Asymptomatic, acute or chronic infections:

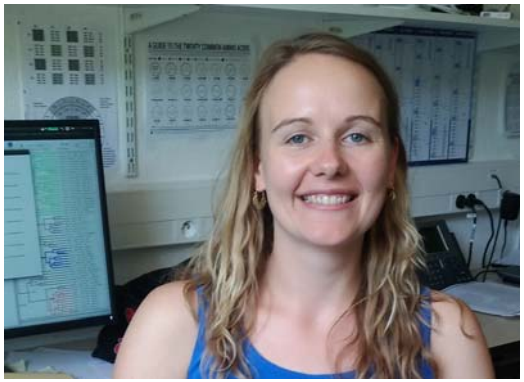
a twisted road from genotype to phenotype in Papillomaviruses

codovirevol

European Research Council

Ignacio G. Bravo
Centre National de la Recherche Scientifique (CNRS)
Montpellier, France
ignacio.bravo@ird.fr

CBGP, June 2018



Anouk Willemsen

Ville Pimenoff

Sara Nicolás-Párraga

Marta Félez-Sánchez

When do we understand a disease?

- when we have an explanation of mechanisms

AND

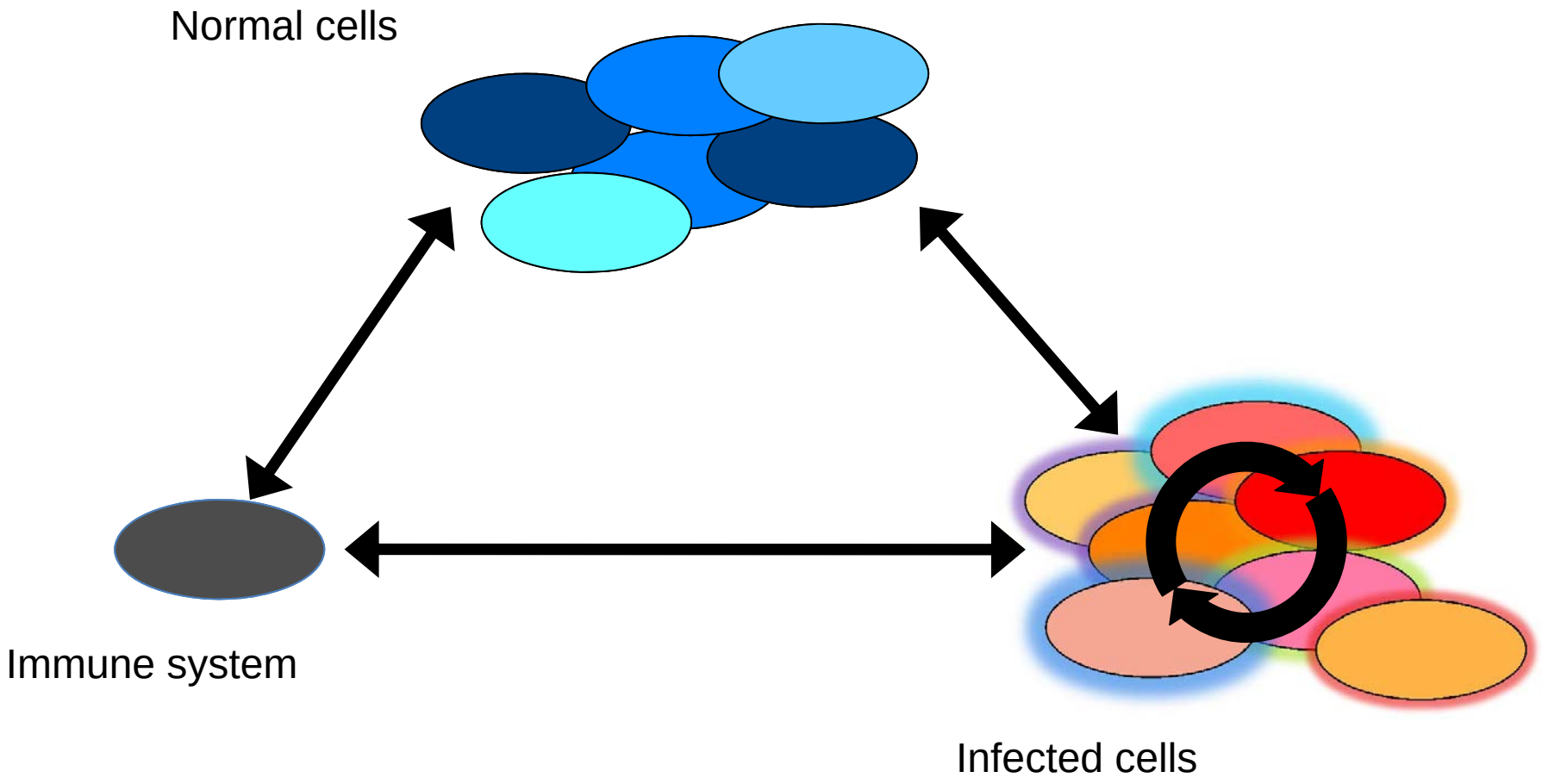
- when we have an evolutionary explanation about how/why the organism is vulnerable to disease despite natural selection

Tinbergen's four categories of questions and explanations

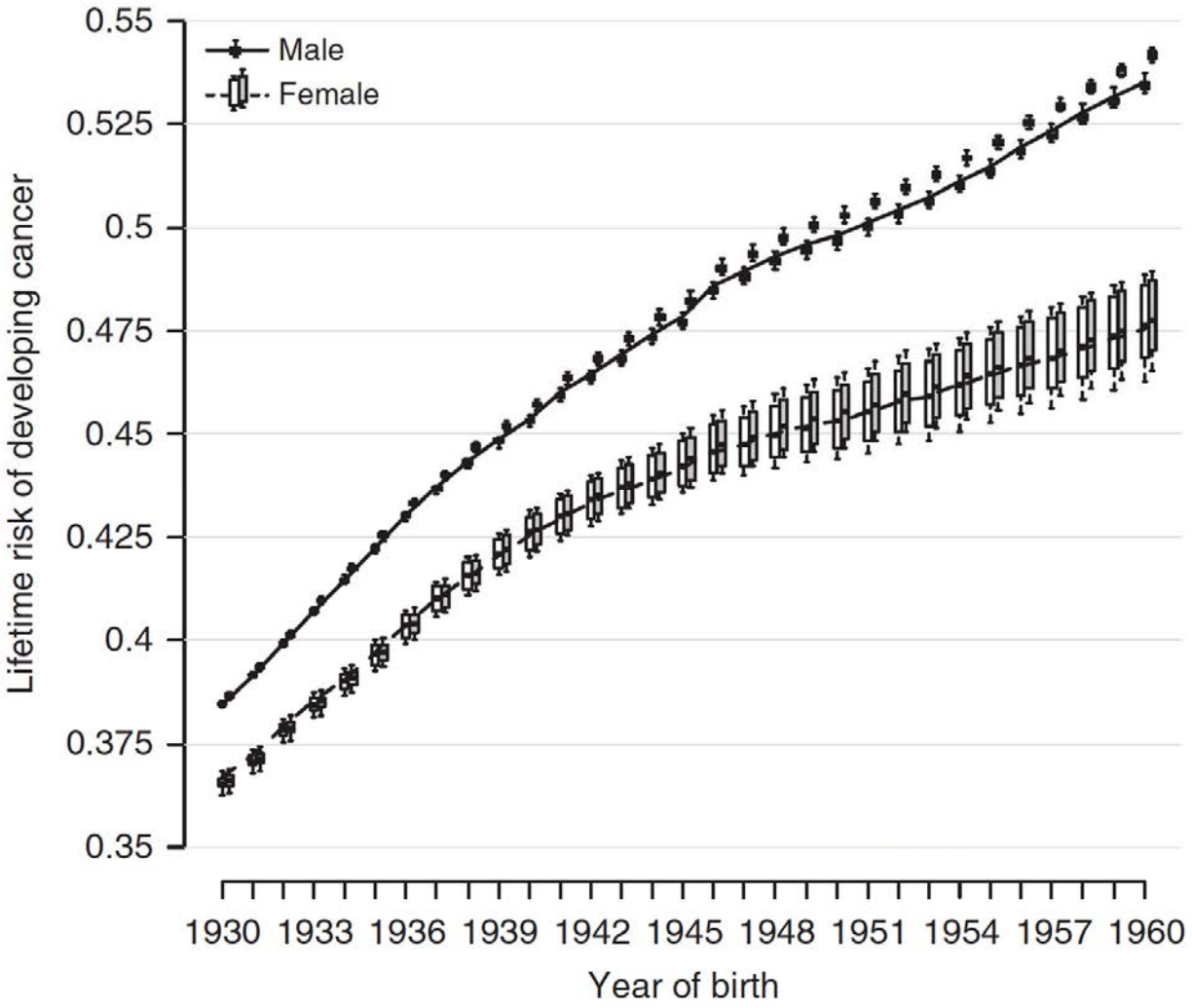
		historic perspective	*contemporary perspective*
How does an individual work?	*proximate explanation*	**ontogeny | plasticity** the changes in genotype expression in an individual	**mechanism** the way a structure works
Why has a species evolved to be so?	*ultimate explanation*	**phylogeny** the history of changes in the species through generations resulting in this structure	**function | adaptation** the problem a structure currently solves and the advantage this structure provides with

Tinbergen *Zeitsch Tierpsych* (1963)

Cancers are eco-evolutionary processes



Cancers are frequent diseases

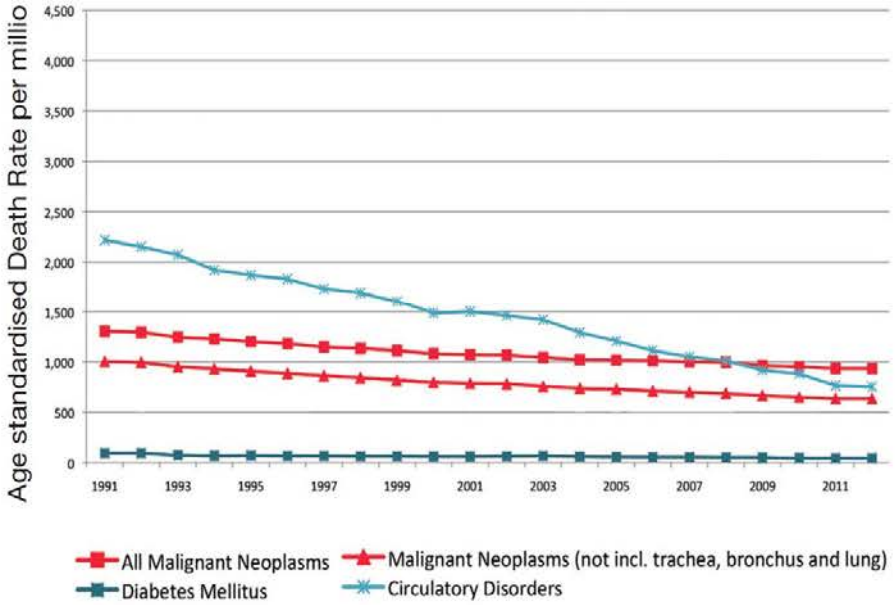


Ashmad et al., 2015, Br J Cancer

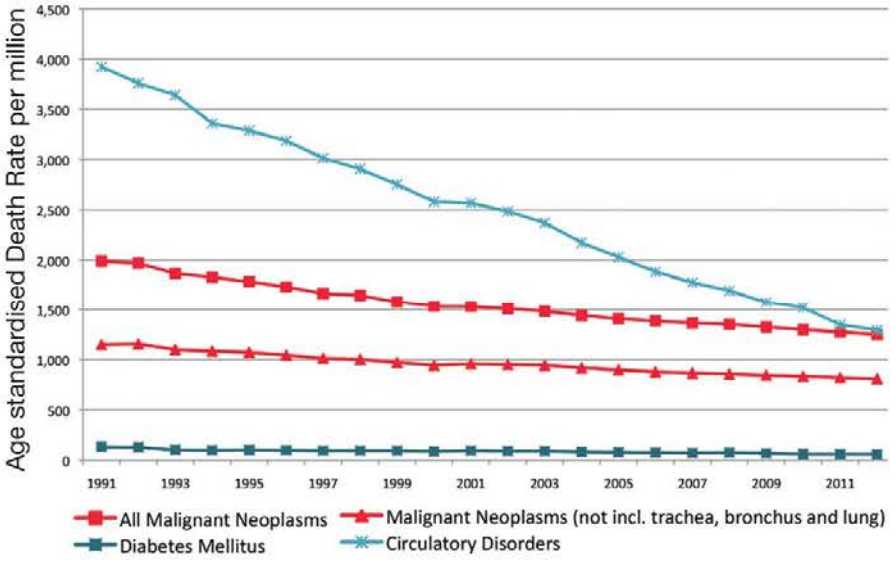
Cancers are frequent diseases

Age standardised death rates for selected causes

females



males



Gill and Sullivan, 2015 (UCL Editions)

Cancers are ancient diseases

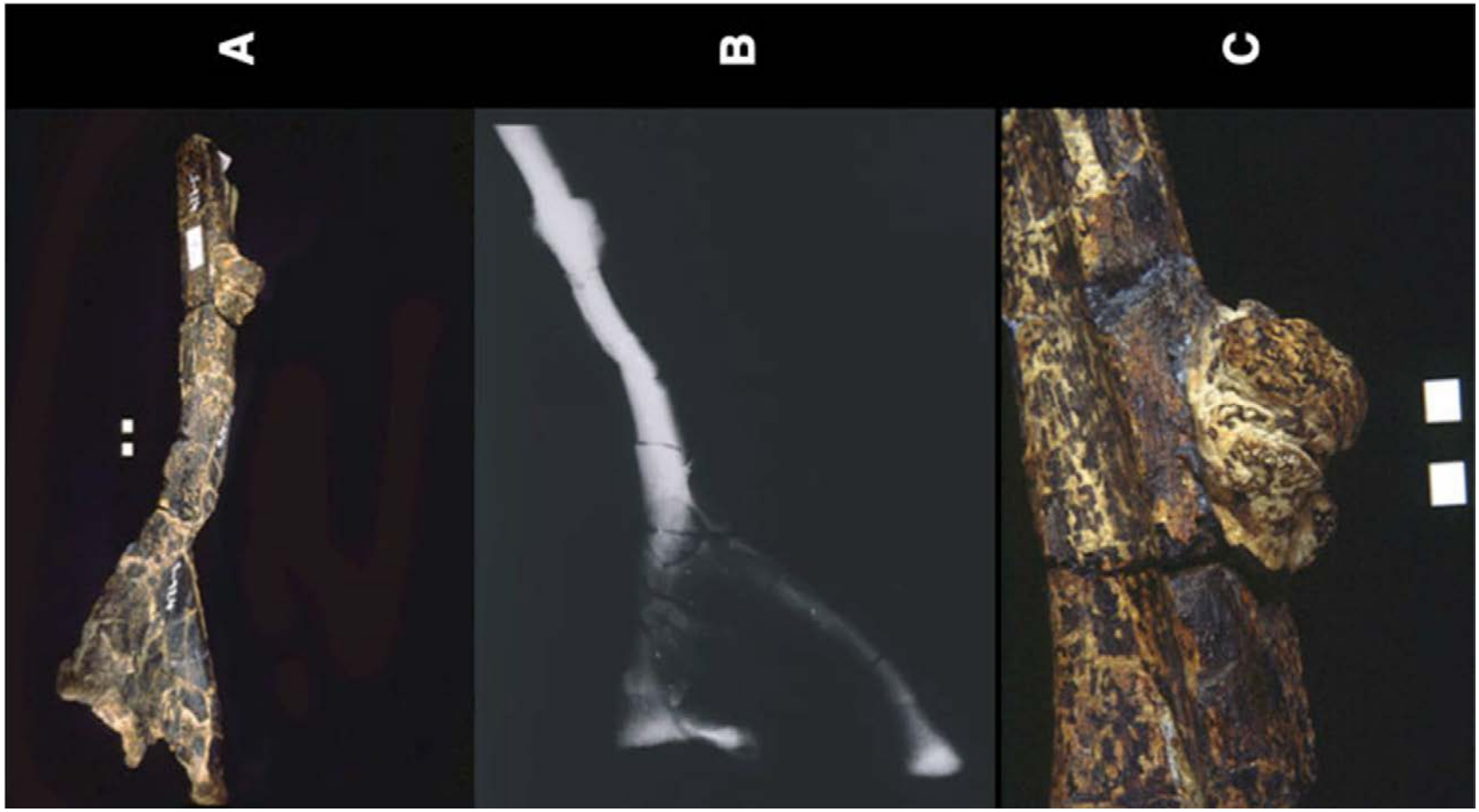
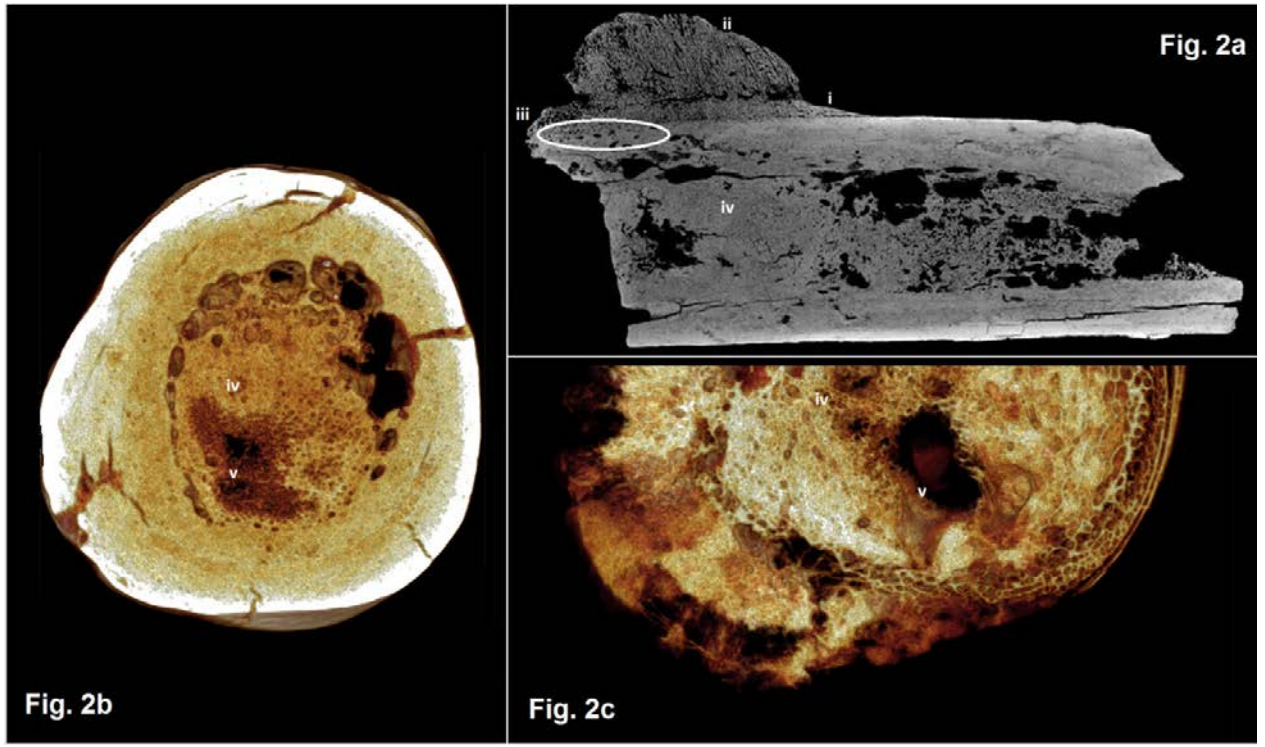


FIGURE 1 – Fragment of a right rib of a large dinosaur, Apatosaurus sp. (a), which presents a sub-rounded mass with multilobulated surface (c), and high radiographic density (b) (Jurassic, Wyoming, USA; sample 323 of the University Museum, Chieti).

Capaso 2005 Int J Cancer

Cancers are ancient diseases

Osteosarcoma of the femur in a native Peruvian dating to ca. 800 BP



Earliest (recorded) hominin cancer: 1.7-million-year old osteosarcoma. (i) reactive new bone formation (ii) ossified exophytic (cauliflower-like) (iii) invasion by the mass into the cortex, (iv) remodelled bone infill

Aufderheide et al. 1997 J Paleopathol

Odes et al. 2016 South African J Sci

Cancer is an eco-evolutionary process

- clonal diversification model

Fidler & Hart 1982; Greaves & Maley 2012

- cancer-stem cell model

Clarke & Fuller 2006

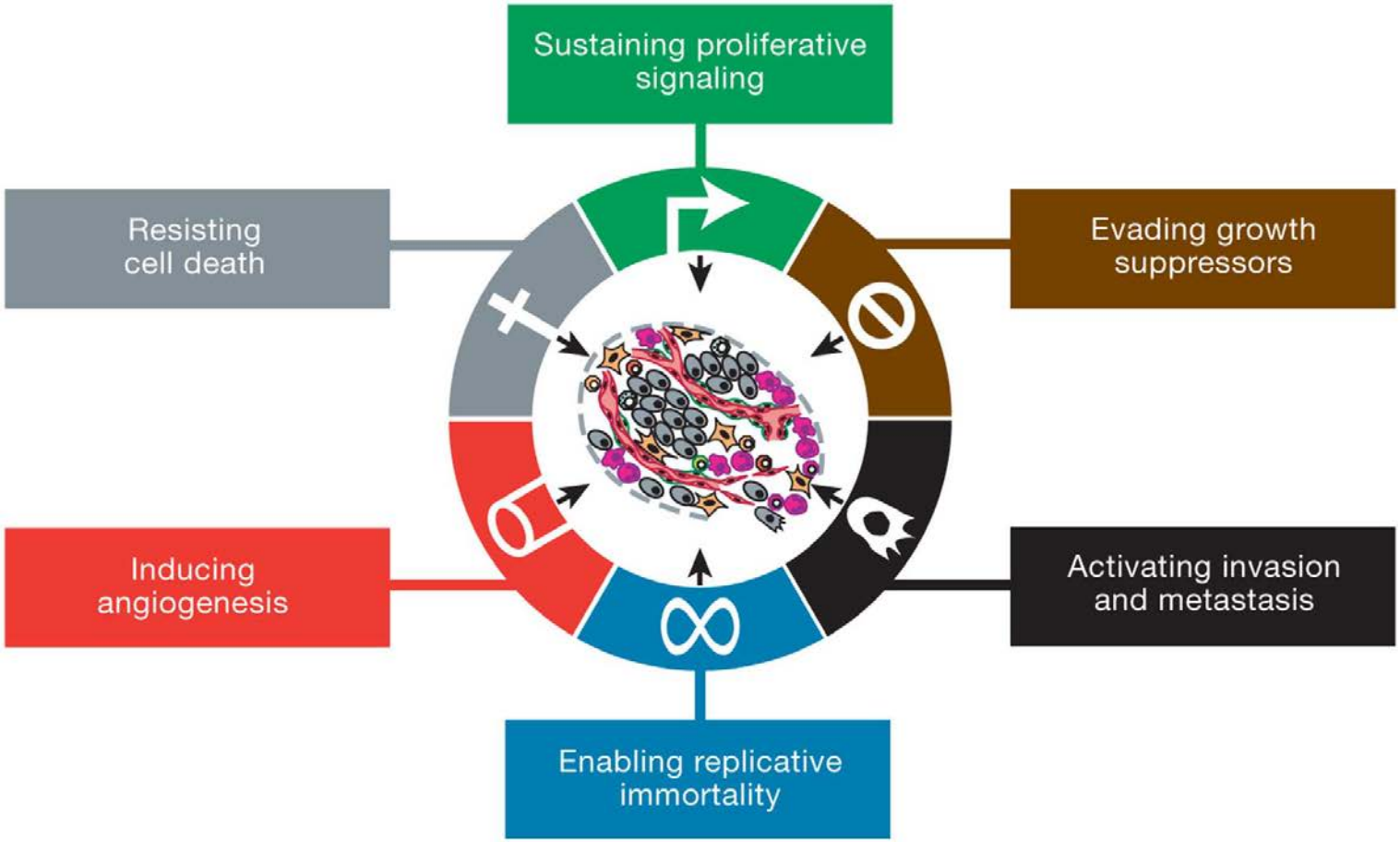
- hallmarks of cancer

Hanahan & Weinberg 2000, 2010

- barrier model of cancer

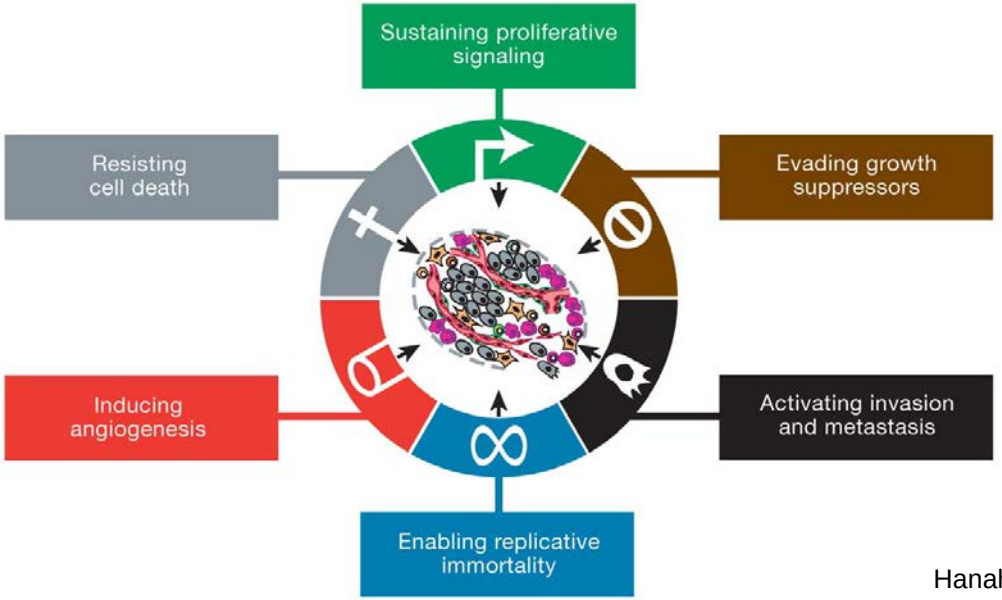
Ewald & Swain Ewald 2012

Cancer is an eco-evolutionary process



Hanahan and Weinberg (2000; 2010) *Cell*

Cancer is an eco-evolutionary process



Hanahan and Weinberg (2000; 2010) *Cell*

Barriers

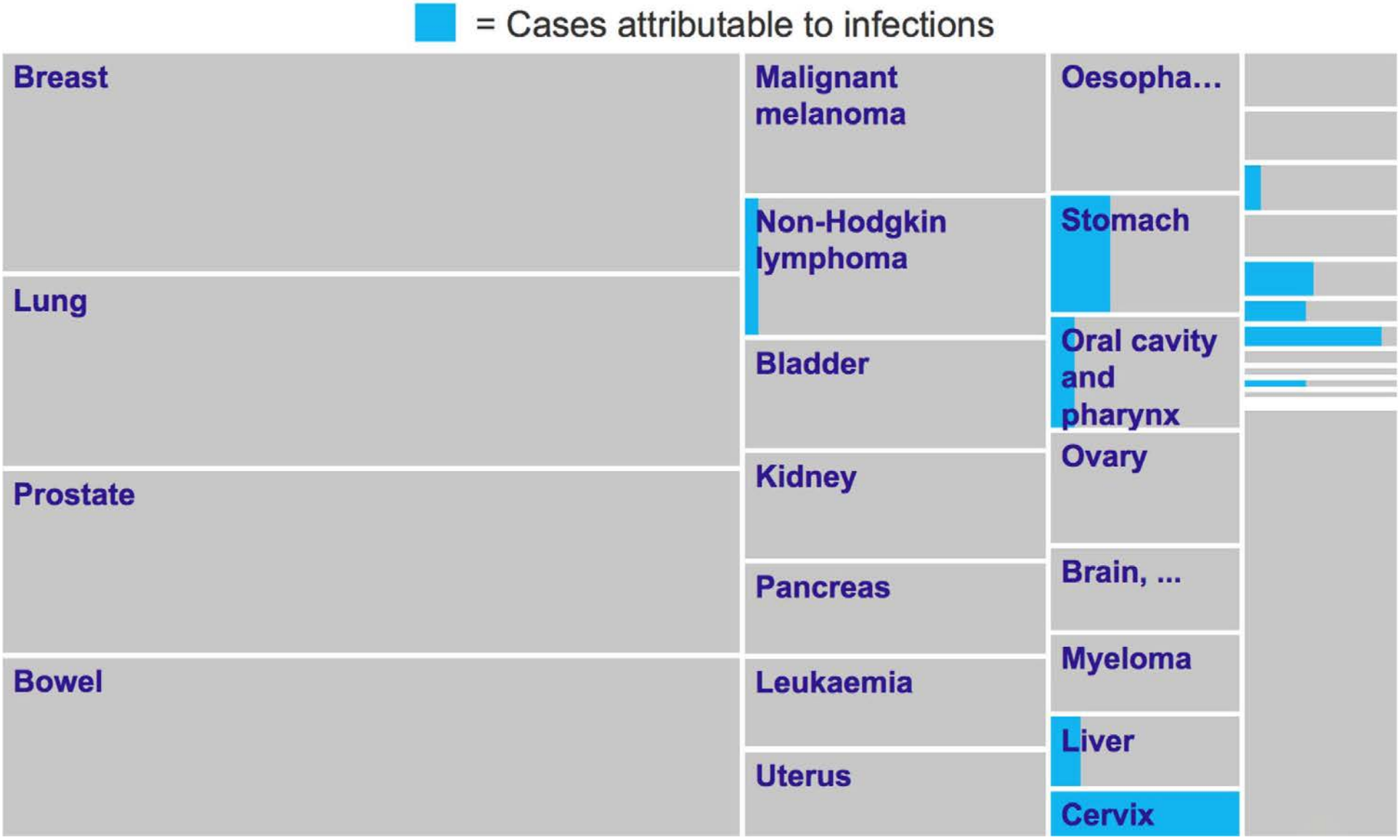
- Cell cycle arrest
- Apoptosis
- Limited division potential
- Cell adhesion
- Asymmetric division

Restraints

- Cell division rate
- Angiogenesis
- Microenvironment manipulation
- Metabolic shifts

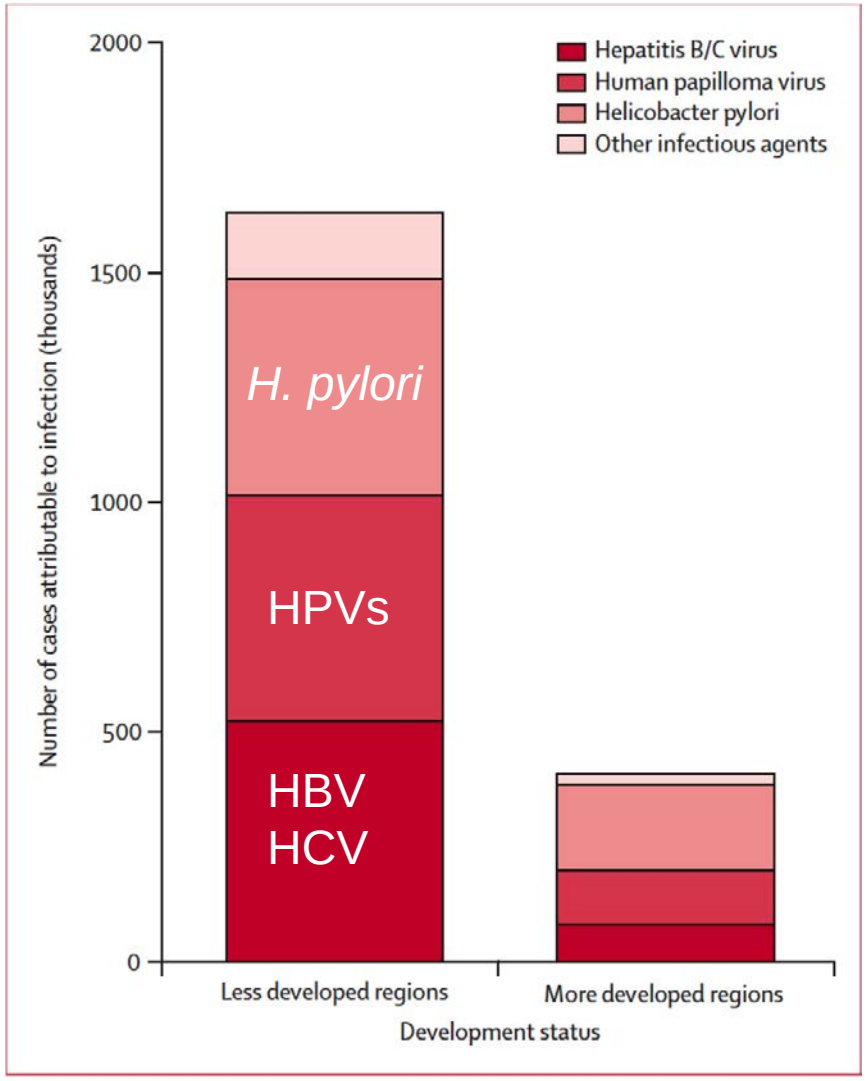
Ewald&Swain Ewald (2012) *Evol App*

Certain infections may cause cancer in humans



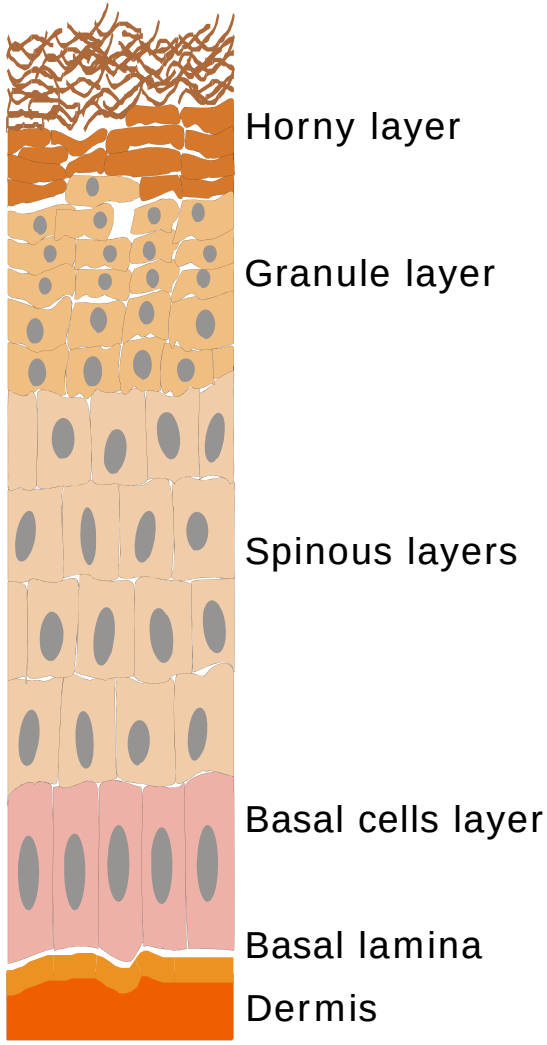
http://www.cancerresearchuk.org/

Certain infections may cause cancer in humans

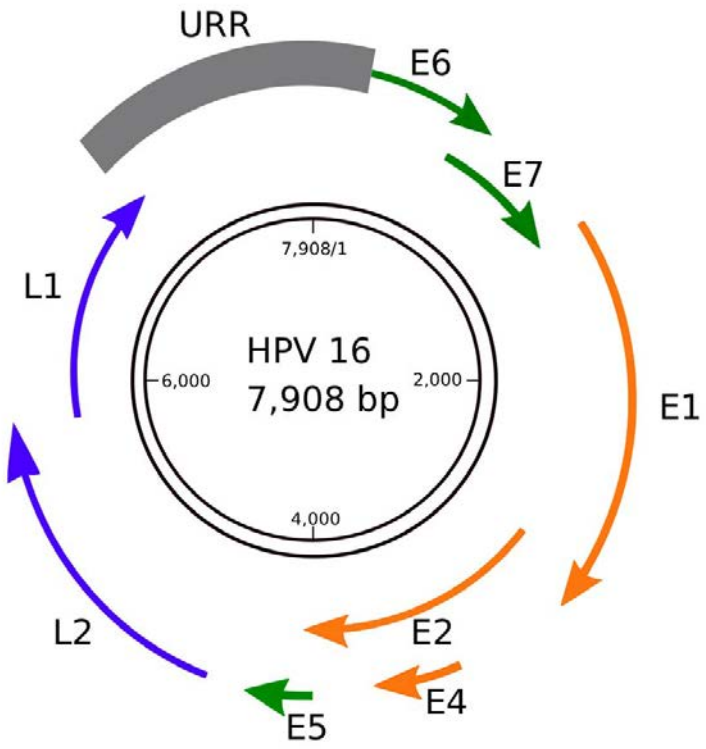


de Mantel (2010) *Lancet*

Papillomaviruses infect epithelia



Papillomaviruses are a simple study system



Locus	Function
URR	-Harbours transcription factor-binding sites -Controls gene expression
E6	-Promotes cellular immortalization by degradation of p53 -Modifies cell adhesion and differentiation by degradation of TAp63 and p73
E7	-Promotes pRb degradation, permitting cell progression to S-phase of cell cycle -Induces chromosomal instability
E5	-Induces cell proliferation -Contributes to evasion of apoptosis -Downregulates MHC expression
E1	-ATP-dependent DNA helicase activity -Role in viral DNA replication
E2	-Coactivator of viral DNA replication -Transcrition repressor of HPV E6 and E7 -Regulates cell cycle and apoptosis -Interacts with chromatin for segregation of viral genome
E4	Binds cytoskeletal proteins and disrupts cytoskeletal structure of the G2 arrested cell
L2	-Minor capsid protein -Recruits L1 protein for virus assembly
L1	-Major capsid protein -Can autoassemble into VLPs

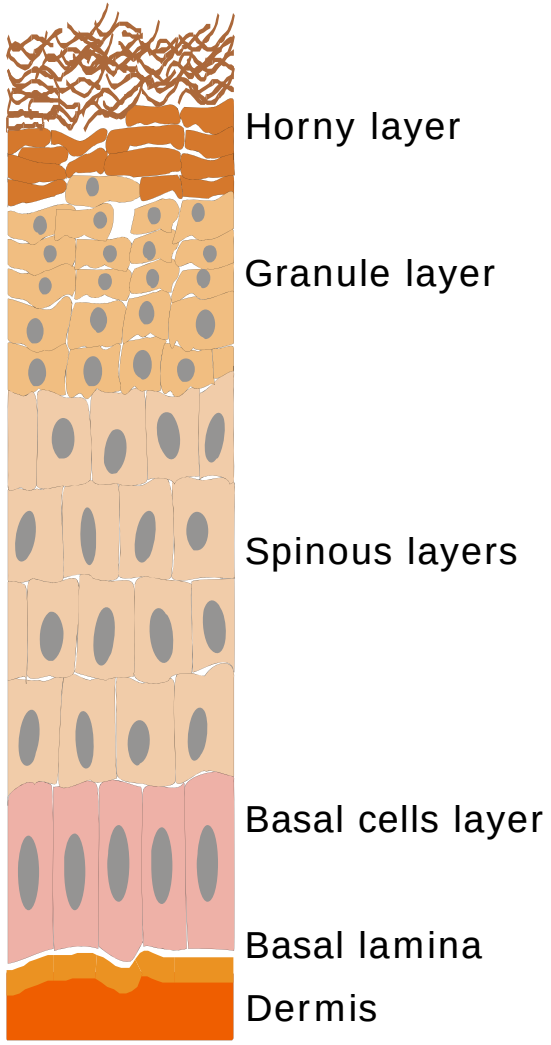
Papillomaviruses life cycle follows keratinocyte differentiation

Virion release

L1 and L2
Encapsidation

E1, E2 and E4 (E5?)
Viral genome replication

E6 and E7 (E5?)
Host cell reprogramming

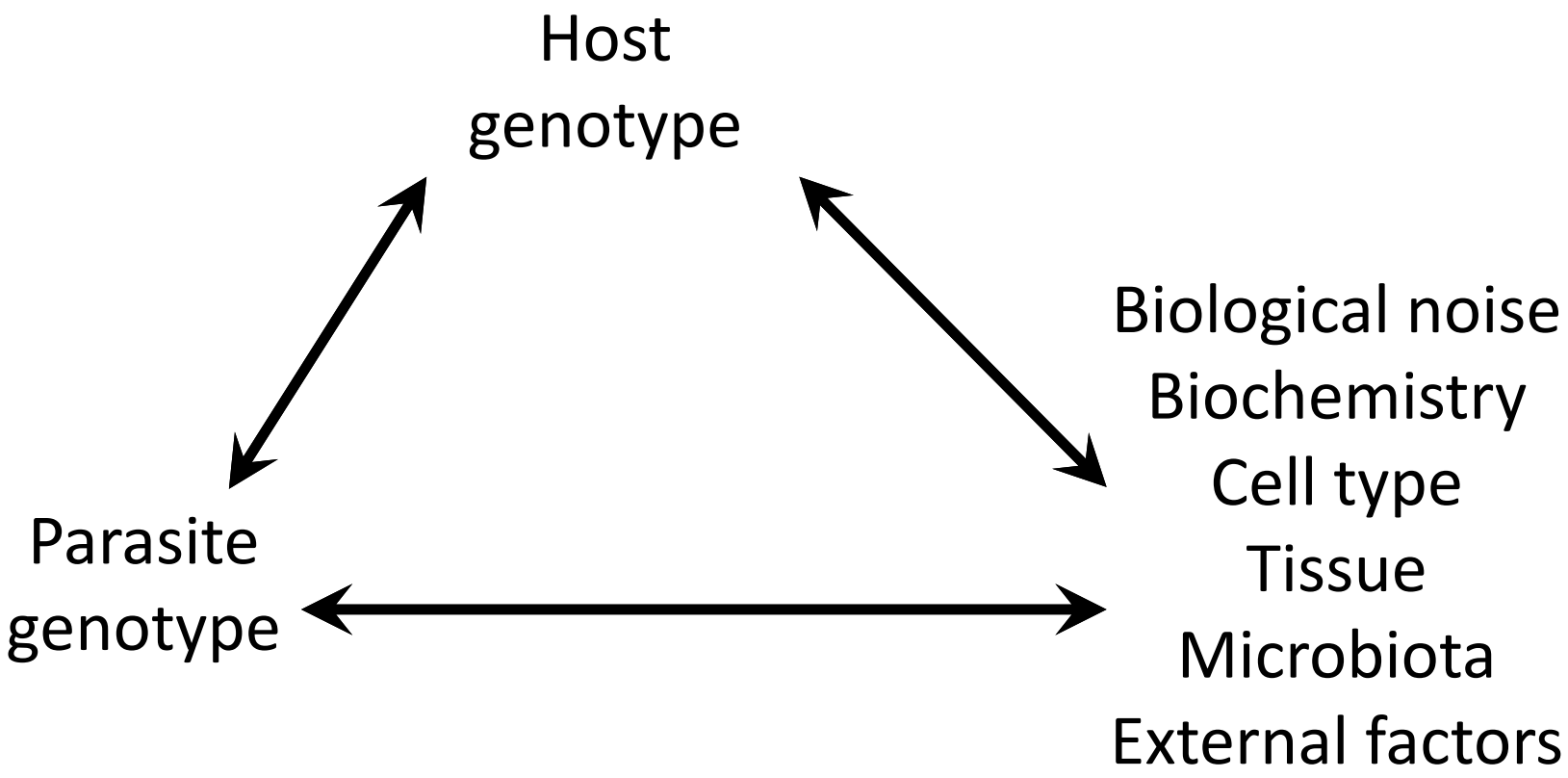


(virtually) All sexually active adults have been exposed to papillomaviruses

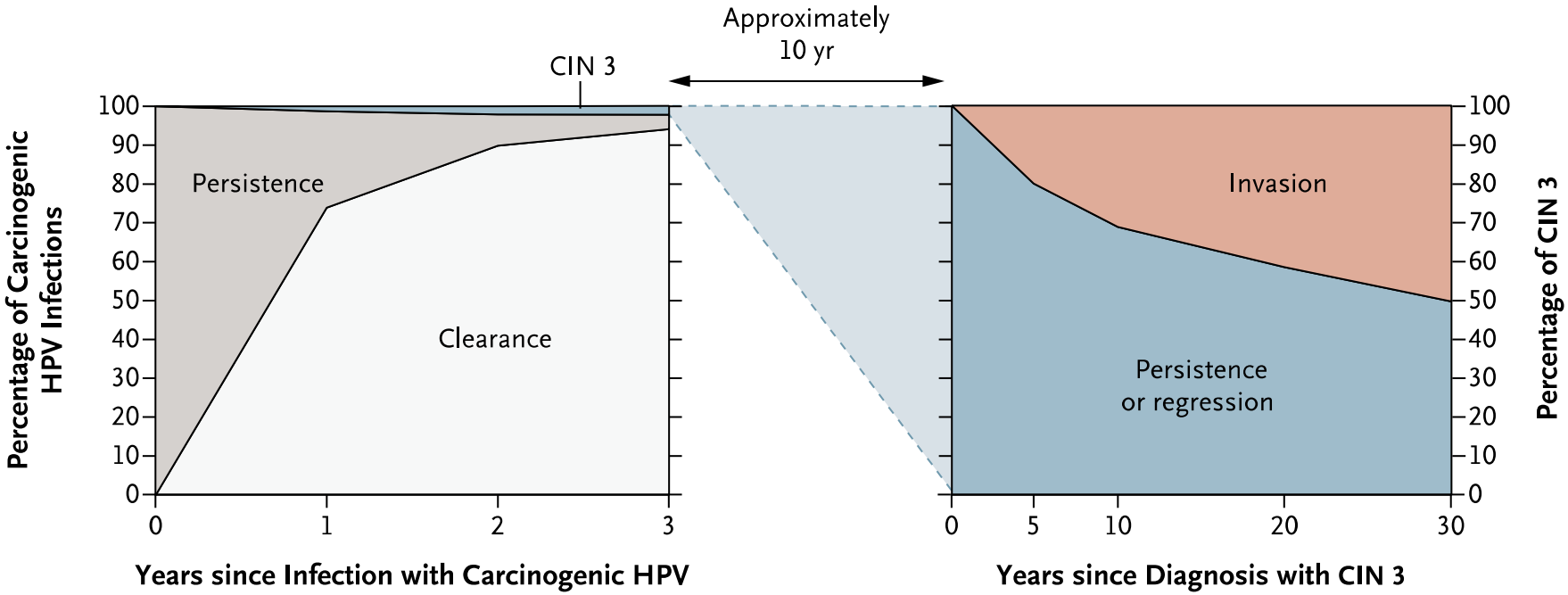
but

only a few develop infection-related cancers

Genotype x Genotype x environment interactions



HPVs-driven cancers appear after years of persistent infection



Schiffman & Solomon (2015) *New Engl J Med*

Cancer burden in France

Table 3: Cervical cancer incidence in France (estimates for 2012)

Indicator	France	Western Europe	World
Annual number of new cancer cases	2,862	9,824	527,624
Crude incidence rate[a]	8.8	10.2	15.1
Age-standardized incidence rate[a]	6.8	7.3	14.0
Cumulative risk (%) at 75 years old[b]	0.6	0.7	1.4

Data accessed on 15 Nov 2015.

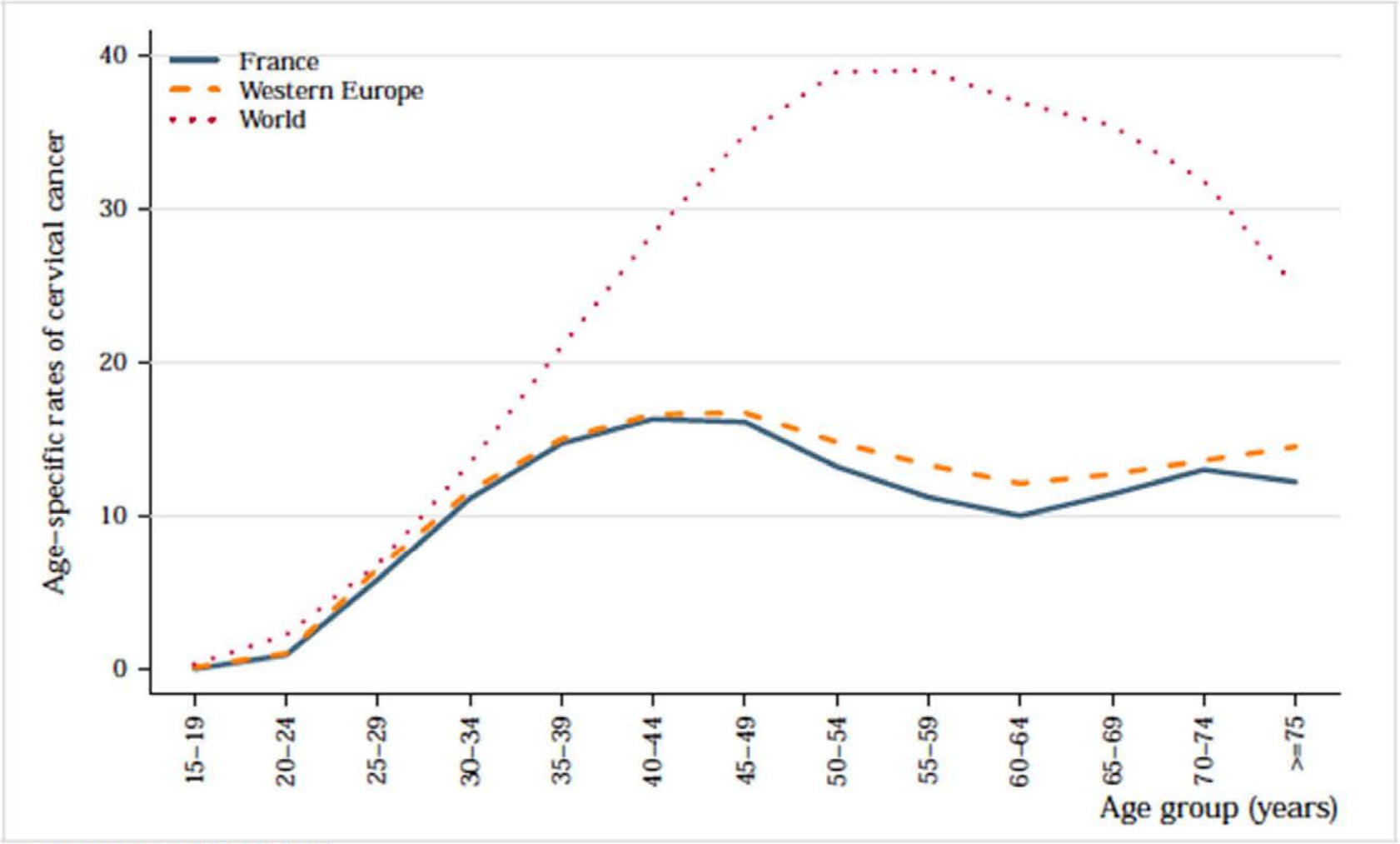
Table 6: Cervical cancer mortality in France (estimates for 2012)

Indicator	France	Western Europe	World
Annual number of deaths	1,167	3,479	265,672
Crude mortality rate[a]	3.6	3.6	7.6
Age-standardized mortality rate[a]	1.9	1.8	6.8
Cumulative risk (%) at 75 years old[b]	0.2	0.2	0.8

Data accessed on 15 Nov 2015.

Cancer burden in France

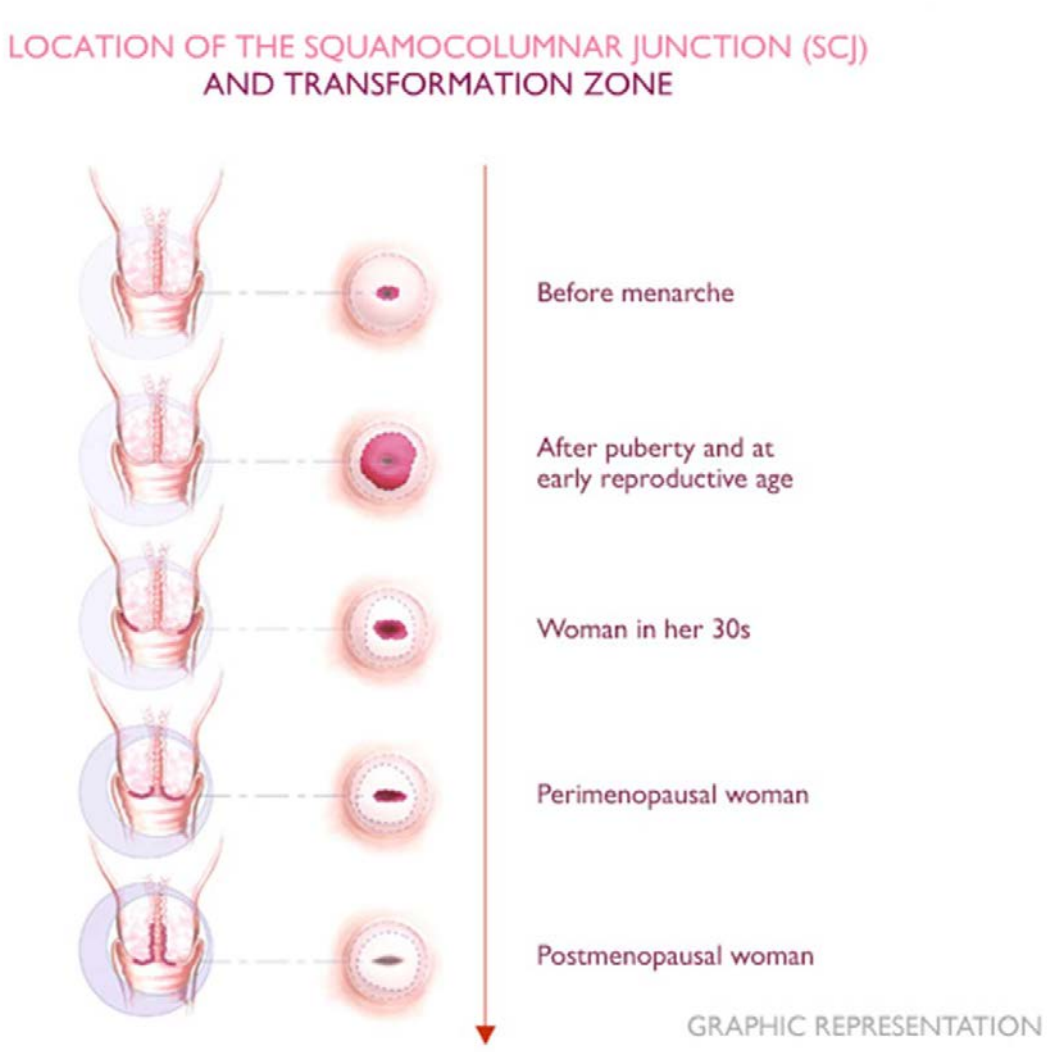
Figure 9: Comparison of age-specific cervical cancer incidence rates in France, within the region, and the rest of world



Data accessed on 15 Nov 2015.

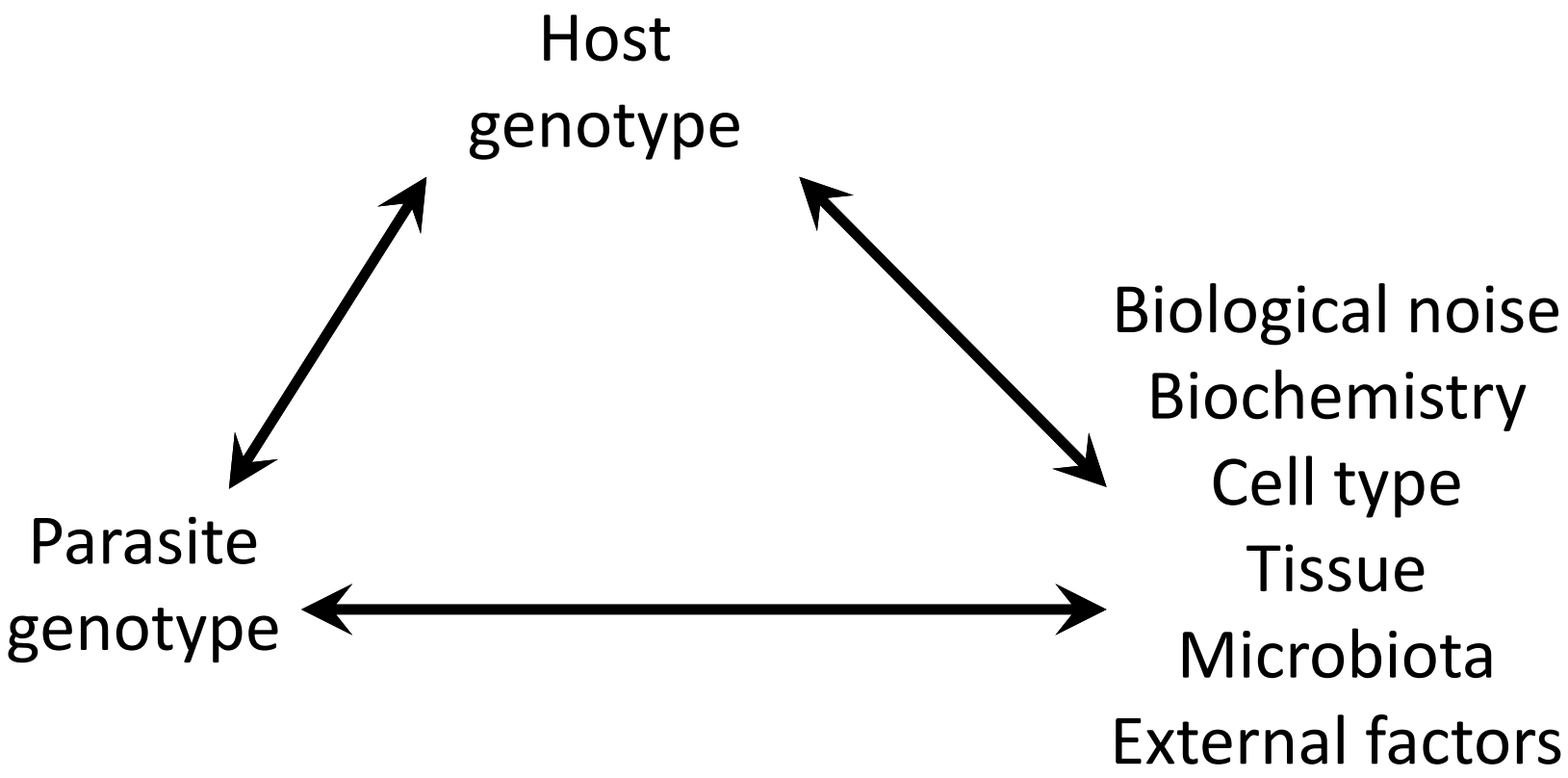
The cervix environment changes through life

(and with it probably also propensity to infection and malignisation)

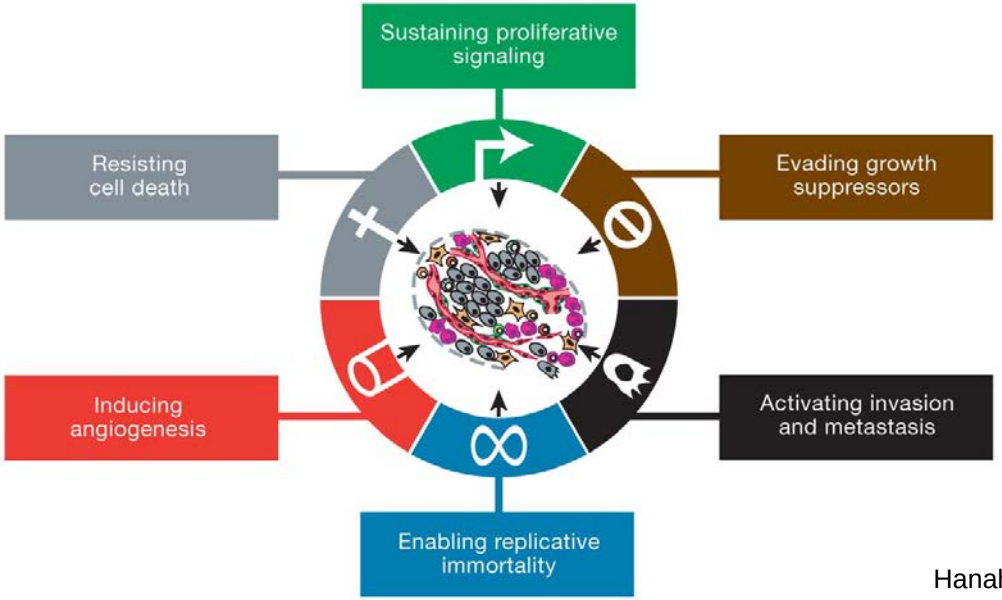


Ferlay et al. (2002) IARC CancerBase

Genotype x Genotype x environment interactions



Cancer is an eco-evolutionary process



Hanahan and Weinberg (2000; 2010) *Cell*

Barriers

- Cell cycle arrest
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Restraints

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Ewald & Swain Ewald (2012) *Evol App*

HPV-related carcinomas have a better prognosis

Taberna, Alemany *et al.* (submitted)

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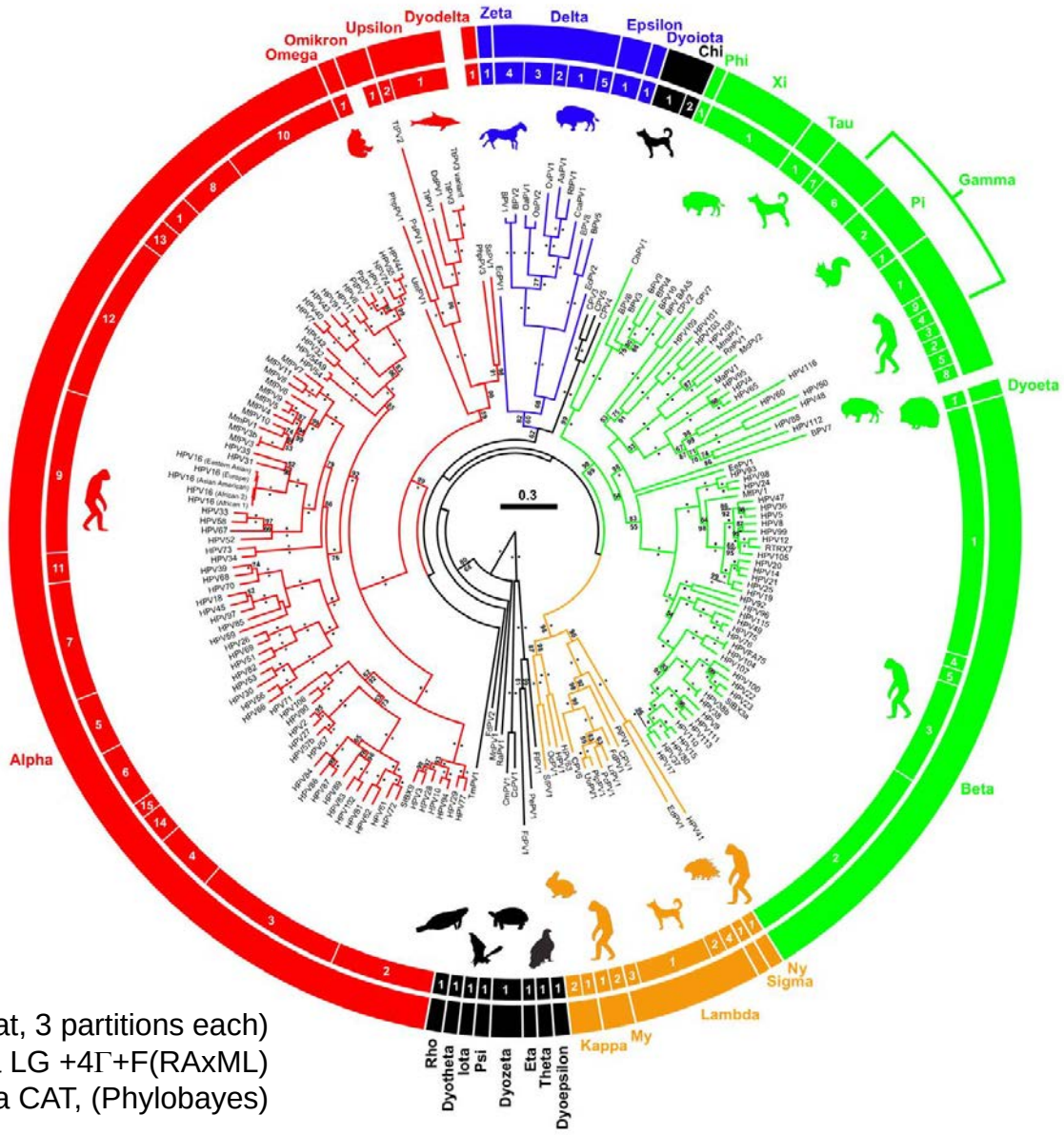
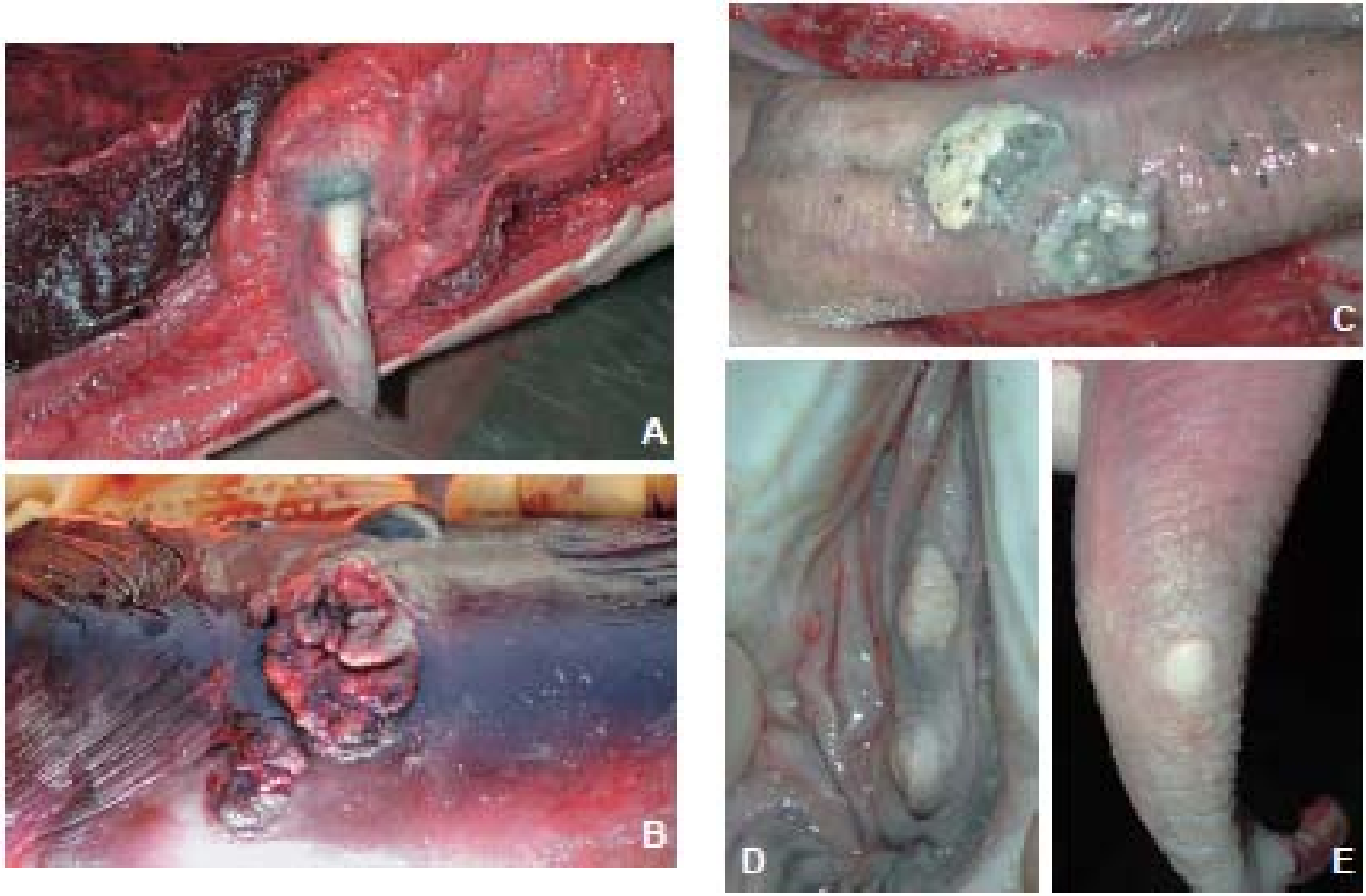
Papillomaviruses infect (probably) all amniotes



3 genes (concat, 3 partitions each)
ML, nt GTR+4Γ ; aa LG +4Γ+F(RAxML)
Bayesian, nt GTR+4Γ ; aa CAT, (Phylobayes)

Why can’t we find cervical cancer outside humans?



Gottschling *et al.* (2011) Mol. Phyl. Evol.

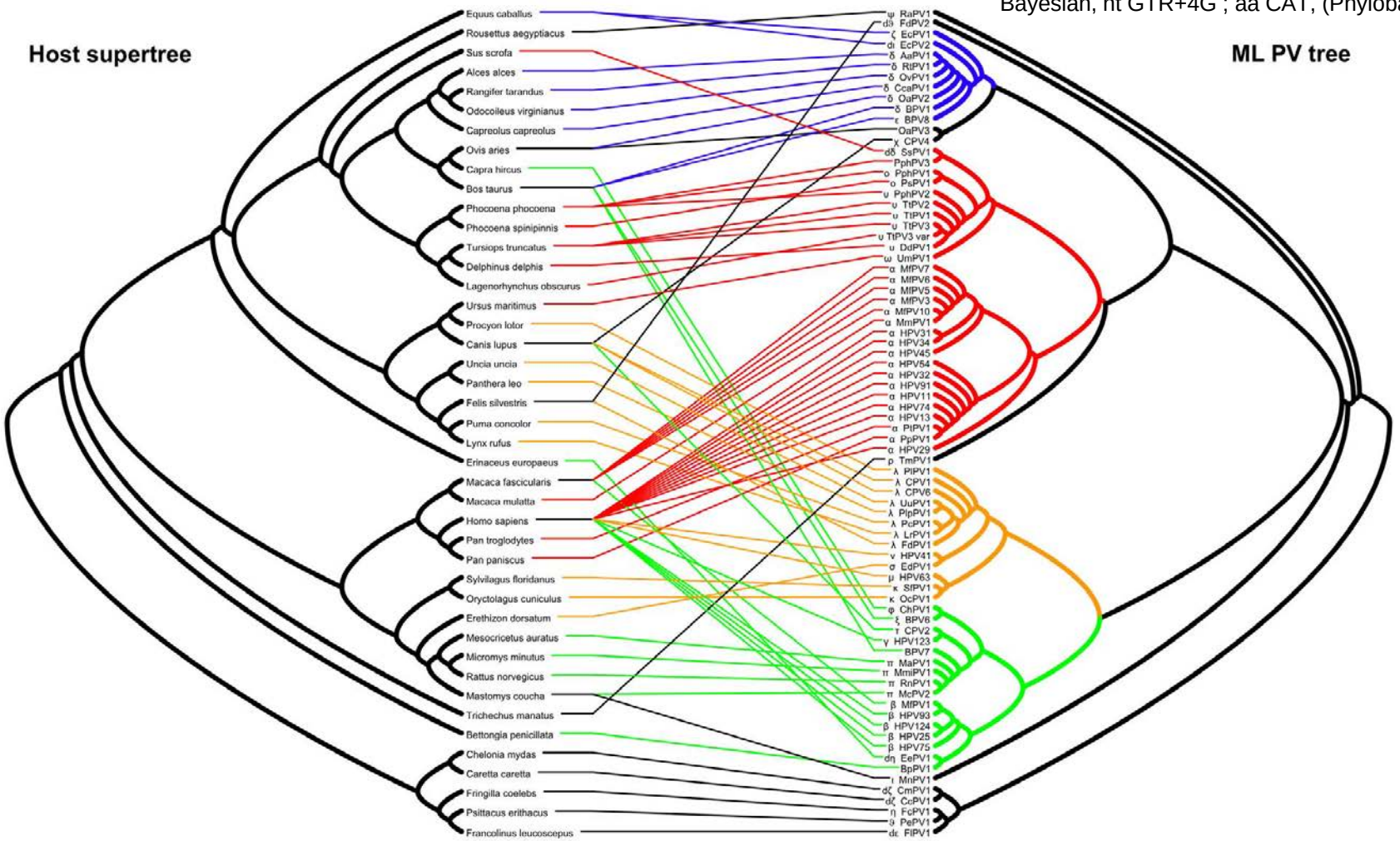
Why do we find so few oncogenic viruses outside humans?

Mengual Chulia *et al.* (2014) *Vet Microbiol*

Virus-host coevolution does not explain PV diversity and evolution

35 genes (supermatrix)
ML; aa LG +4G+F(RAxML)

3 genes (concat, 3 partitions each)
ML, nt GTR+4G ; aa LG +4G+F(RAxML)
Bayesian, nt GTR+4G ; aa CAT, (Phylobayes)



Gottschling *et al. Mol. Biol . Evol.* (2011)

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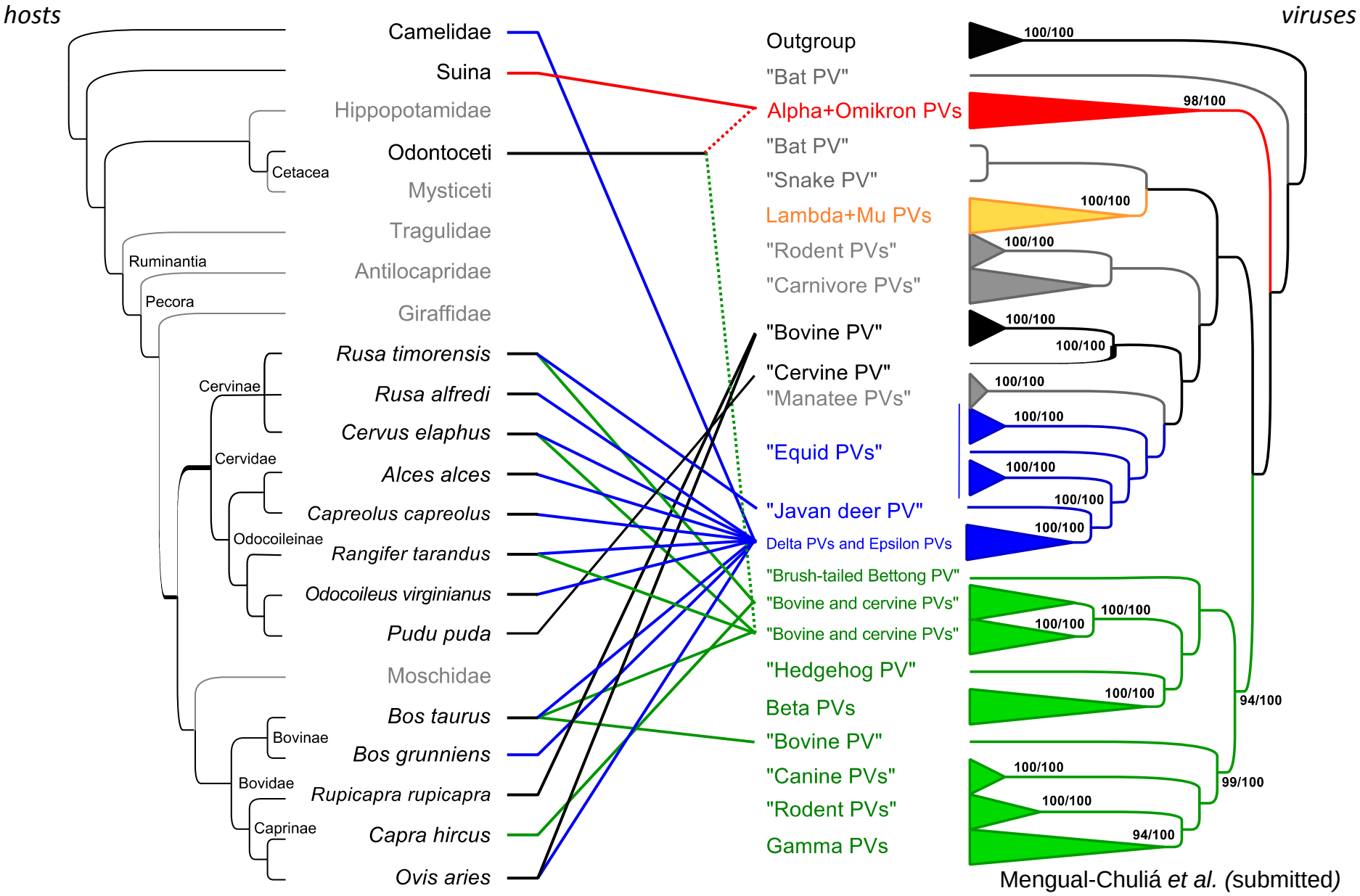
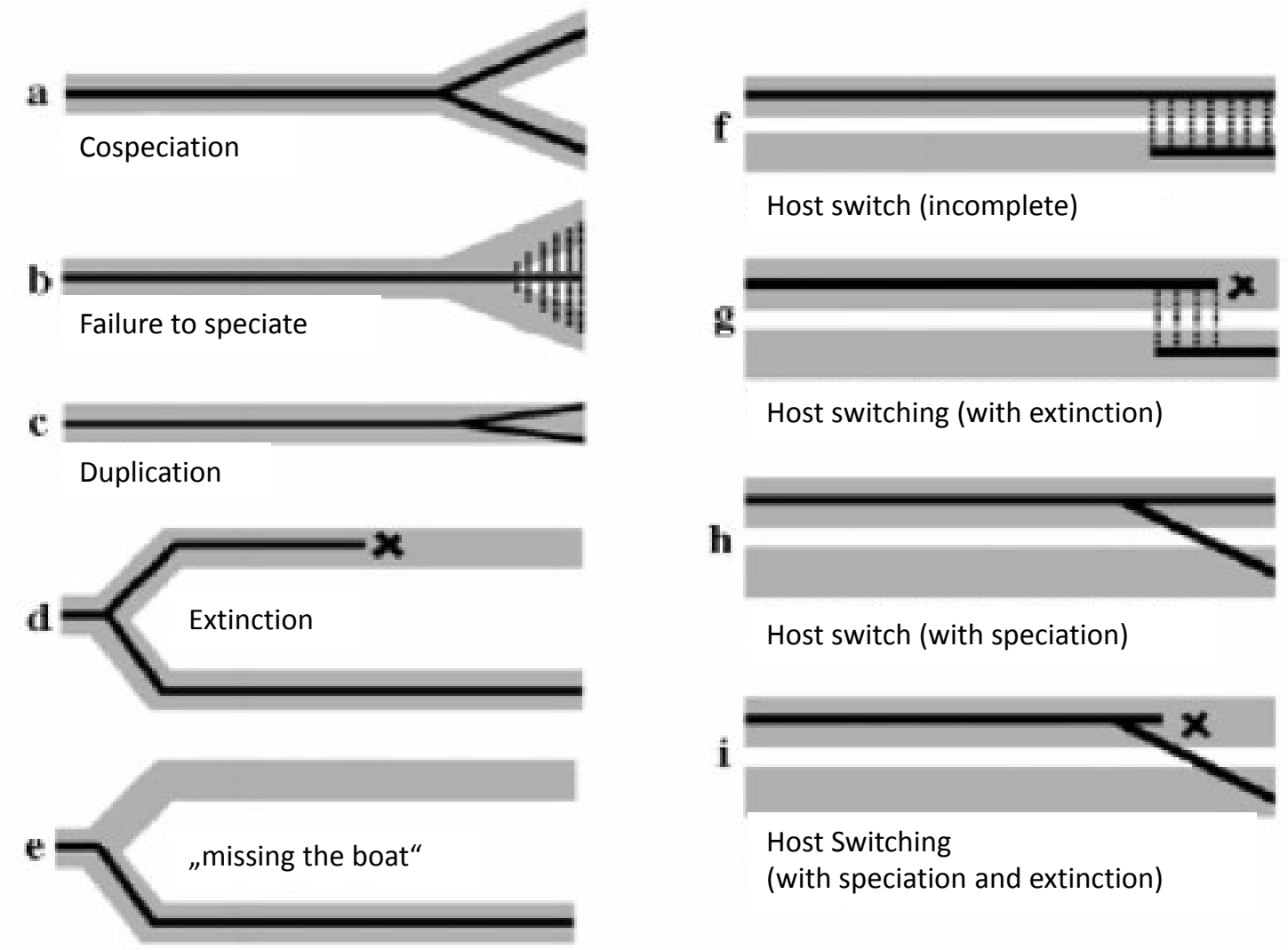


Mengual-Chuliá *et al.* (submitted)

Multiple mechanisms may contribute to the virus-host evolutionary history



Johnson *et al. Syst. Biol* . (2003)

PVs interspecies transmission is not that rare

- heterologous infection by BPV1 and BPV2

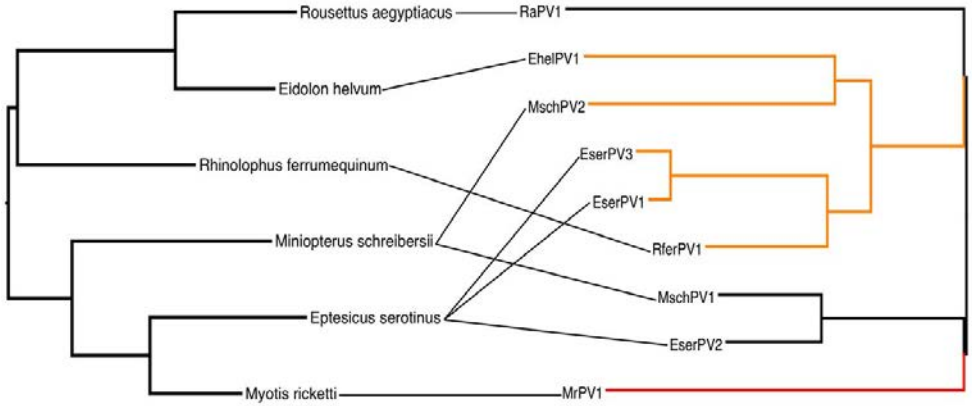
Van Dyk *et al.* (2009) *J. Virol. Methods*

Kidney *et al.* (2007) *ESDV*

PVs can be transmitted between species

Eptesicus serotinus

Eptesicus isabellinus



3 mitochondrial genes, 1 nuclear (concat)
Bayesian, nt GTR+4Γ (BEAST)

4 genes (concat, 3 partitions each)
ML, nt GTR+4Γ ; aa LG +4Γ+F(RAxML)
Bayesian, nt GTR+4Γ ; aa CAT, (Phylobayes)

García-Pérez *et al. Genome Biol Evol* (2014)

PVs interspecies transmission is not that rare

Virus Research 144 (2009) 315–317

Contents lists available at ScienceDirect

Virus Research

journal homepage: www.elsevier.com/locate/virusres

Short communication

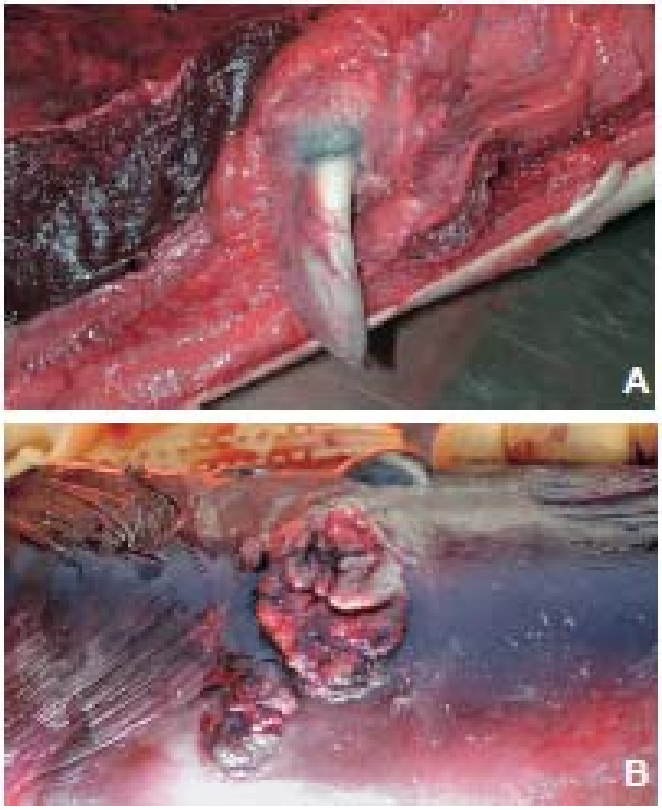
The detection of Bovine Papillomavirus type 1 DNA in flies

Margaret Finlay[a], ZhengQiang Yuan[a], Faith Burden[b], Andrew Trawford[b], Iain M. Morgan[a], M. Saveria Campo[a], Lubna Nasir[a,*]

[a] Institute of Comparative Medicine, Faculty of Veterinary Medicine, University of Glasgow, Bearsden Road, Glasgow G61 1QH, Scotland, UK
[b] The Donkey Sanctuary, Sidmouth, Devon EX10 0NU, UK

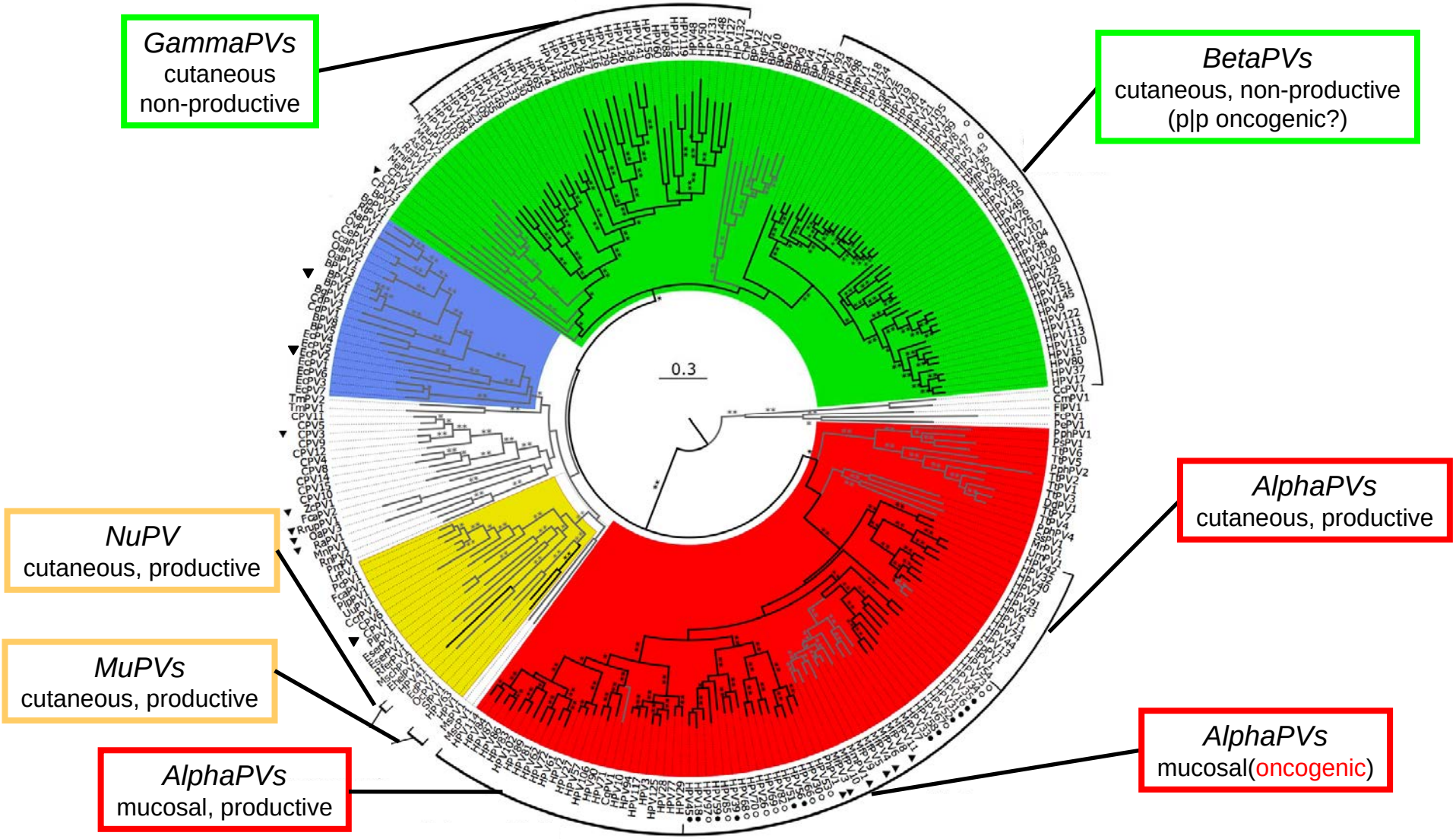
PVs interspecies transmission is not that rare

- mucosotropic PVs in cetaceans

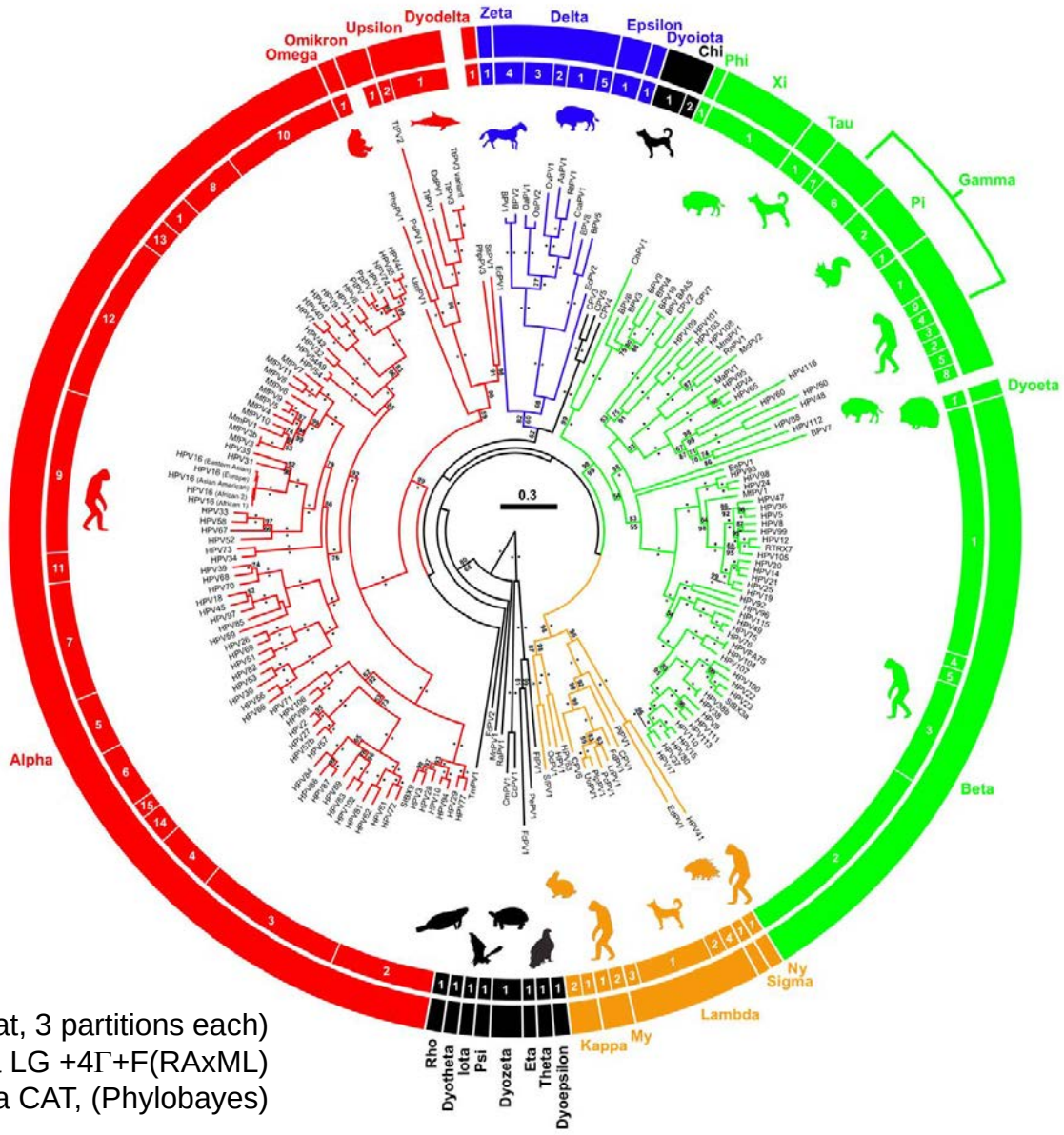


Gottschling *et al.* (2011) *Mol. Phyl. Evol.*

Papillomaviruses show multiple lifestyles



Papillomaviruses infect (probably) all amniotes



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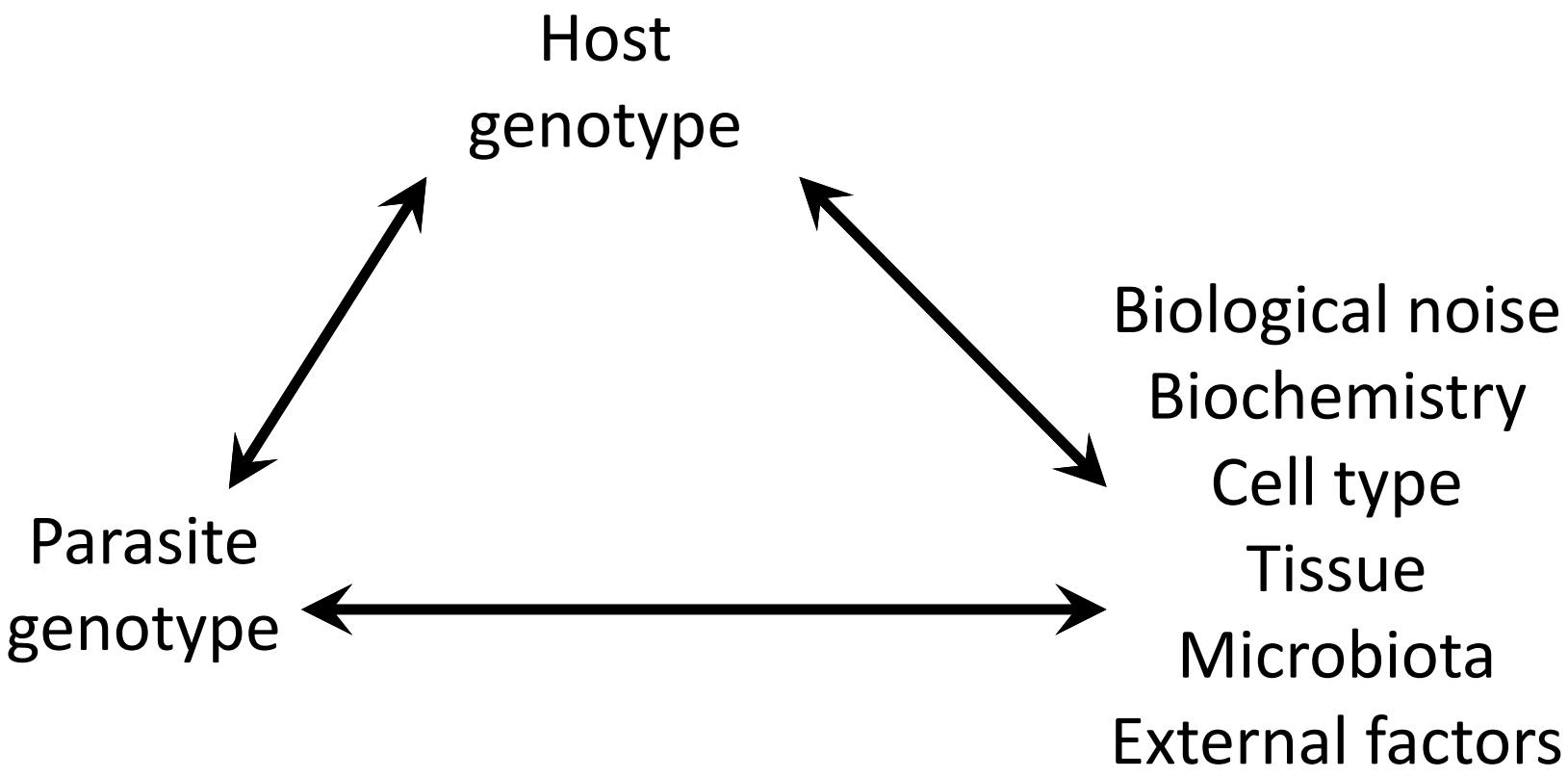
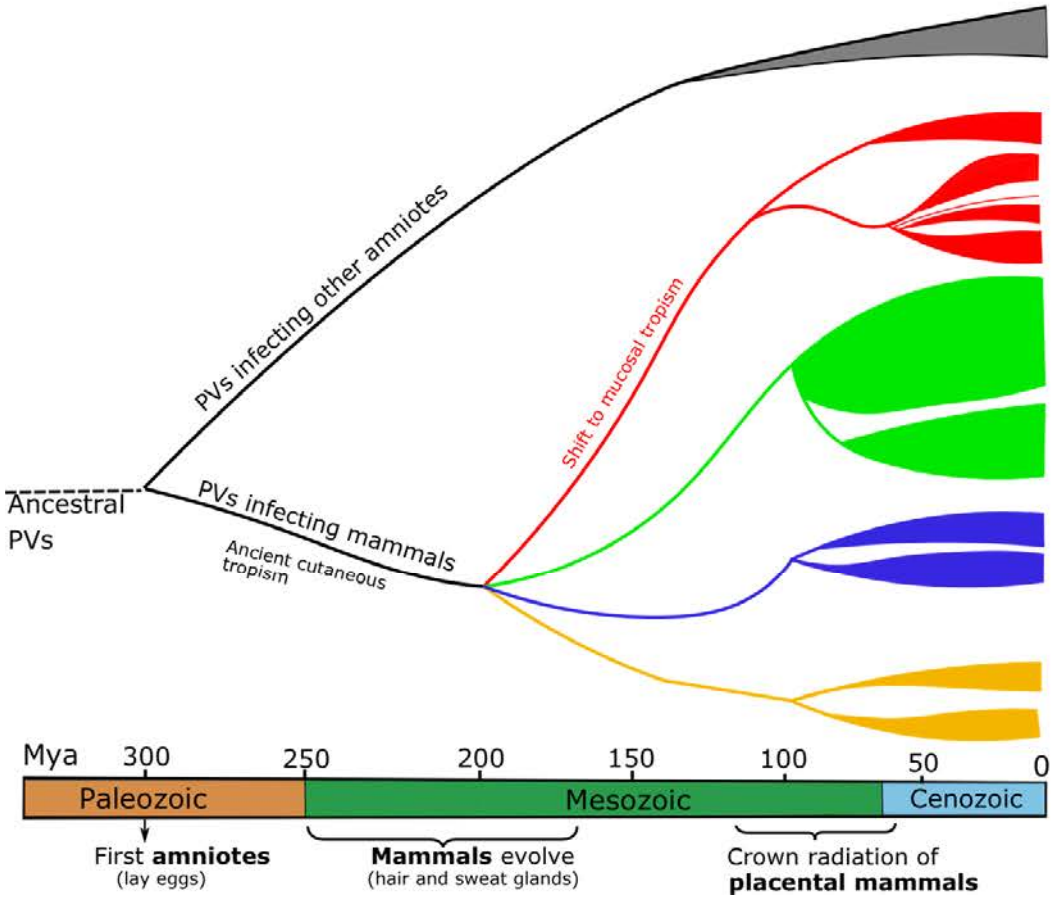
Genotype x Genotype x environment interactions



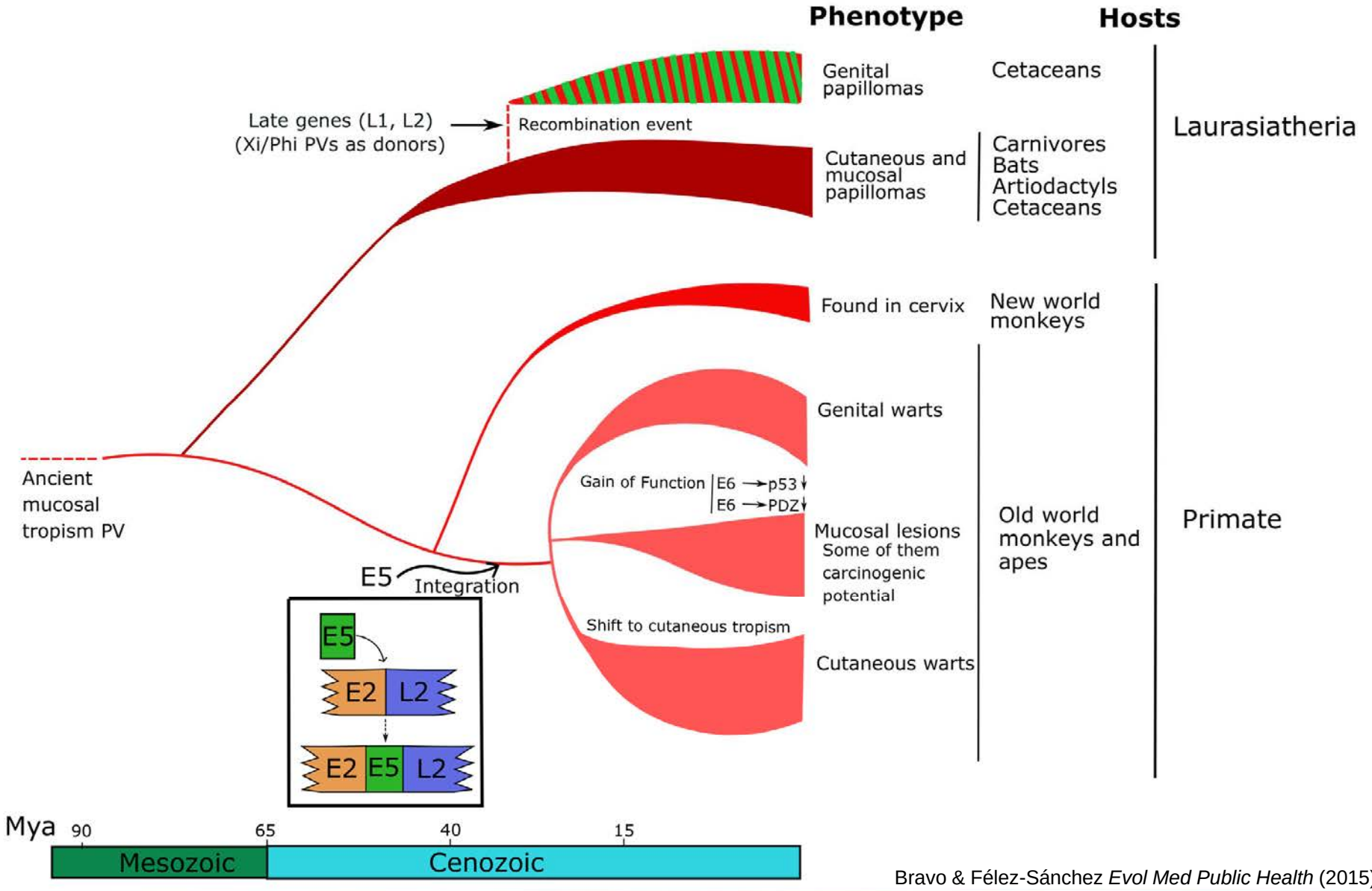
How PV diversification may have happened



Crown-group	Characteristics of the Phenotype
	-Essentially cutaneous PVs -Productive and asymptomatic infections
Alpha-Omikron	-Essentially mucosal PVs -Productive infections -Some of them carcinogenic potential (e.g. HPV16 and HPV18)
Beta-Xi	-Cutaneous PVs -Mainly asymptomatic -Some of them proliferative infections (e.g. HPV4)
Delta-Zeta	-Cutaneous PVs -Productive infections -Some of them carcinogenic potential (e.g. EcPV2)
Lambda-Mu	-Mucosal and cutaneous PVs -Productive and asymptomatic infections

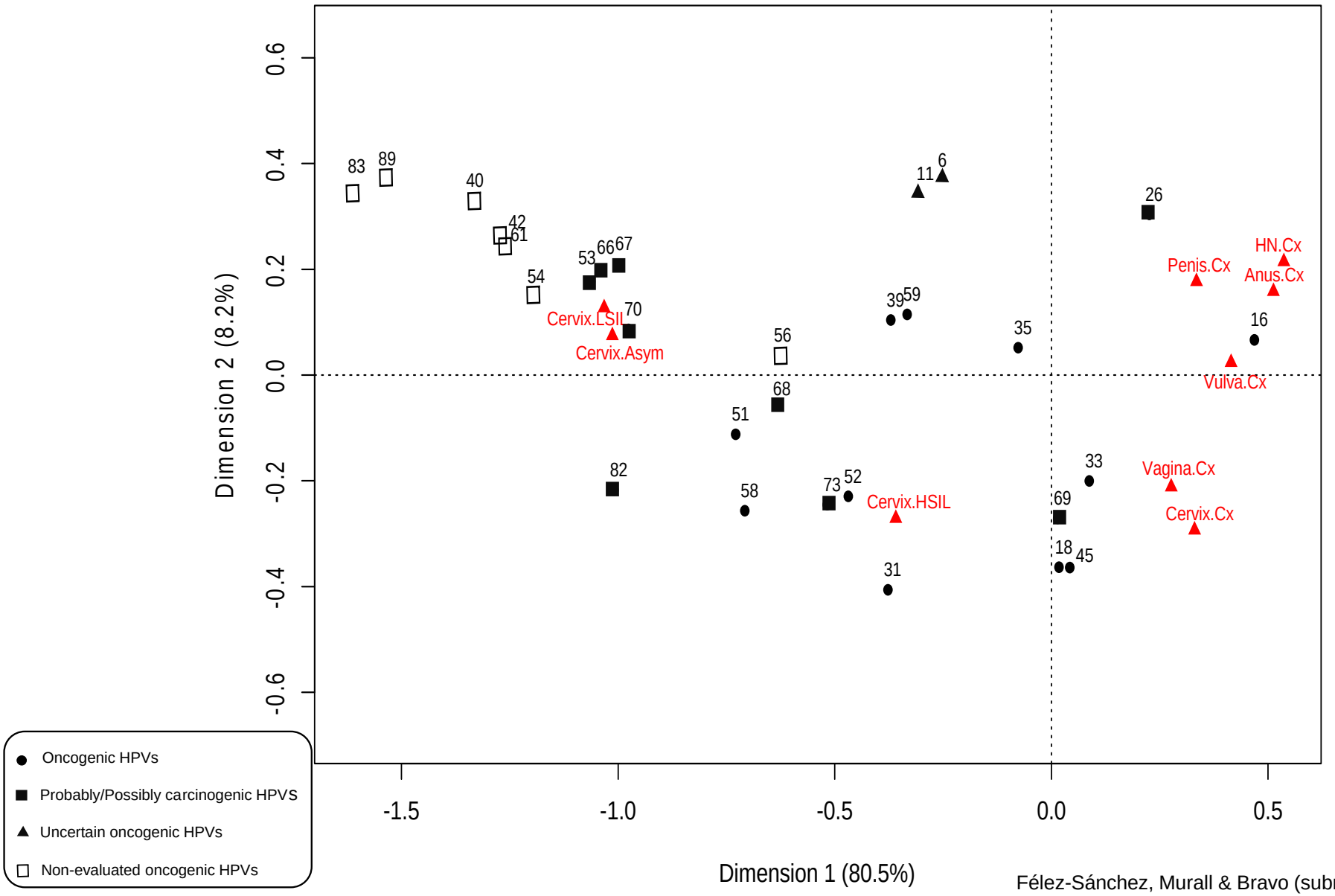
Bravo & Félez-Sánchez *Evol Med Public Health* (2015)

How PV diversification may have happened



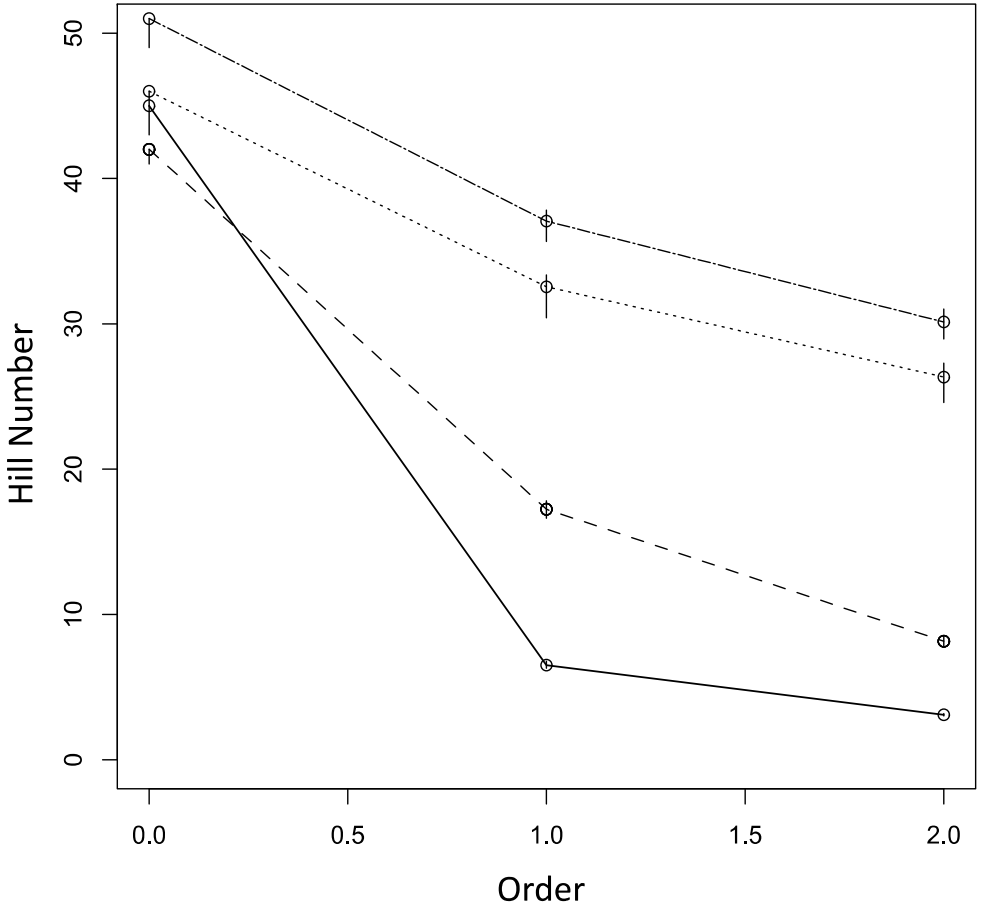
Bravo & Félez-Sánchez *Evol Med Public Health* (2015)

All PVs are not born equal



Félez-Sánchez, Murall & Bravo (submitted)

All PVs are not born equal



Asymptomatic

Low-grade lesions

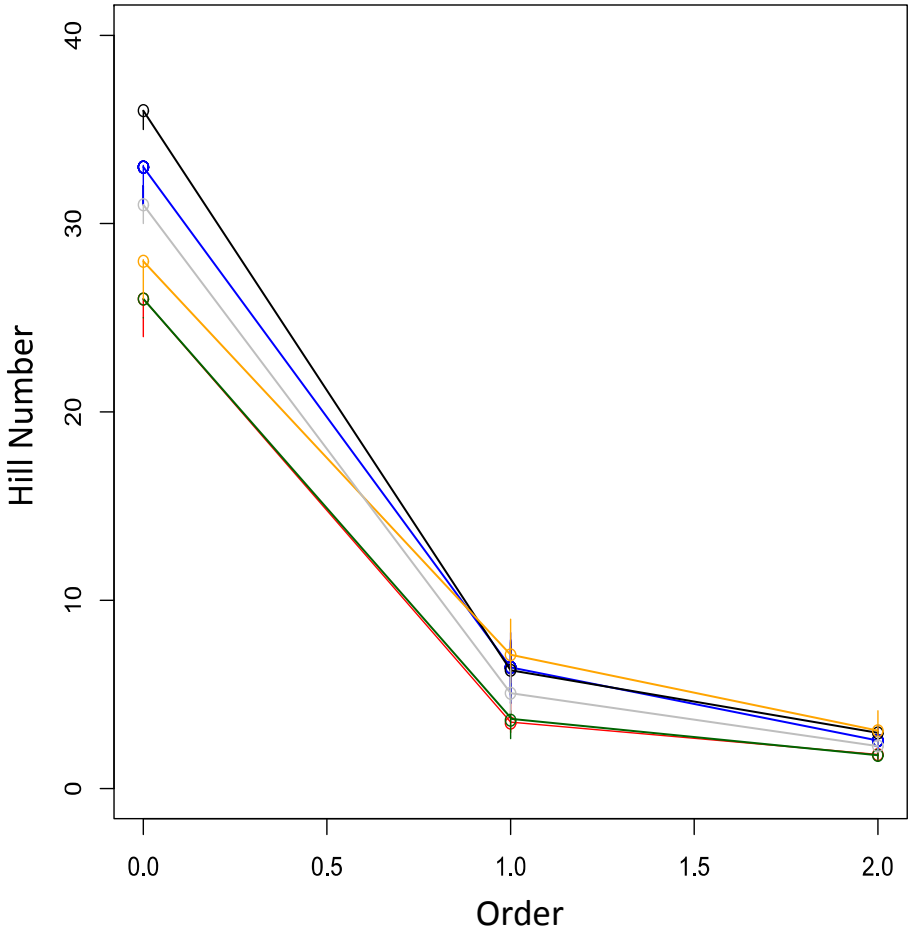
High-grade lesions

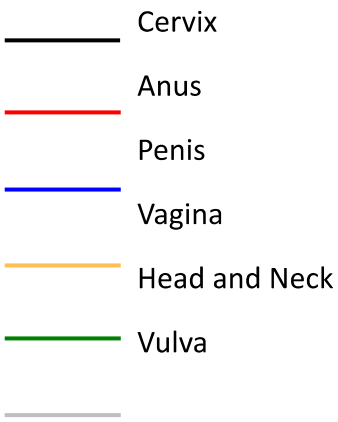
Cancer

Hill number	Equivalence	Asymptomatic	Low-grade lesion	High-grade lesions	Cancer
0	Species richness	51	46	42	45
1	Exponential shannon	37.06	32.54	17.24	6.51
2	Inverse simpson	30.12	26.34	8.15	3.09

Félez-Sánchez, Murall & Bravo (submitted)

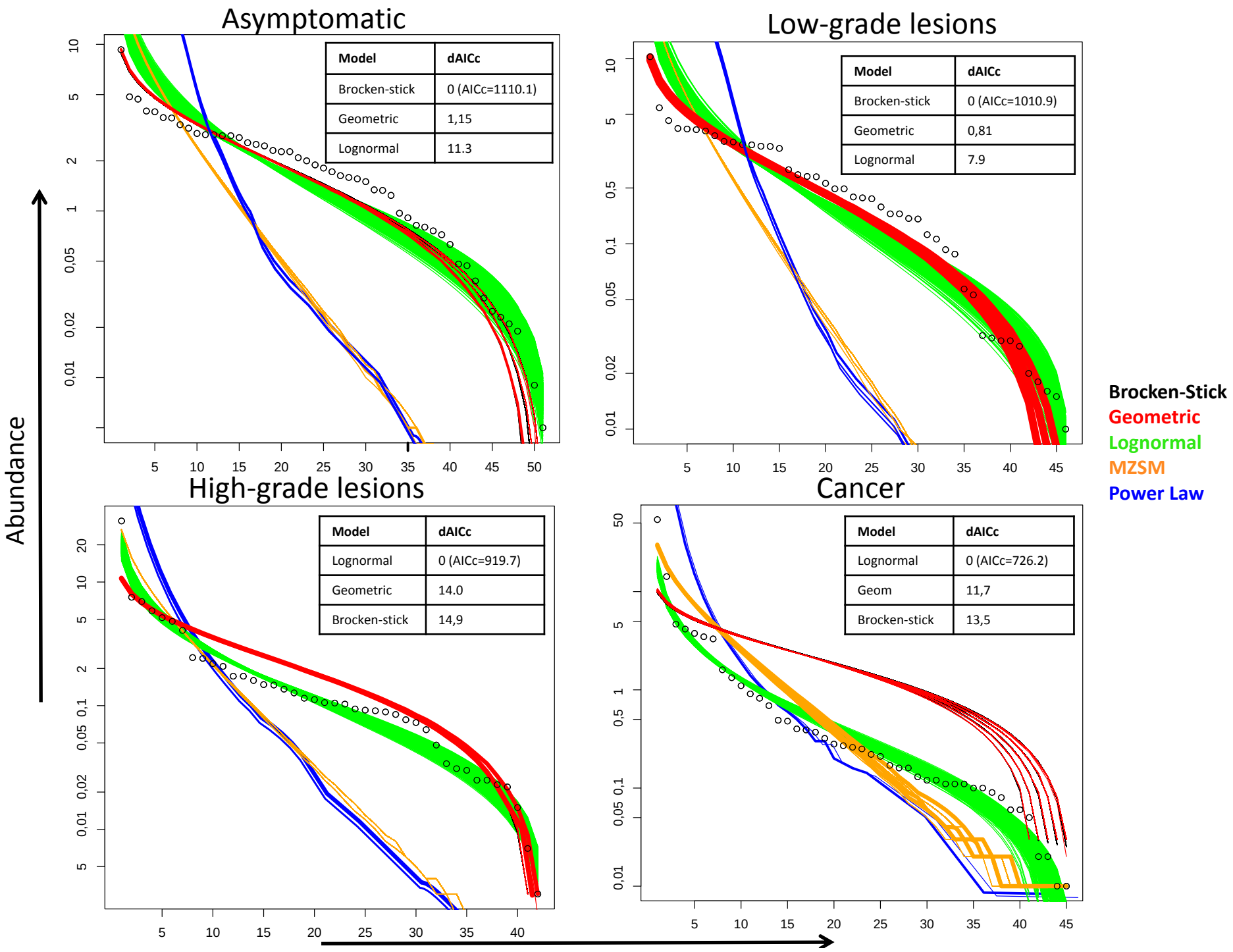
All PVs are not born equal





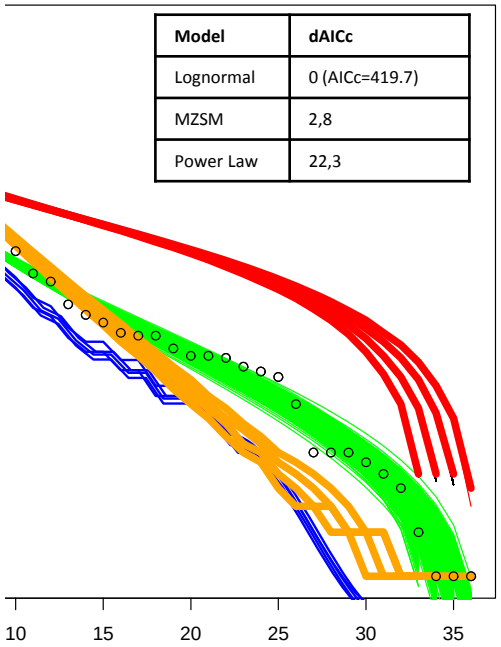
Hill number	Equivalence	Cervix	Penis	Vulva	Vagina	Anus	Head and neck
0	Species richness	36	33	31	28	26	26
1	Exponential shannon	6.29	6.43	5.07	7.11	3.66	3.63
2	Inverse simpson	2.96	2.55	2.26	3.08	1.81	1.77

Félez-Sánchez, Murall & Bravo (submitted)


Asymptomatic
Model | dAICc
Brocken-stick | 0 (AICc=1110.1)
Geometric | 1,15
Lognormal | 11.3
Low-grade lesions
Model | dAICc
Brocken-stick | 0 (AICc=1010.9)
Geometric | 0,81
Lognormal | 7.9
High-grade lesions
Model | dAICc
Lognormal | 0 (AICc=919.7)
Geometric | 14.0
Brocken-stick | 14,9
Cancer
Model | dAICc
Lognormal | 0 (AICc=726.2)
Geom | 11,7
Brocken-stick | 13,5
Abundance
Brocken-Stick
Geometric
Lognormal
MZSM
Power Law

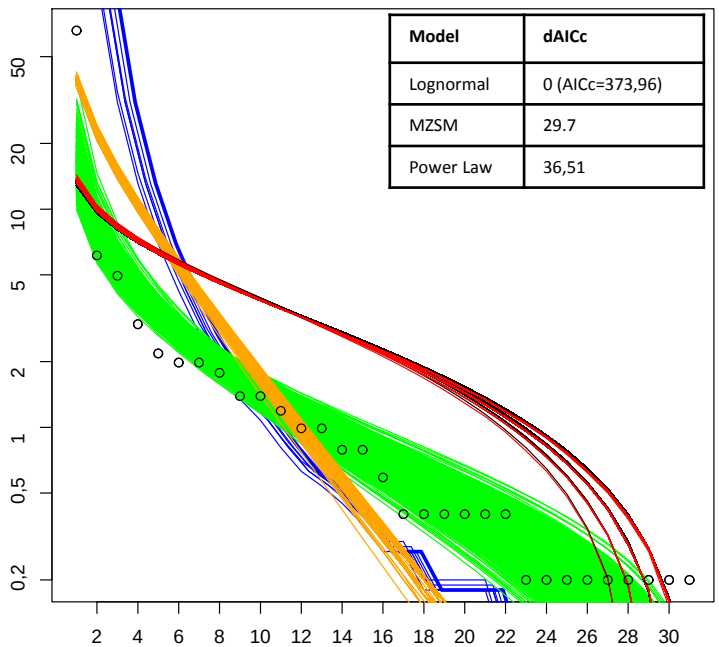
Cervix

Model	dAICc
Lognormal	0 (AICc=419.7)
MZSM	2,8
Power Law	22,3



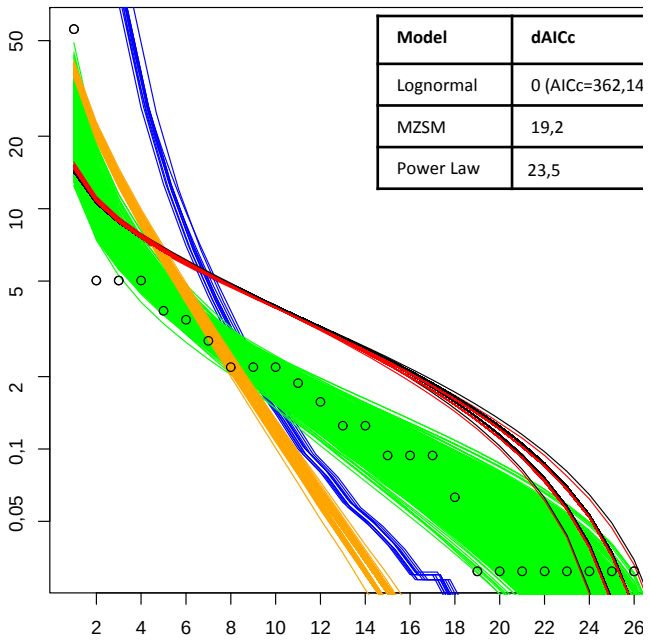
Vulva

Model	dAICc
Lognormal	0 (AICc=373,96)
MZSM	29.7
Power Law	36,51



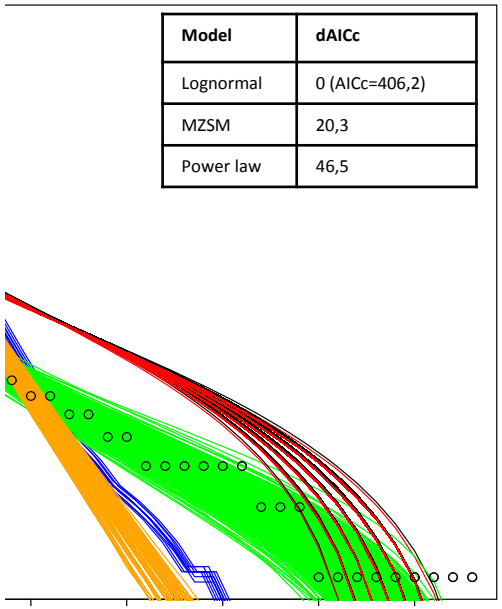
Vagina

Model	dAICc
Lognormal	0 (AICc=362,14
MZSM	19,2
Power Law	23,5



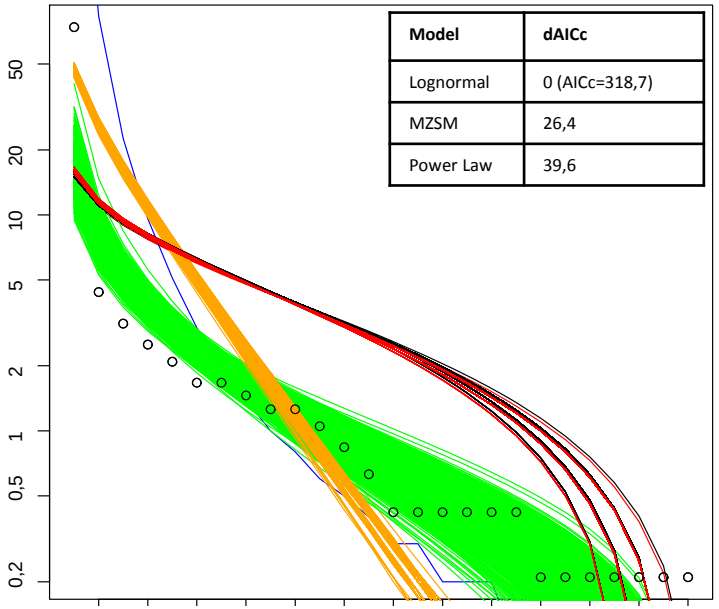
Penis

Model	dAICc
Lognormal	0 (AICc=406,2)
MZSM	20,3
Power law	46,5



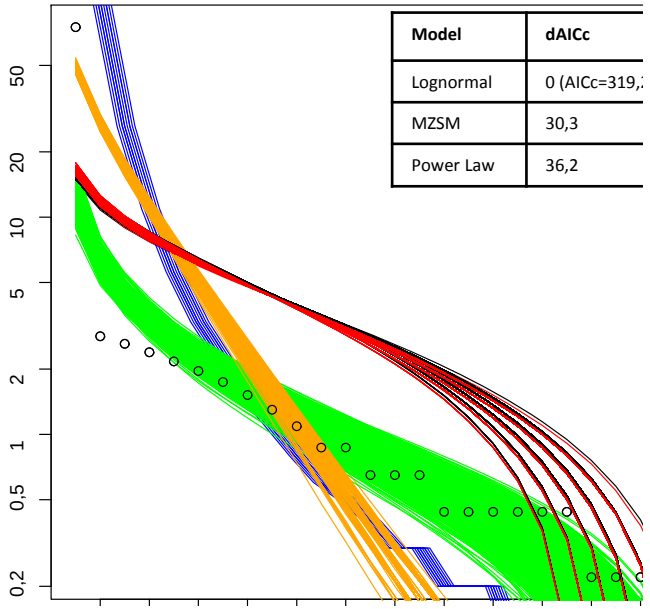
Anus

Model	dAICc
Lognormal	0 (AICc=318,7)
MZSM	26,4
Power Law	39,6



Head and neck

Model	dAICc
Lognormal	0 (AICc=319,3
MZSM	30,3
Power Law	36,2

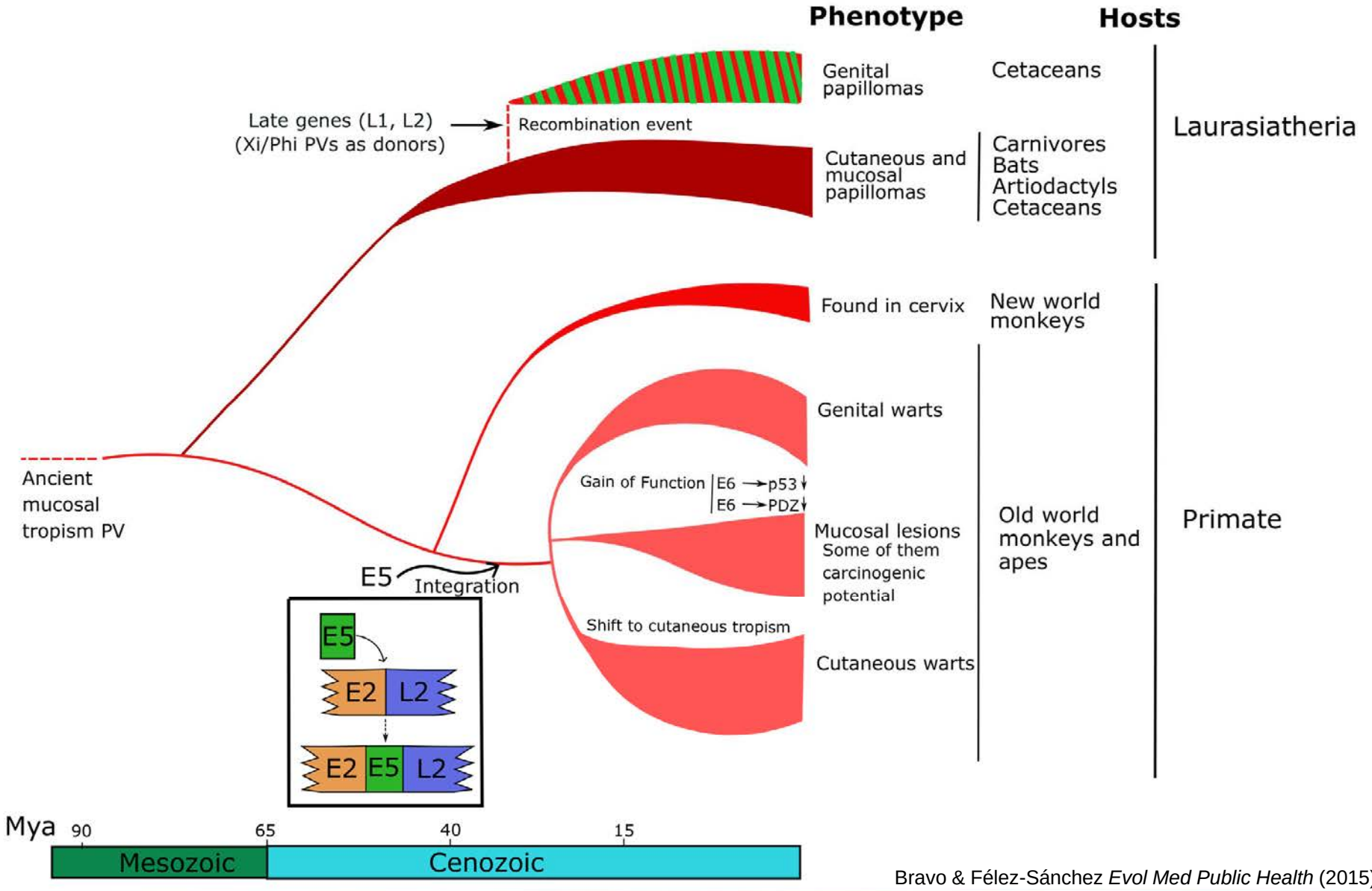


HPV16 is the most oncogenic human PV

	Fraction attributable to HPVs	Fraction attributable to HPV16
Cervix	99-100%	61%
Vagina	70-80%	73%
Vulva	30-35%	58%
Anus	85-95%	81%
Penis	25-35%	69%
Oropharynx	15-20%	83%

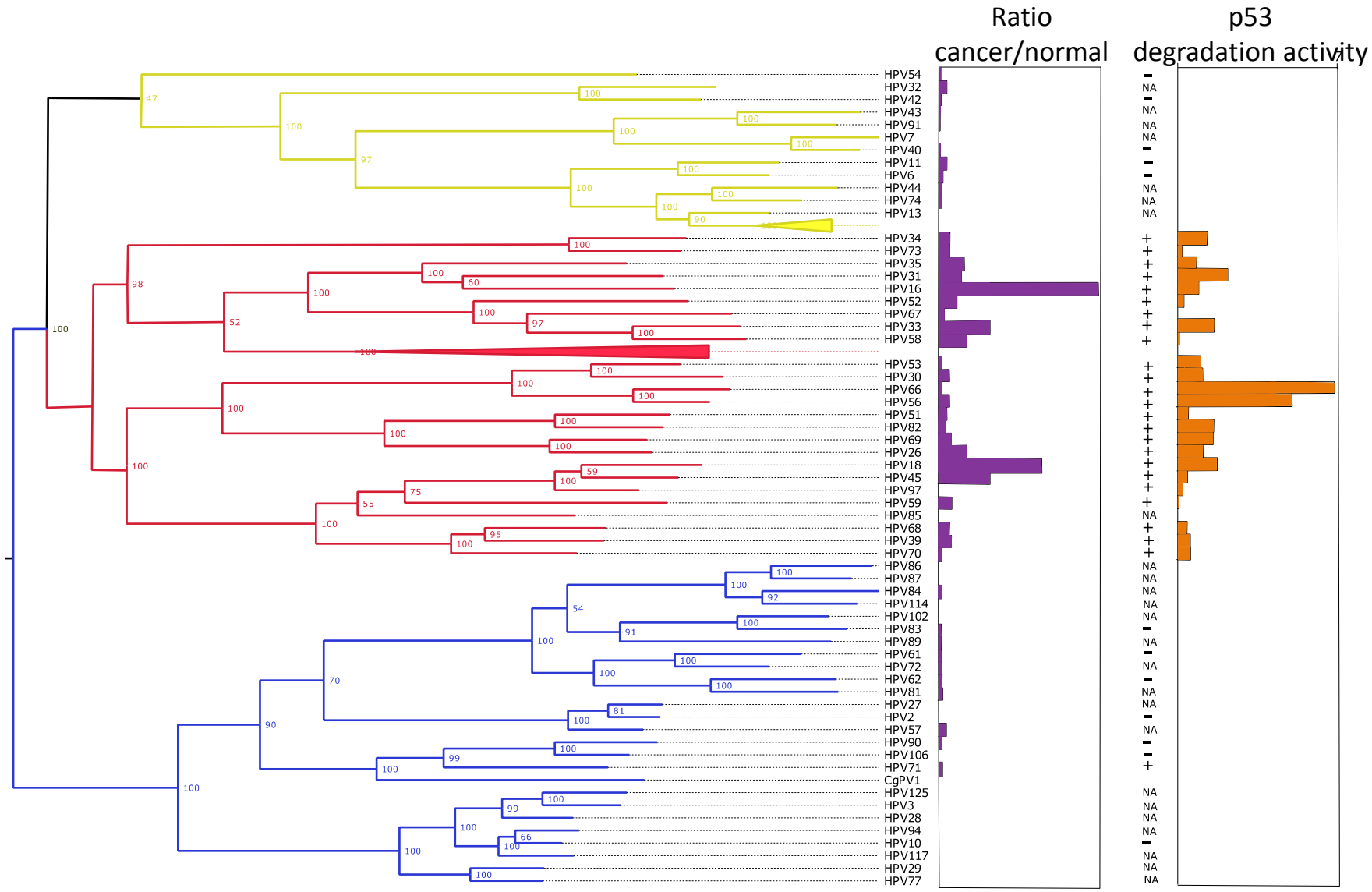
Sanjose *et al.*, (2010) *Lancet Oncol*
Alemany *et al.*, (2014) *Eur J Cancer*
Sanjose *et al.*, (2013) *Eur J Cancer*
Alemany *et al.*, (2014) *Int J Cancer*
Alemany *et al.*, (2016) *Eur Urol*
Castellsagué *et al.*, (2016) *J Natl Cancer Inst*

How PV diversification may have happened



Bravo & Félez-Sánchez *Evol Med Public Health* (2015)

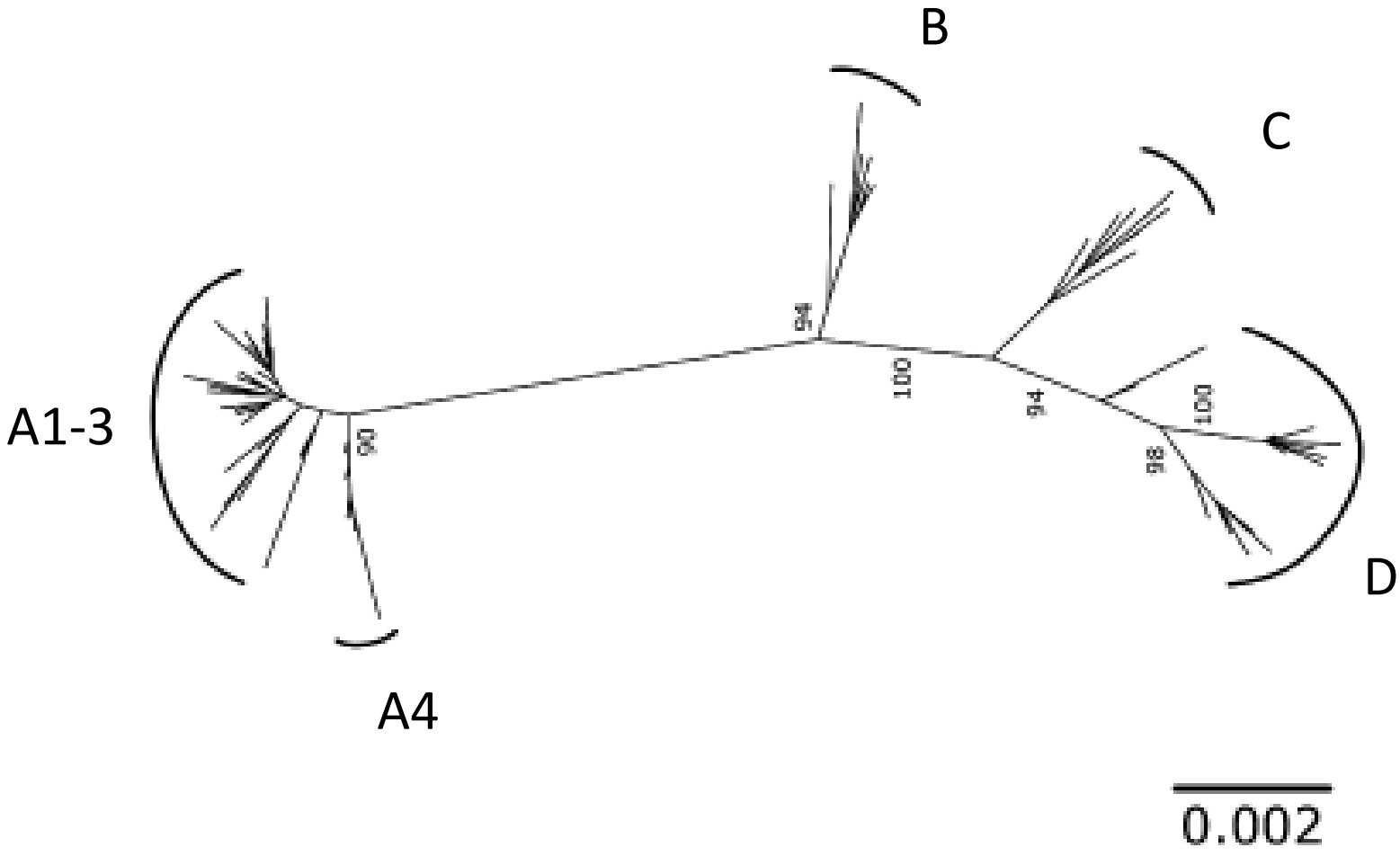
Phylogeny and biochemistry alone do not explain HPV16 differential oncogenic potential



6 genes (concat, 3 partitions each)
ML, nt GTR+4G ; aa LG +4G+F(RAxML)

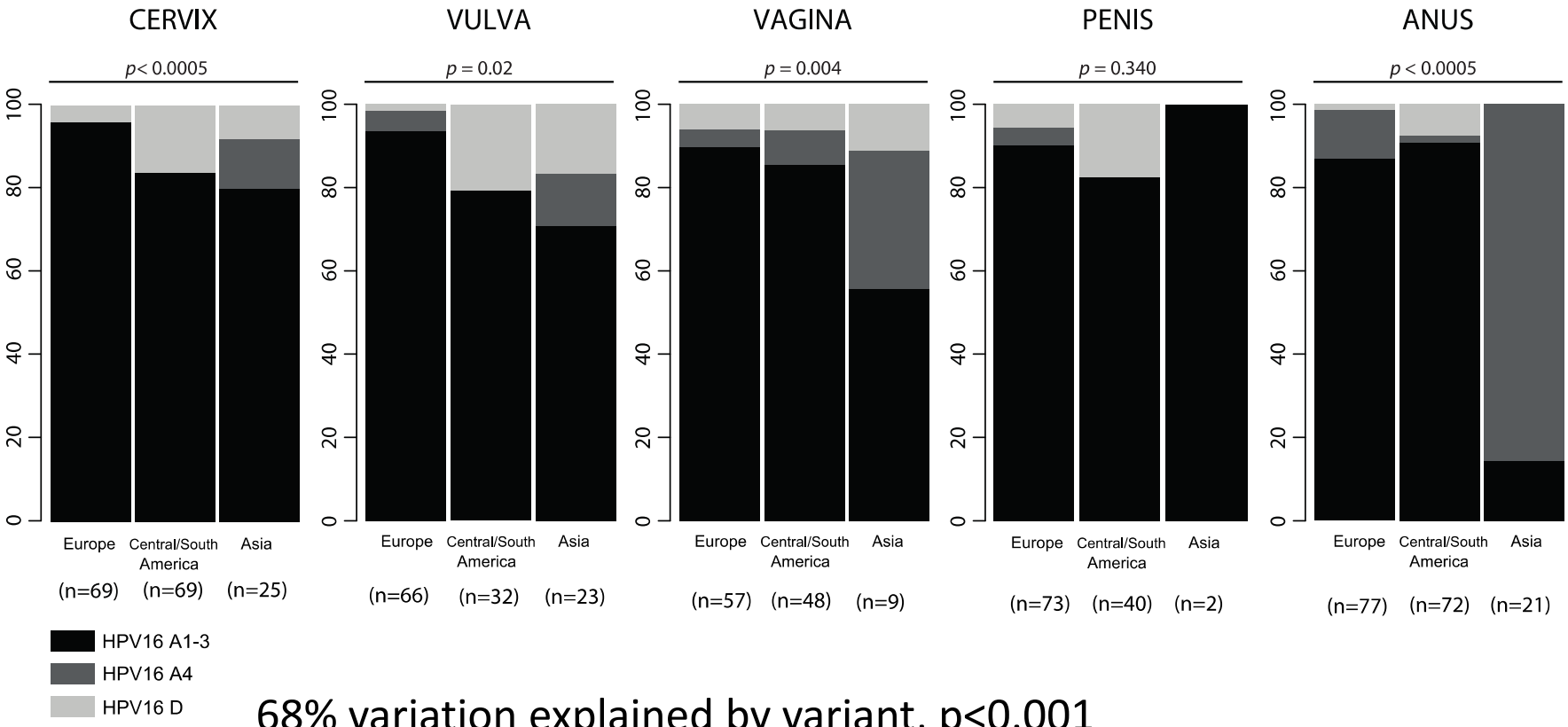
Fu *et al.*, (2010) *PLoS ONE*
Mesplede *et al.*, (2012) *J Virol*

There is ample standing variation in HPV16



Full genome, unpartitioned
ML, nt GTR+4G

Impact of geography and anatomical location on HPV16 lineage prevalence in squamous carcinomas



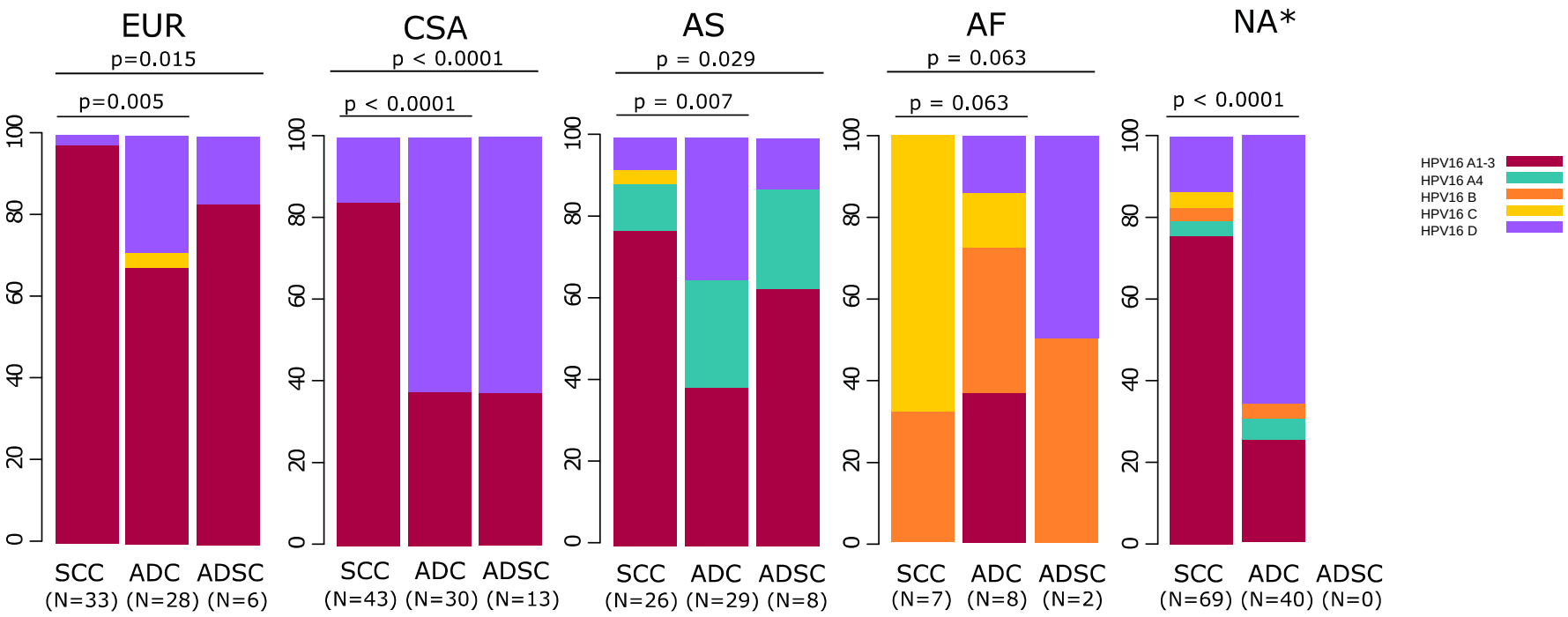
68% variation explained by variant, p<0.001

9% variation explained by variant-geography interaction, p<0.001

3% variation explained by variant-anatomy interaction, p<0.001

Nicolás-Párraga *et al.* (2016) *Cancer Med*

Impact of geography and tissue tropism on HPV16 lineage prevalence in cervical carcinomas



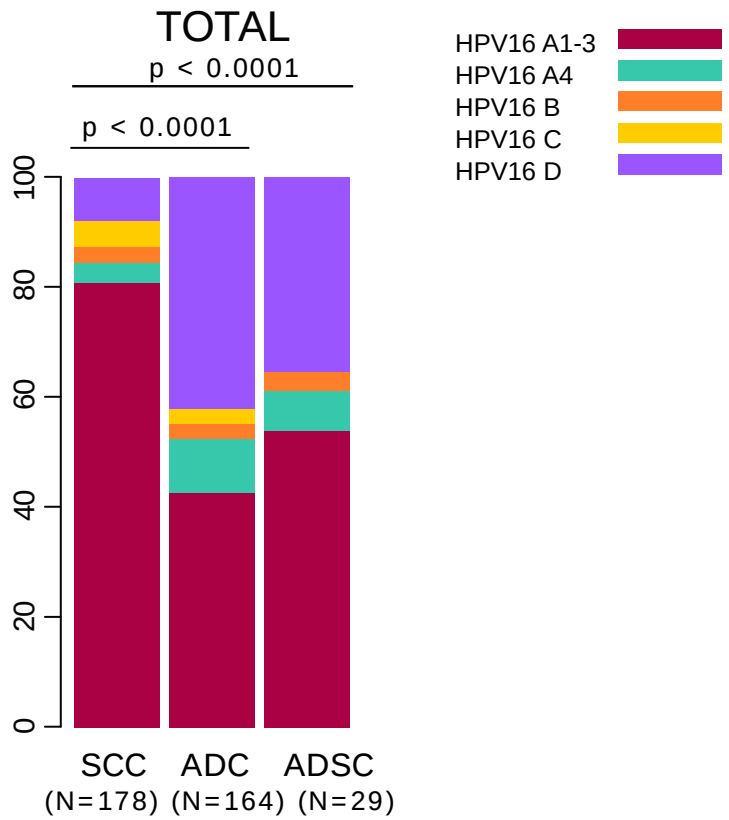
78% variation explained by variant, p<0.0001

10% variation explained by variant-geography interaction, p<0.0001

9% variation explained by variant-histology interaction, p<0.0001

Nicolás-Párraga *et al.* (2017) *Int J Cancer*

Large differences in phylogeography and prevalence among HPV16 lineages in cervical SCCs and ADCs



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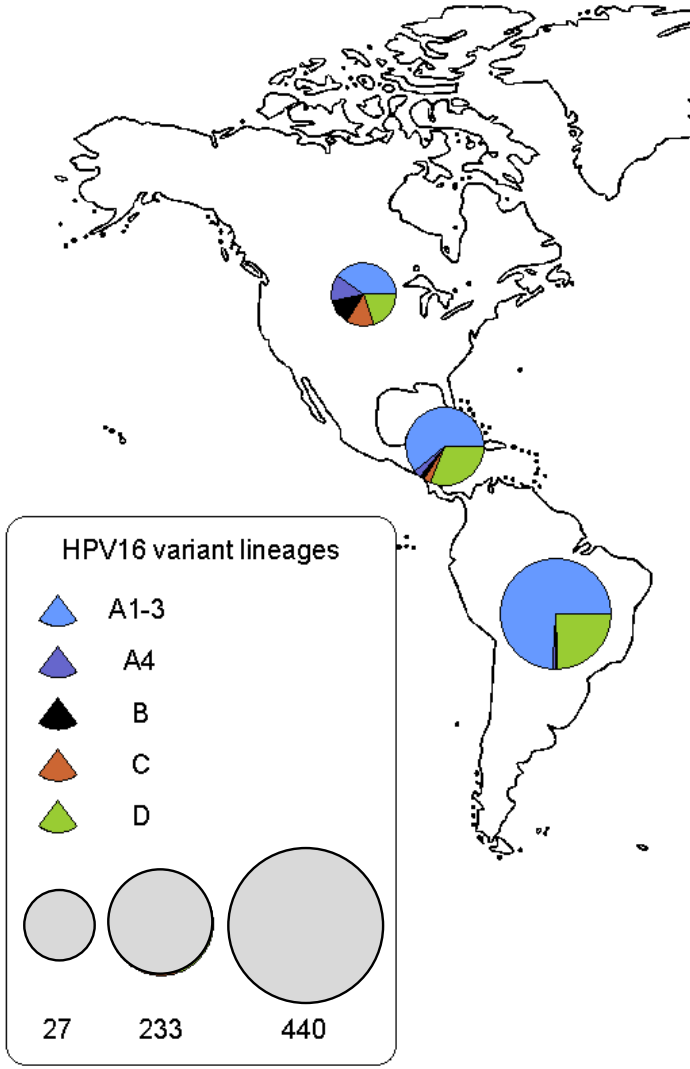
Nicolás-Párraga *et al.* (2017) *Int J Cancer*

Large differences in phylogeography and prevalence among HPV16 lineages



HPV16 lineage	Isolate	%
A1-3	983	58.4
A4	159	9.4
B	141	8.4
C	206	12.2
D	190	11.3
unclassified	5	0.3
total	1684	100

Pimenoff *et al*, *Mol Biol Evol* (2017)

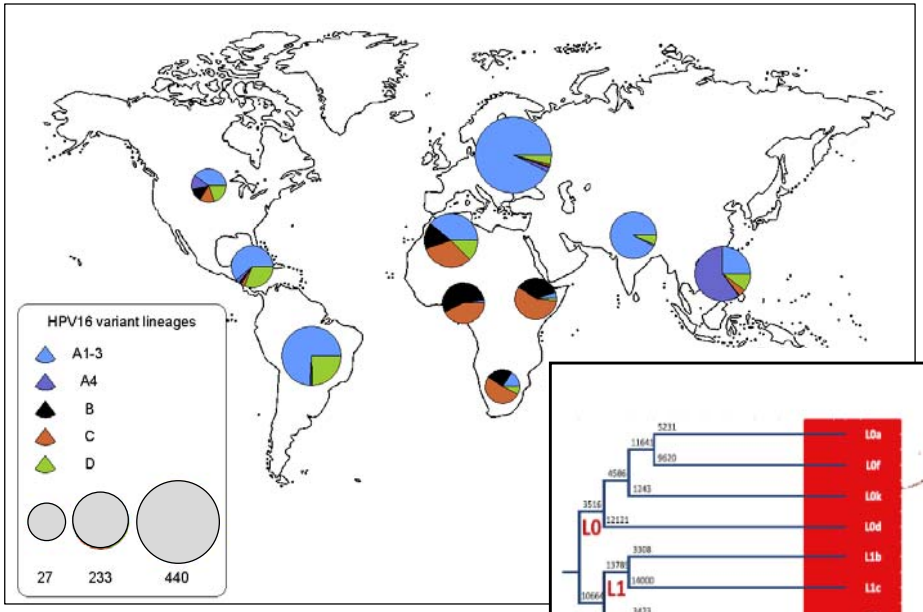
Assessment of HPV16 phylogeography

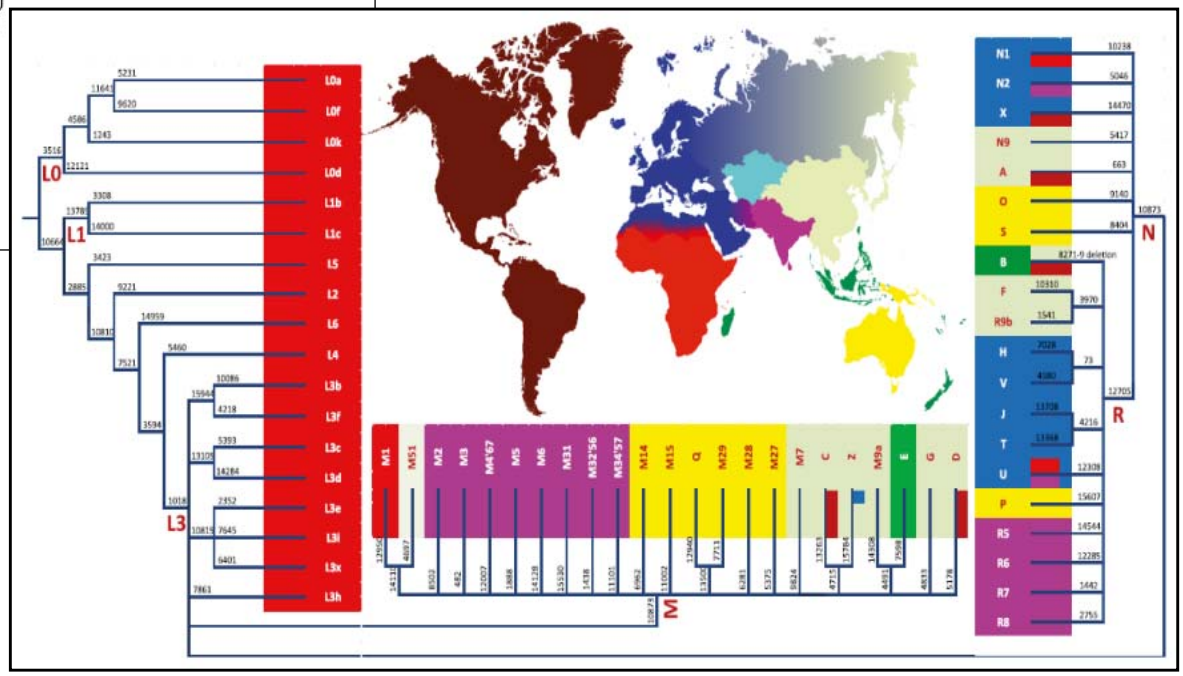
- Two alternative scenarios explored:
  - Recent out of Africa expansion (ROOA)
  - Hominin-Host-Switch (HHS)
- Observational features:
  - Highest viral diversity in East Asia
  - Highest prevalence of HPV16A outside Sub-Saharan Africa
  - Distant relationship HPV16A vs HPV16BCD
  - Preference for HHS for rooting the HPV16 tree

Assessment of HPV16 phylogeography

- Viral data: 140 HPV16 genomes + 1684 partial seqs
- Human genomic data (#938, 51 pops):
  - mtDNA (#875), Y-chrm (551), autosomal SNPs (12k Afr| 111k Eur)
- Accounting for selection in the HPV16 genome
  - Sites under selection: 26 diversifying, 115 purifying
- Different clock models and demographic models
  - Strict/relaxed clock uncorrelated log normal
  - Constant pop | exp growth | Bayesian skyline
- Different priors for evol rate
  - PVs gnm; Mammalian gnm; Human mitochondria; uninformative flat prior
- Calibrations
  - Sapiens out-of-Africa; mrca archaic|modern humans

Human phylogeography explains only partially the distribution and prevalence of HPV16 lineages





Kivisild (2015) *Invest. Genet.*

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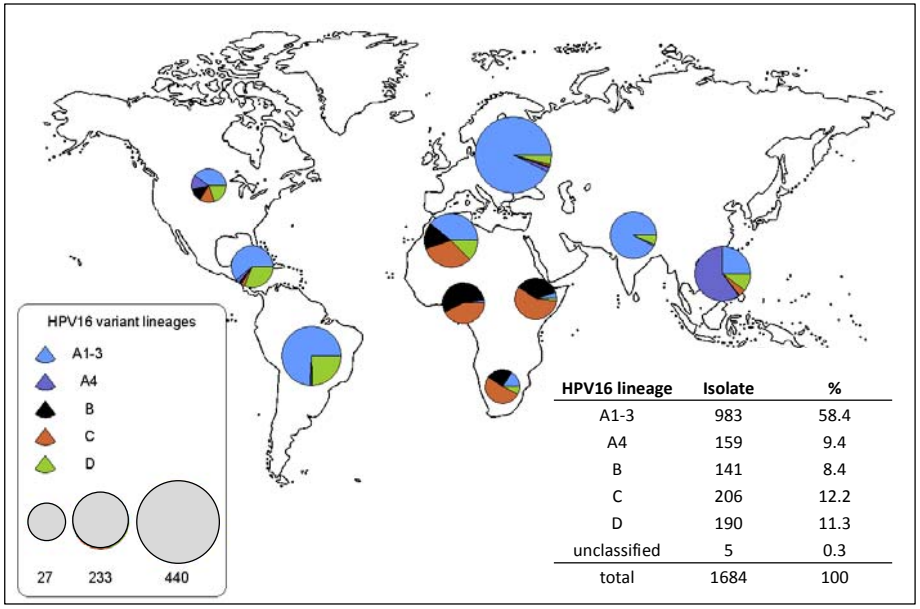

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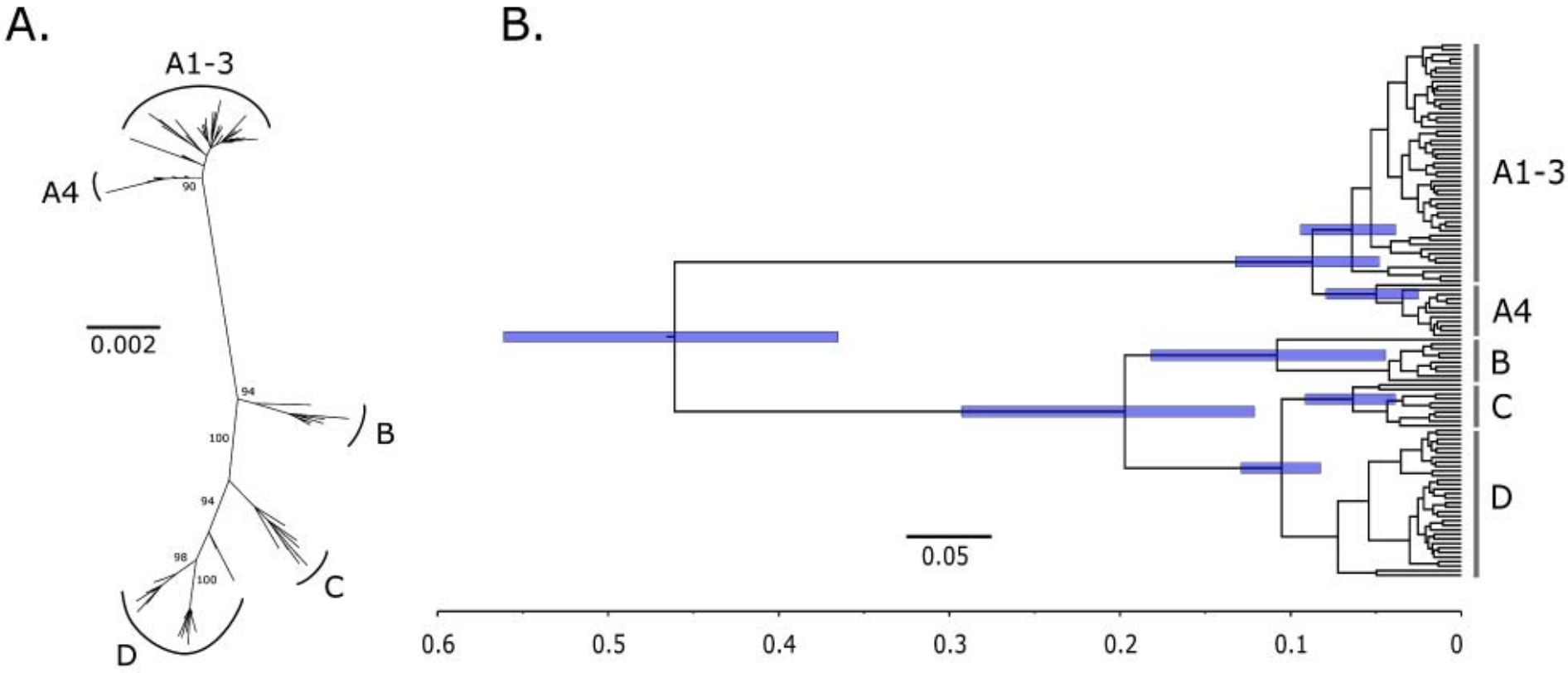
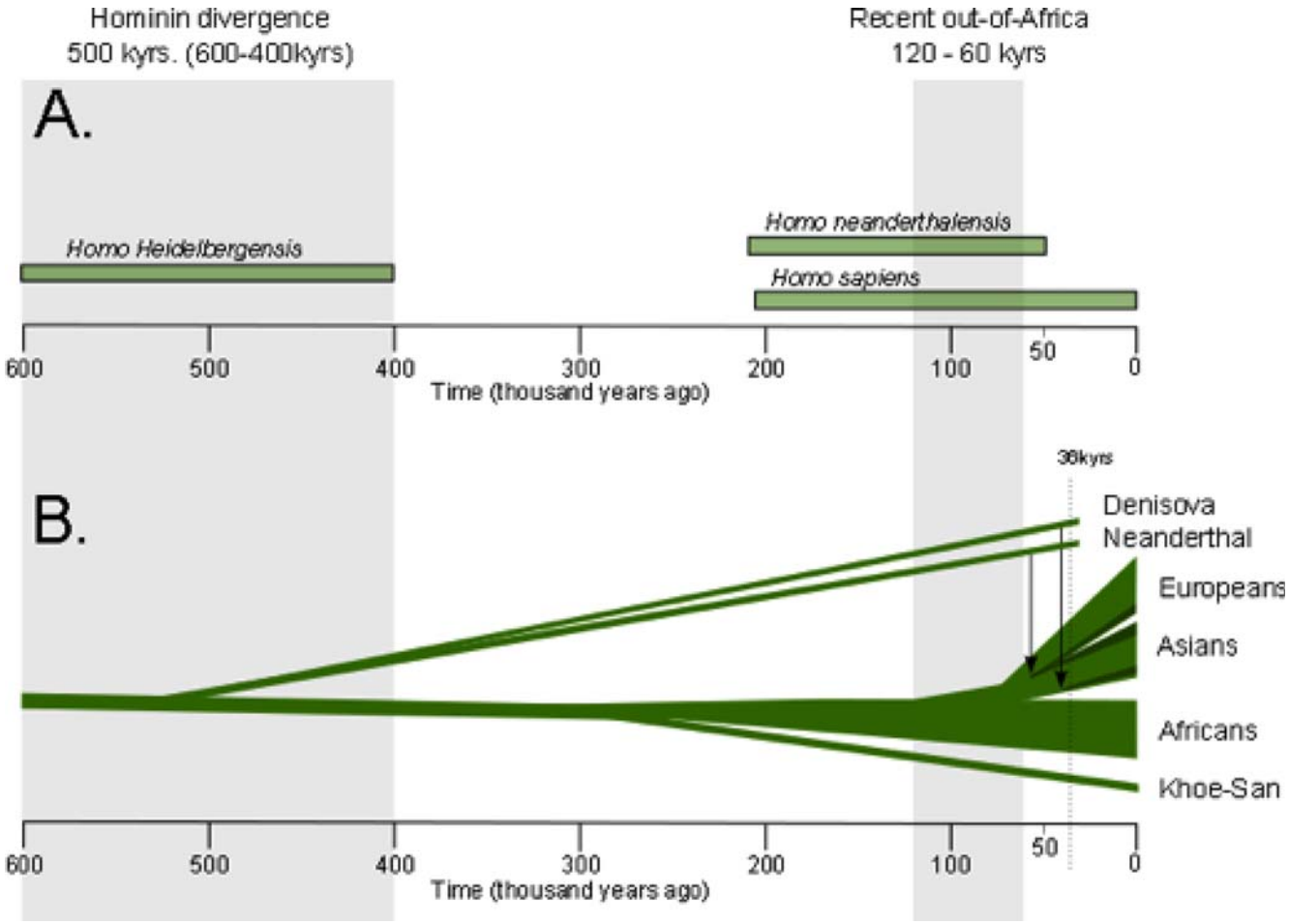
Taxa	loci	region	N	Selection-filtered		full HPV16 genome	
				Correlation (%)[2]	p-value[3]	Correlation (%)[2]	p-value[3]
mtDNA	1	16kb genome	875	12.4	0.037	15.8	0.026
NRY	1	5000kb	551	8.5	0.062	11.8	0.044
Chr1[4]	6353	SNPs	773	8.8	0.062	12.7	0.033
Chr1[5]	729	SNPs	773	15.5	0.024	19.5	0.016

There is ample standing variation in HPV16



Full genome, unpartitioned
ML, nt GTR+4G

The model explaining best the current distribution of HPV16 variants implies transmission between ancient human populations



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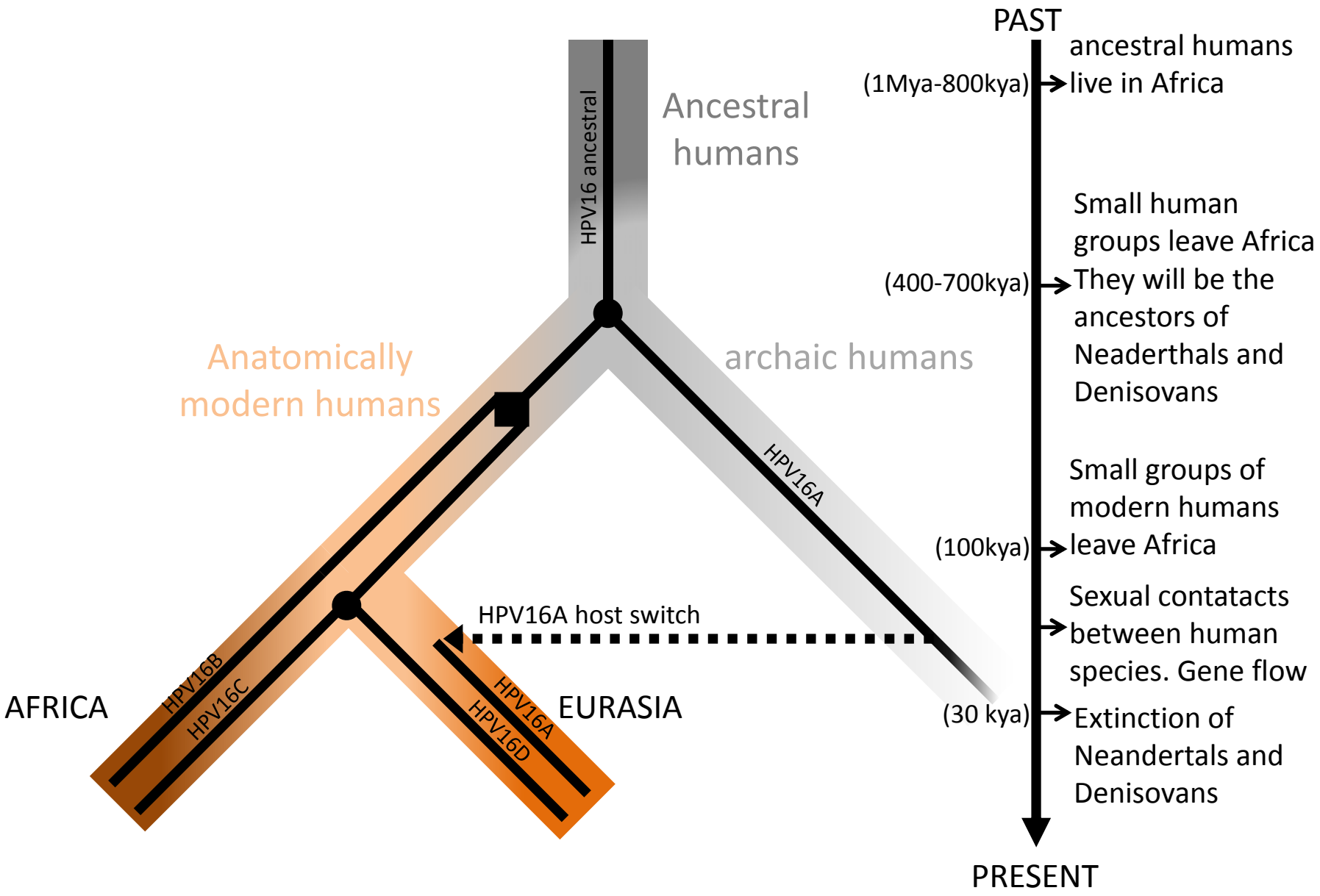
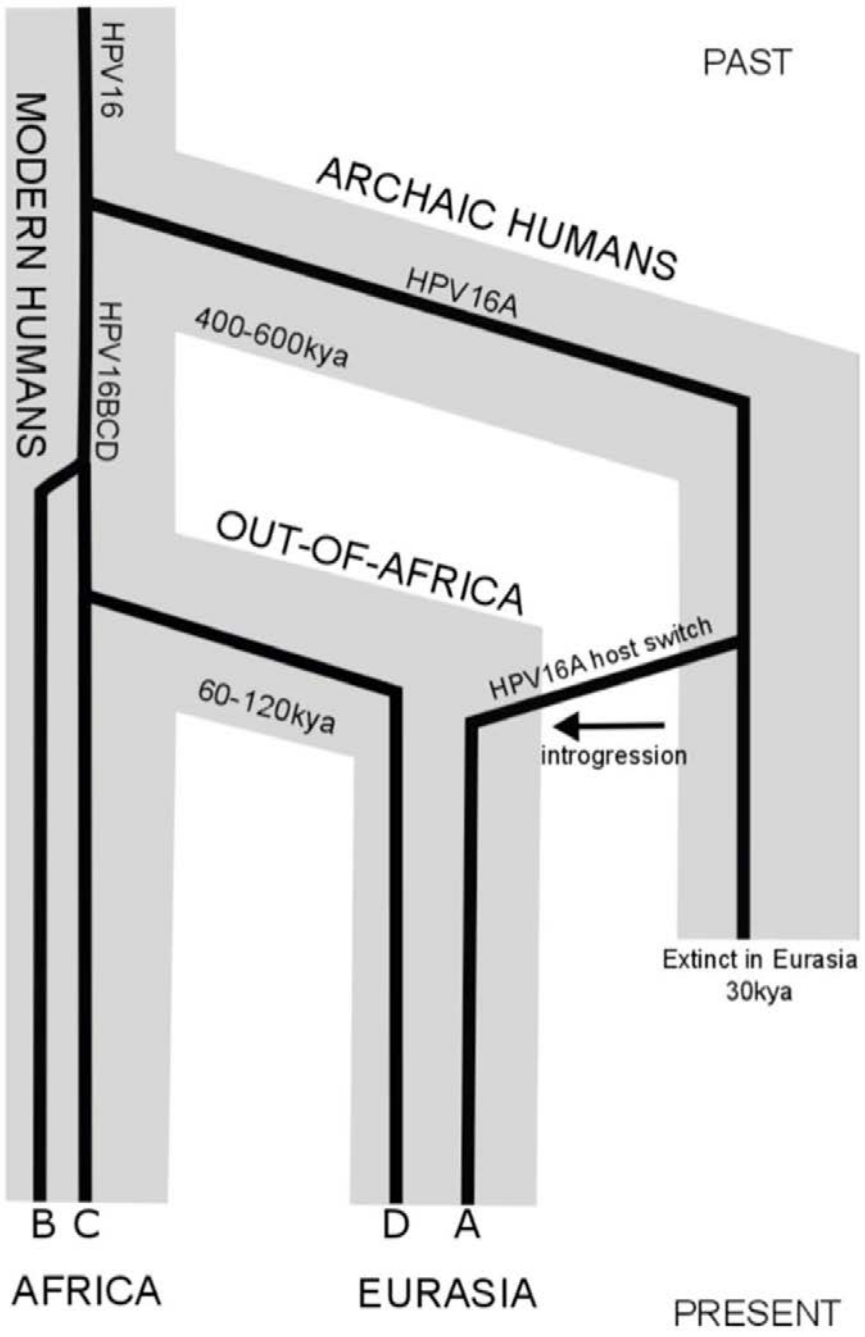


Pimenoff *et al. Mol Biol Evol* (2017)



The model explaining best

the current distribution of

HPV16 variants

implies transmission between ancient human populations

Pimenoff *et al. Mol Biol Evol* (2017)

Introgressed archaic loci are enriched in immune and keratinocyte differentiation genes

Human papillomavirus 16A lineages	sub-Saharan Africa	West Eurasia	East Eurasia	Ref.
HPV16A1-3	< 5%	93%	25-91%	this study
HPV16A4	absent	~ 1%	60%	this study
Introgressed archaic alleles/haplotypes				
Neanderthal ancestry loci	absent	64%	62%	(Sankararaman et al. 2014)
Loci involved in keratinocyte differentiation	absent	40%-70%	40%-66%	(Vernot and Akey, 2014)
HLA I loci (innate immunity)	< 7%	52%-59%	72%-82%	(Abi-Rached et al. 2011)
Toll-like receptor loci (innate immunity)	absent	15%-39%	17%-51%	(Dannemann et al. 2015)
APOBEC3A deletion (innate immunity)	< 1%	7%	14-93%	(Kidd et al. 2007)

Conclusions

- PV evolution is coupled to the apparition of novel niches during the evolution of mammalian skin

- The most prevalent HPV16 lineage outside Sub-Saharan Africa may have reached humans through interbreeding with ancestral populations (Neandertals and Denisovans)

Merci !

European
Research
Council

ignacio.bravo@ird.fr