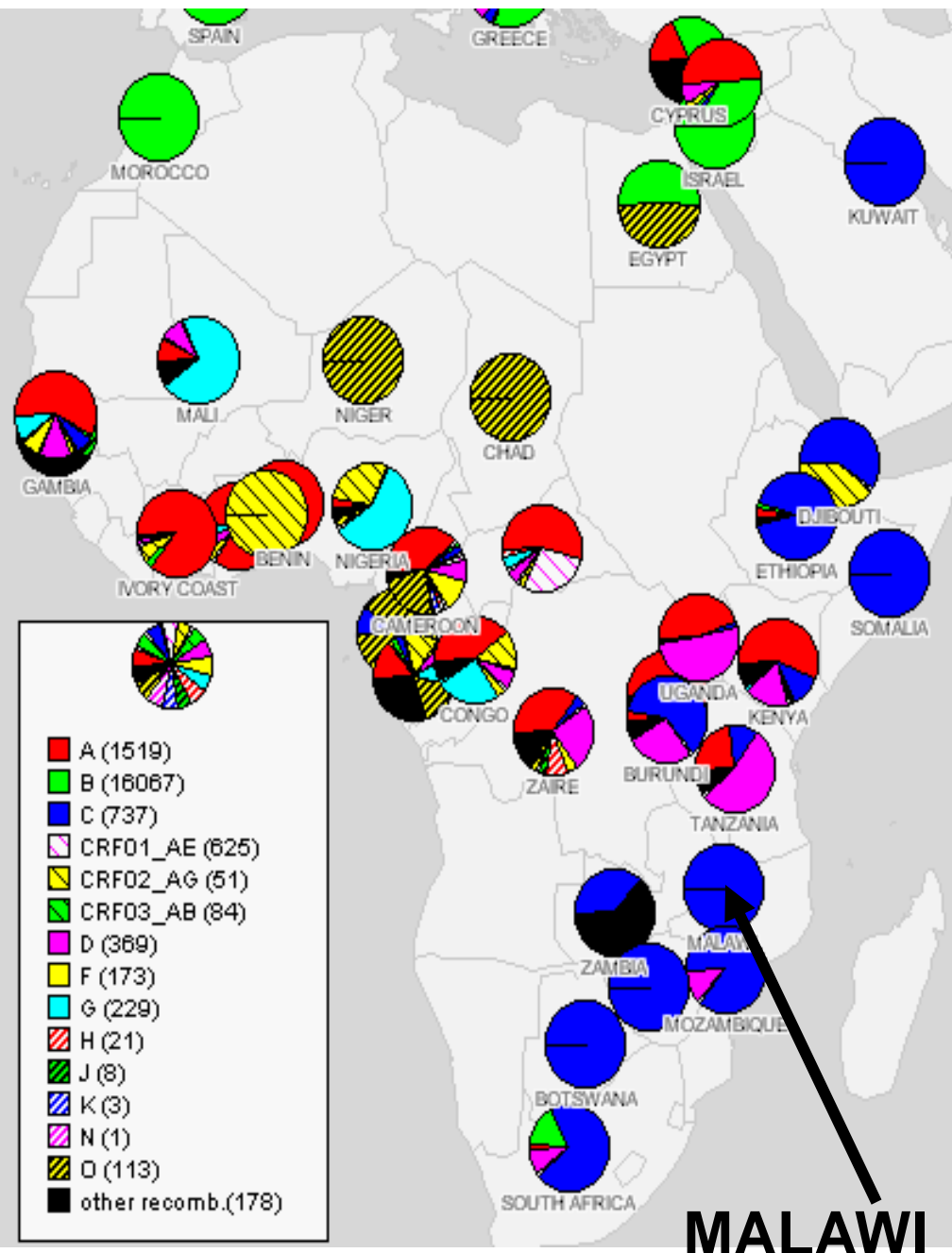
High diversity consistent with its origins

Subtype C

60% worldwide infections

90% sub-Saharan infections

Different to the HIV-1 in Western world (subtype B) but how different???

Malawi is a small country in sub-Saharan Africa.

>25% of young people in cities are HIV-1 positive and about 15% of those in rural areas

Legend:
- A (1519)
- B (16067)
- C (737)
- CRF01_AE (625)
- CRF02_AG (51)
- CRF03_AB (84)
- D (369)
- F (173)
- G (229)
- H (21)
- J (8)
- K (3)
- N (1)
- O (113)
- other recomb.(178)

MALAWI

Karonga District

Aims

- What subtypes ?
- How many introductions ?
- How diverse ?
- How is it changing ?
- Exchange between spouses
- Effect of immigrants
- Effect of culture
- Vaccine implications

Subtypes

1982-1984	11 samples	prevalence of 0.1%
Subtypes	C	55%
	D and A	9% each
	unclassifiable	27%

1986-1989	192 samples	prevalence of 2%
Subtypes	C	90%
	A + D	
	3 recombinant types (AD, AC and CD)	
	One unclassifiable	

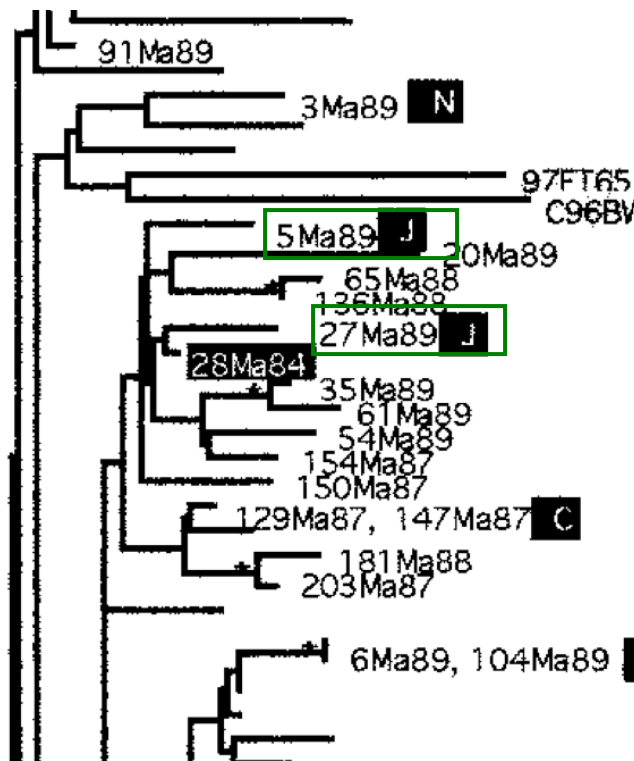| 1997-2001	210 samples	prevalence of *c.*15%
Subtype	C	91%
	A & D	
	recombinants	
	No new unclassifiables	

McCormack et al J. Virol. 2002

TABLE 3. Pairwise comparisons of sequences from epidemiologically linked individuals from the Karonga 1986-to-1989 survey, with regard also to the clusters in which they fall
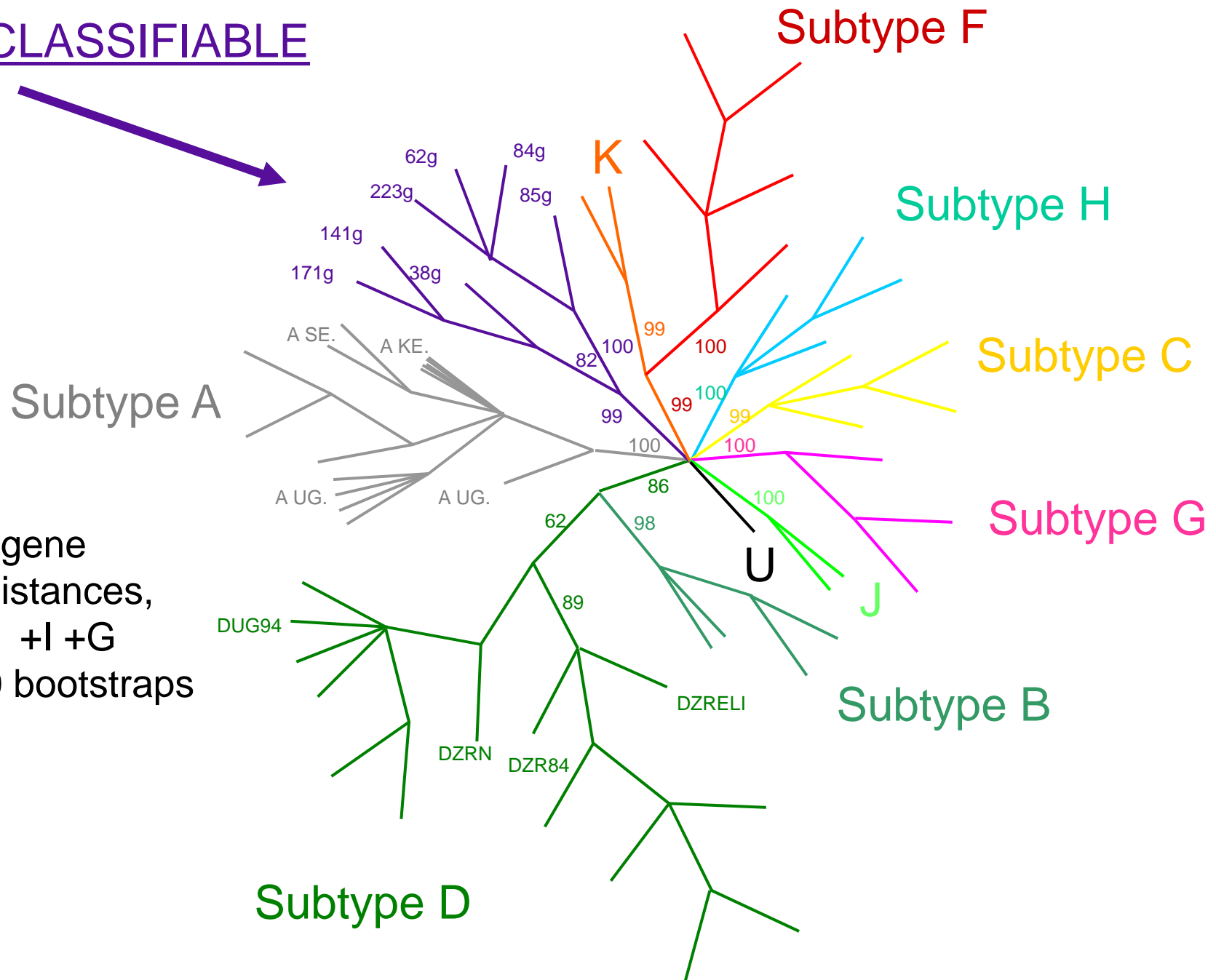
Sequences compared (spouse pair)	Cluster[a] or subtype	Mean genetic distance (%) for:	
		gag	*env*
Spouses			
84 and 85 (a)	Unclassifiable	1.82	7.44
107 and 114 (b)	2	0	0.5
107 and 145 (B)	2	0.48	0.5
129 and 147 (c)	2	0.8	
89 and 201 (d)	2		5
89 and 86 (d)	2	0	0
87 and 81 (e)	1	0.64	
76 and 82 (f)	1	1.64	
7 and 63 (g)	4	0.32	1
6 and 104 (h)	1	0	
194 and 195 (i)	1		1
5 and 27 (j)	1	2.29	5
51 and 90 (k)	2	2	
110 and 56 (l)	1		3.8
190 and 49 (m)	2	1.65	0.5
3 and 98 (n)	1/2		
53 and 182 (p)	2	2.36	
130 and 144 (q)	D	0.63	
Siblings			
91 and 92	3	1.2	7.43
91 and 115	3/4	3.2	11.26
92 and 115	3/4	3.9	10.81
Mother and daughter			
154 and 155	3/4	5.4	4

TABLE 3. Pairwise comparisons of sequences from epidemiologically linked individuals from the Karonga 1986-to-1989 survey, with regard also to the clusters in which they fall
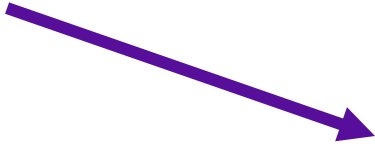
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UNCLASSIFIABLE

Subtype F

K

Subtype H

62g   84g
223g   85g

141g

171g   38g

A SE.   A KE.

Subtype A

82   100   99

100   99   100

99

Subtype C

99

100

Subtype G

A UG.   A UG.

100

Gag gene
ML distances,
GTR +I +G
1000 bootstraps

86

U

98   100

J

62

89

Subtype B

DUG94

DZRELI

DZRN   DZR84

Subtype D

McCormack *et al.* (2003) *Aids Research and Human Retroviruses*

Are these unclassifiable sequences CRFs?

AE

AG

Window: 300 bp, Step: 10 bp, GapStrip: On, Kimura + Neighbor-Joining

Window: 200 bp, Step: 20 bp, GapStrip: On, Reps: 100, Kimura, T/t: 2.0, NEIGHBOR
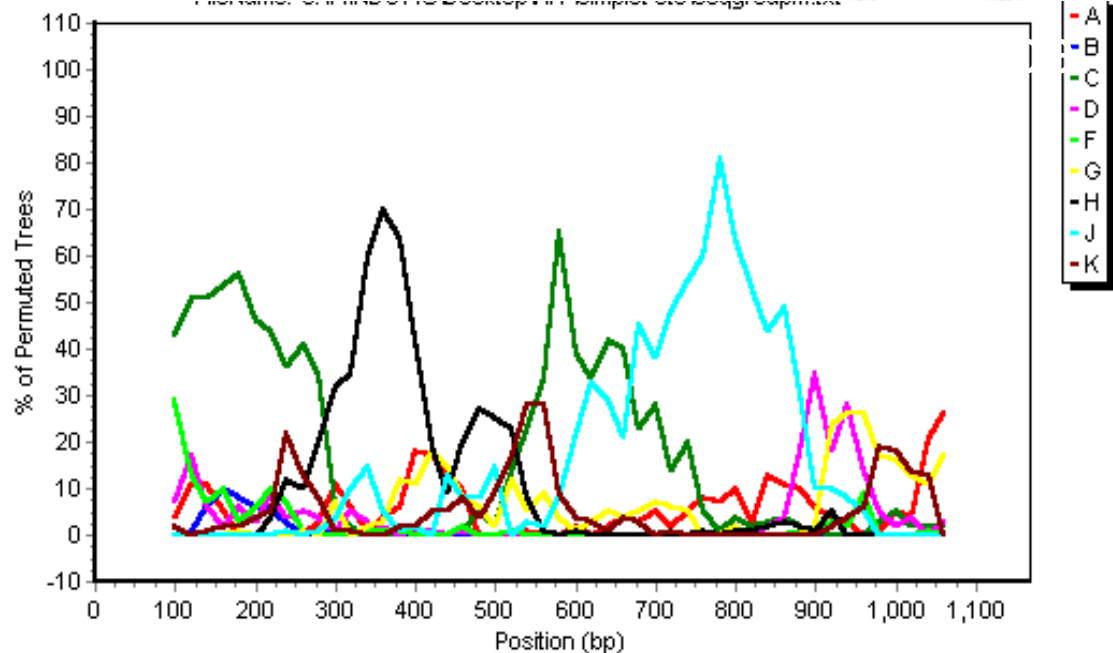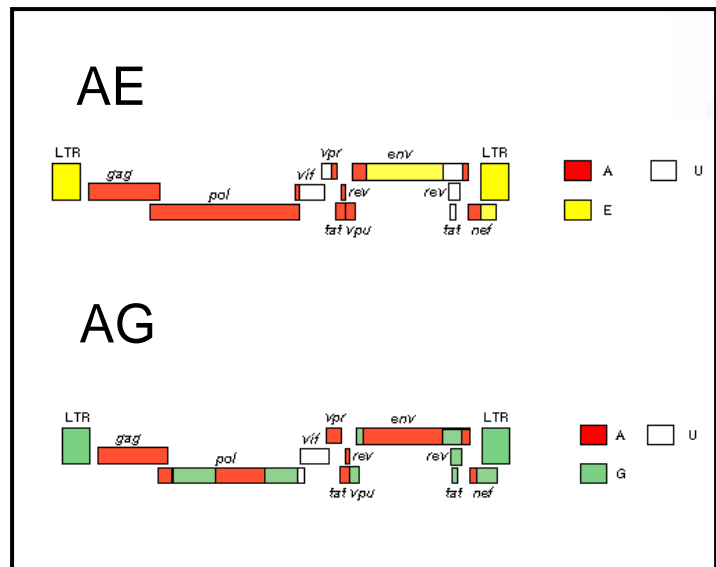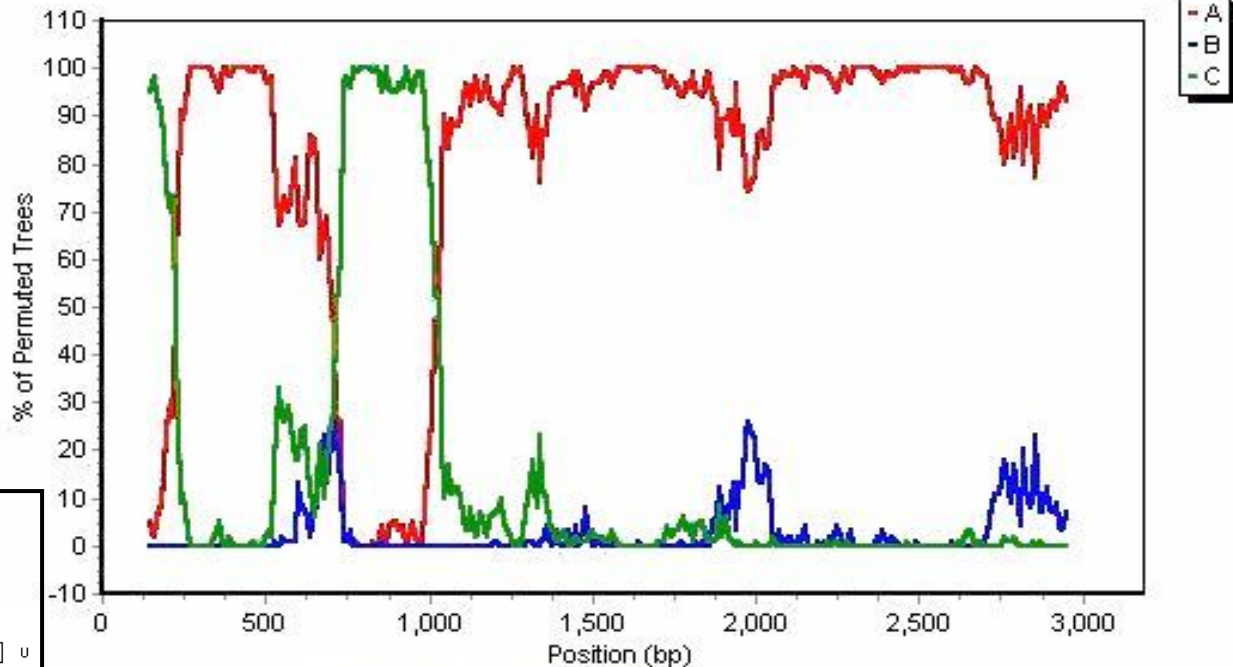
Dating the origin of subtype C

Other subtypes
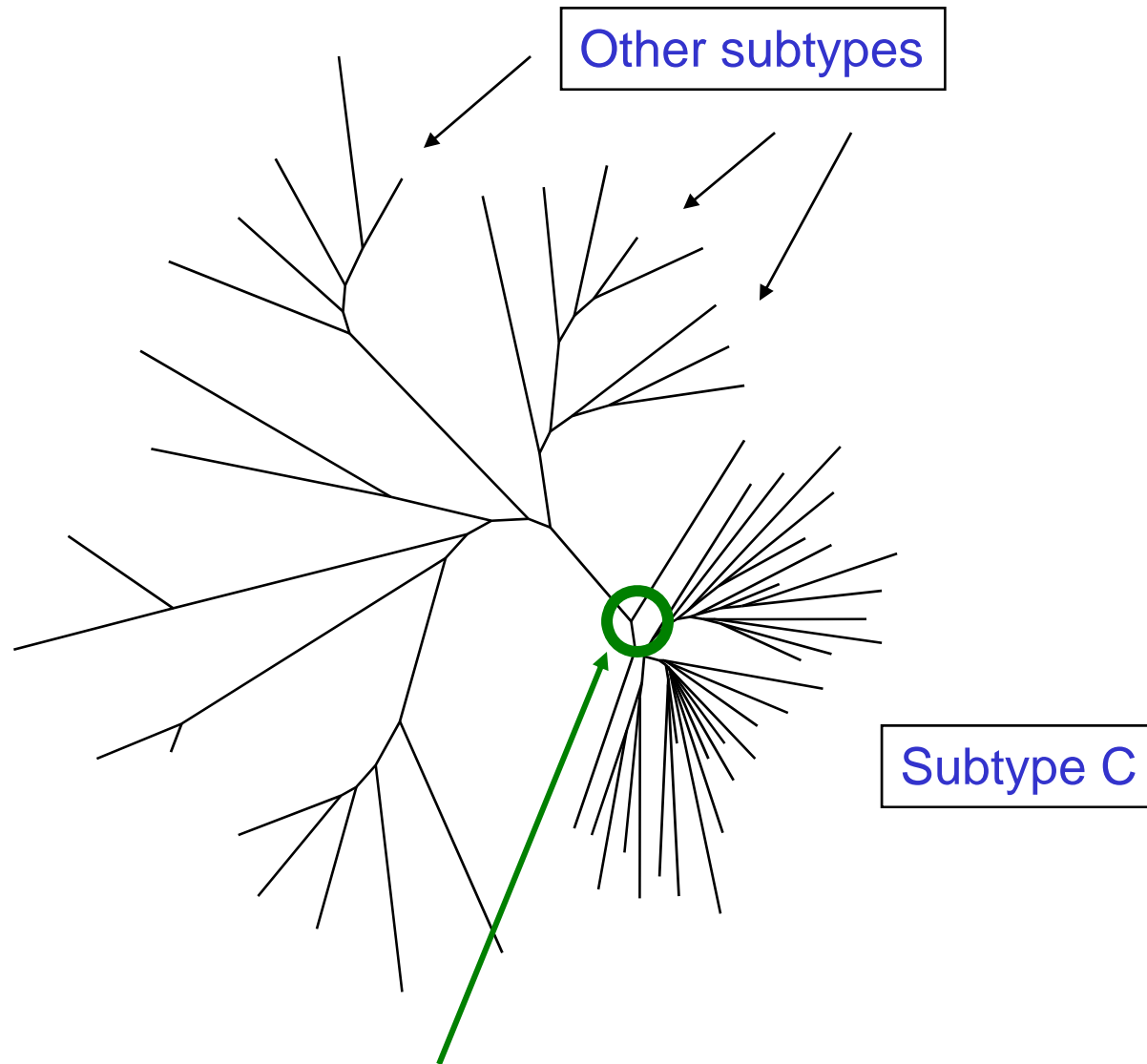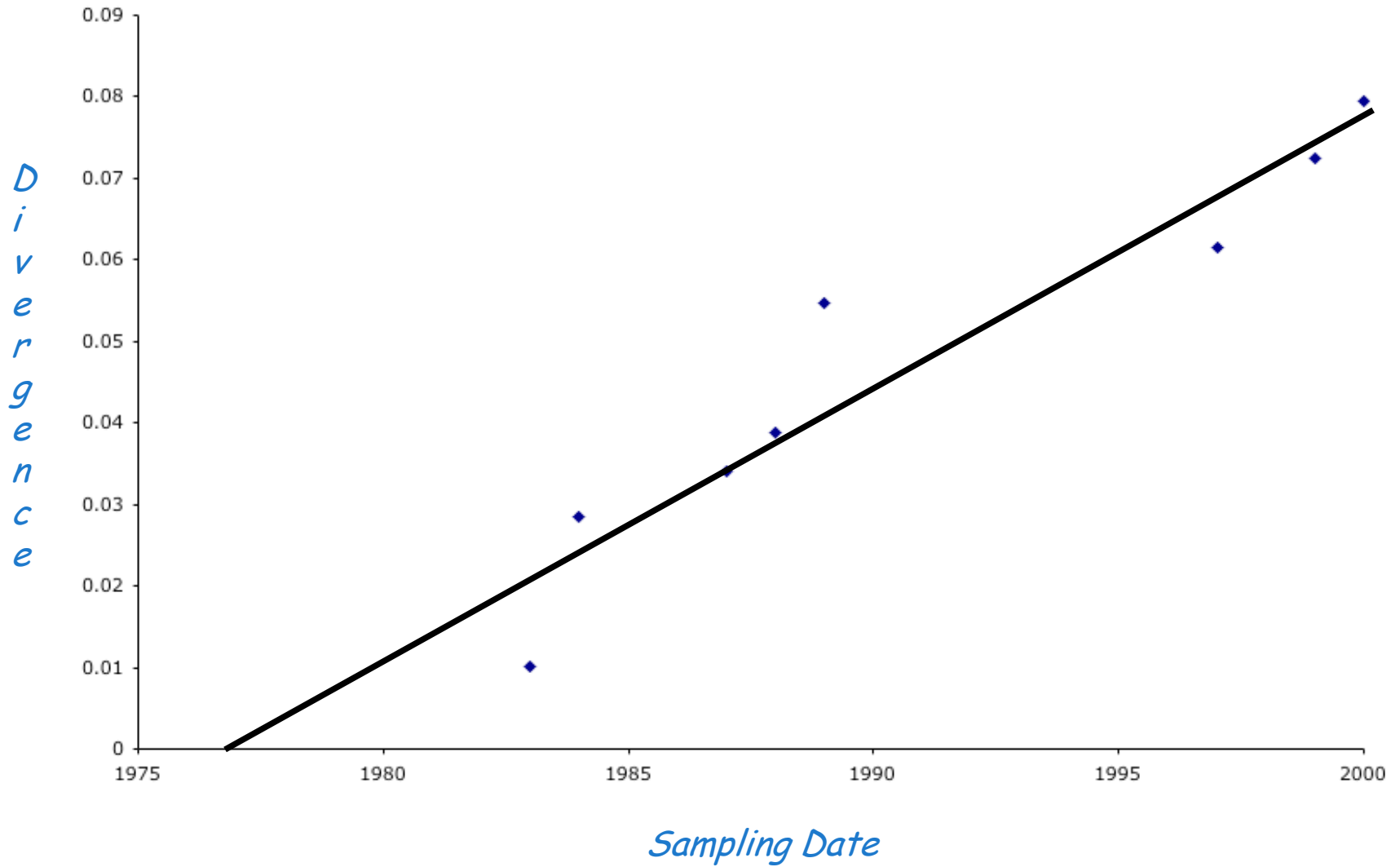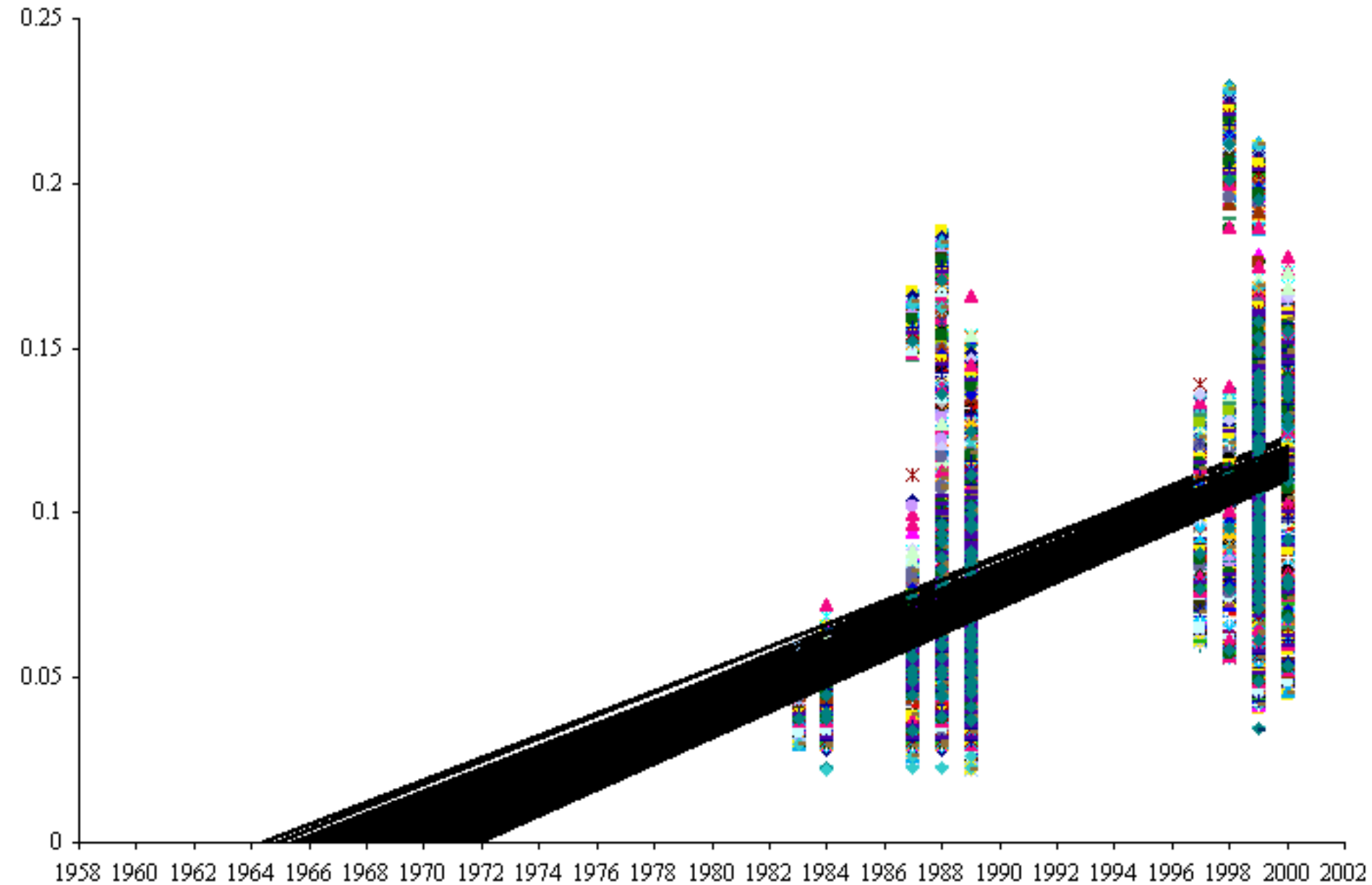
Subtype C

**Root Node =   putative common ancestor
                 of all subtype C viruses**

Dating Methods

Linear regression analysis

Timing and Reconstruction of the Most Recent Common Ancestor of the Subtype C Clade of Human Immunodeficiency Virus Type 1

Simon A. A. Travers,[1] Jonathan P. Clewley,[2] Judith R. Glynn,[3] Paul E. M. Fine,[3]
Amelia C. Crampin,[3,4] Felix Sibande,[4] Dominic Mulawa,[4]
James O. McInerney,[1] and Grace P. McCormack[1*]

Datasets Used	Estimated Dates
gag	
•**Karonga** (208 sequences, 618bp)	➢**1960–1969**
•**Entire** (376 sequences, 660bp)	➢**1966–1969**
env	
•**Karonga** (125 sequences, 417bp)	➢**1966–1975**
•**Entire** (299 sequences, 435bp)	➢**1962–1972**

Method Confirmation

Estimate the date of a "Malawi Clade"

      -introduced to Northern Malawi from one/few individuals c. 1980

   **-thought to have spread to *c.* 40% of infected persons sampled by**

late 1980s

	"Malawi' clade	
	All Sequences	Mean
Method	**Date** [95% CI]	**Date** [95% CI]
PAML dS Distances	**1979** [1973-1983]	**1979** [1975-1981]
PAUP* Distances	**1979** [1975-1982]	**1979** [1976-1981]
ML Opt. BLens	**1979** [1976-1983]	**1979** [1977-1982]
MrBayes BLens	**1981** [1979-1983]	**1980** [1978-1985]

Travers et al., J. Virol. 2004

Malawi Long Term Survivors

- 192 HIV positive individuals sampled in 1980s

- Follow up studies in late 1990s identified 38 of these individuals again, 32 of whom supplied blood samples (DNA extraction successful for 31).

- 1990s: 31 infected with HIV for a minimum of 10 years

- 2004: ~23 confirmed still alive (more than 15-16 years after seroconversion)

Pairs of samples collected from same person 1980s and 1990s

→	44gMa89	GTCAGCAA---AAAACACAGCAAGCAGAAGCGGCTGAC		
→	210gMa99	gtcagcaR---aaaacacagcaa--------gctgac	*
→	4gMa89	--CAACAA---AAAACACAGCAGGCAGGAGCGGCTGAC	
→	224gMa99	gccagcaa---aaagcacagcag--------gctgac	*
→	173gMa89	GTCAGCAA---AAAACACAGCAGGCAGAAGCGGCTGAC	
→	238gMa98	gtcagcaa---aaaacacagcag--------gctgac	*
→	178gMa89	GTCAGCAA---AAAACACAGCAGGCAAAAGAGGATGAC	
→	231gMa99	--cggcaa---aaaacacagcag--------gatgac	*
→	184gMa88	GTCAGCAA---AAAACACAGCAGGCAAAAGCGACTGAC	
→	208gMa99	GCCAGCAA---AAAACACAGCAGGCAAAAGCAACTGAC	*
→	35gMa89	GTCAGCAA---AAAACACAGCAAGCAGAAGCGGCTGAC	
→	237gMa98	gtcagcaa---aaaacacagcag--------gctgac	*
→	36gMa89	GCCAGCAA---AAAACACAGCAGGCAGAAGCGGCTGAC	
→	242gMa98	gtcagcaa---aaaacacagcag--------gctgac	
→	46gMa89	GTCAGCAA---AAAACACAGCAAGCAGAAGCGGCTGAC	
→	225gMa99	gtcagcaa---aaaacacagcaa--------gctgac	*
→	47gMa89	GTCAGCAA---AAAACACAGCAAGCAGAAGCGGCTGAC	
	236gMa99	gtcagcaa---aaagcacagcag--------gctgct	
	48gMa89	GTCAGCAA---AAAGCACAGCAGGCAAAAATGGCTGAC	
	235gMa99	gtcagcaa---aaagcacagcag--------gctgaa	*
	60gMa89	GTCAGCAA---AAAACACAGCAGGCAGAAGCGGCTGAC	
	241gMa98	gtcagcaa---aaaacacagcag--------gctgac	*
	75gMa88	GTCAGCAA---AAAACACAGCAAGCAGAAGCGGCTGAC	
	221gMa99	gtcagcaa---aaaacacagcaa--------gctggc	*
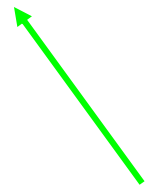
Deletion in 1990s sequence

Identified in 1990s samples of 11 of 15 (74%) patients with sequences available in both the late 1980s and late 1990s.

Gag p17 deletion

Not observed in any sequences from Malawi prior to 1990s.

Only observed in 3 other published subtype C sequences (1 BW, 2 ZA).

However observed in 36 of 53 (68%) sequences collected as local controls in Malawi in 1990s.

Extremely unlikely that all Malawi survivors were infected with the same strain.

More likely that this strain emerged independently in each long-term survivor.

This viral mutation may be associated with long survival and has spread from survivors to the general population.

Current Work on Long Term Survivors

- Follow up 15 LTS:
  - 10 dried blood spots (2004) - 7 amplified
  - Plasma samples from 2007

- 36 general population samples sequenced (2007)

- Original deletion not observed but new indels were seen.

2007 Sequences

Current Work on Long Term Survivors

- Follow up 15 LTS:

  – 10 dried blood spots (2004) - 7 amplified

  – Plasma samples from 2007

- 36 general population samples sequenced (2007)

- Original deletion not observed but new indels were seen.

- Competitive fitness assays comparing sequences with the 3 amino acid deletion to sequences without the deletion

- Study presence of indel strains as minor variants in the population in 80s/90s/00s.

Evolution of drug resistance in HIV

- Anti Retroviral Treatment (ART) in Karonga started in late 2006.

- RT inhibitors only

- Aug 2008,130,000 people started ART

    Hosseinipour et al, 2008

- Baseline drug resistance data

- Ultra deep sequencing for detection of minor variants from Malawi samples

- Detection of transmission of drug resistance.

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LSHTM, London, UK
Judith Glynn,
Paul Fine

CPHL, London UK
Jonathan Clewley

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Amelia Crampin,
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