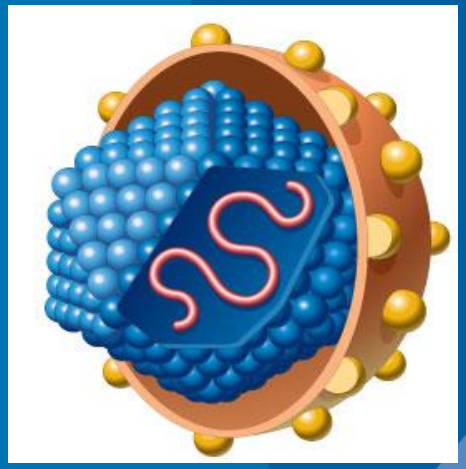


Molecular Epidemiology of HCV among PWID: New Insights into HCV Transmission

INHSU Oct 7, 2015

BC Centre for Disease Control
An agency of the Provincial Health Services Authority

Mel Krajden MD, FRCPC
Acting Medical Director,
Public Health Laboratory
Medical Head, Hepatitis
BC Centre for Disease Control
Canada

Professor, Pathology &
Laboratory Medicine
The University of British Columbia, Canada

Disclosures

- Research contracts/grants
  - Roche
  - Siemens
  - Merck
  - Hologic (Gen-Probe)
  - Boerhinger Ingelheim

- Honoraria donated to the BCCDC Foundation for Population & Public Health

Pathogen Genomics

- Many HCV epidemiological tools
  - Social and sexual networks
  - Require lots of data collection
- Sintchenko & Holmes (BMJ 2015) – posit that whole genome sequencing (WGS) will transform molecular epidemiology:
  1. High-throughput, rapid, accurate and affordable,
  2. Compare data locally/nationally/internationally,
  3. Link genomics with clinical and epidemiological metadata.

Transform outbreak management!

Existing

Sacks-Davis et al. PloS One 2012

- Phylogenetic clustering is associated with social networks and injecting relationships

Jacka et al. Hepatology 2014

- 1/3 of PWID in the VIDUS cohort demonstrated phylogenetic clustering

Sacks-Davis et al. PLoS One 2013 and JID 2015

- Reinfections and spontaneous clearance

Bretana et al. 2015

- Ongoing HCV transmissions amongst high-risk prisoners (HITS-p team)

Lamoury et al. 2015

- Genomic region used is important for transmission cluster discrimination

Jacka et al. submitted 2015

- Young injectors are seeded from many transmission events between HCV-infected older and younger injectors

7 Things Molecular Epidemiology Can do for You!

1. Recreate the transmission dynamics of an outbreak without fully knowing the traditional surveillance epidemiology
2. Determine if infection is incident or prevalent & time the infection
3. Determine transmission directionality: privacy implications
4. Determine if a transmission cluster is expanding or stable
5. Identify: mixed infection; treatment relapse vs reinfection
6. Detect Resistance Associated Variants (RAVs)
7. Study early infection events – vaccine design?

SEQUENCING TECHNIQUES

Cloning – precise – labour intensive

GACTGACT

GACTTTACT

PCR-based – average sequence – miss admixtures if <20%

GACTGACT
GACTTTACT
GACTGACT

NGS of a single PCR product – detect "all" the variants in the sample + artifacts

GACTGACT
GACTTTACT
GACTGACT
GACTGACT
GACTTTACT
GACTGACT

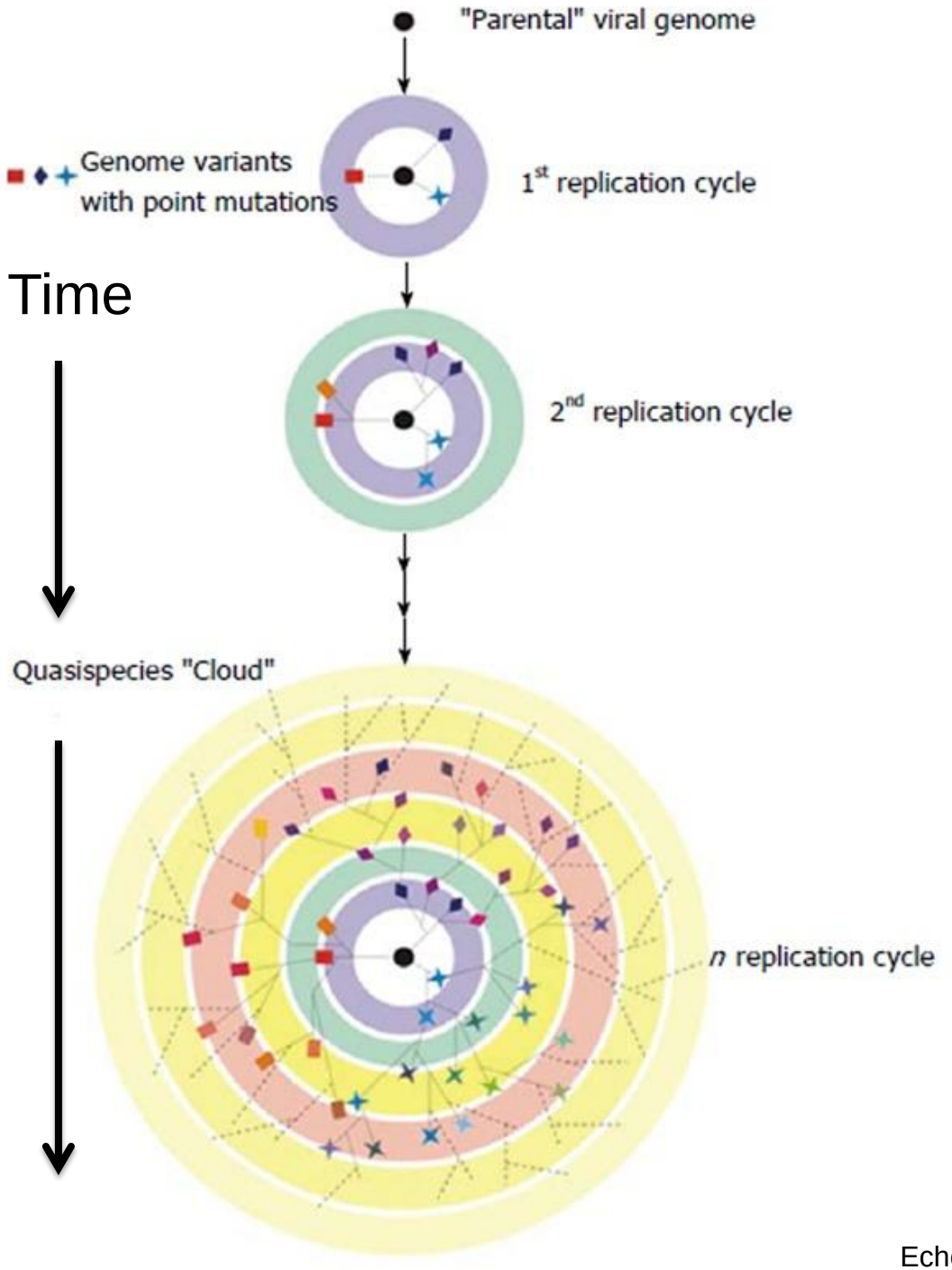
WGS (NGS) – sequencing multiple fragments – assembled by computer – "all" variants + artifacts

GACTG ACT
GACTTTA T
GAC TGACT
GACT GACT
GACTTT ACT
GACTGAC T

Long read whole genome – early days - errors

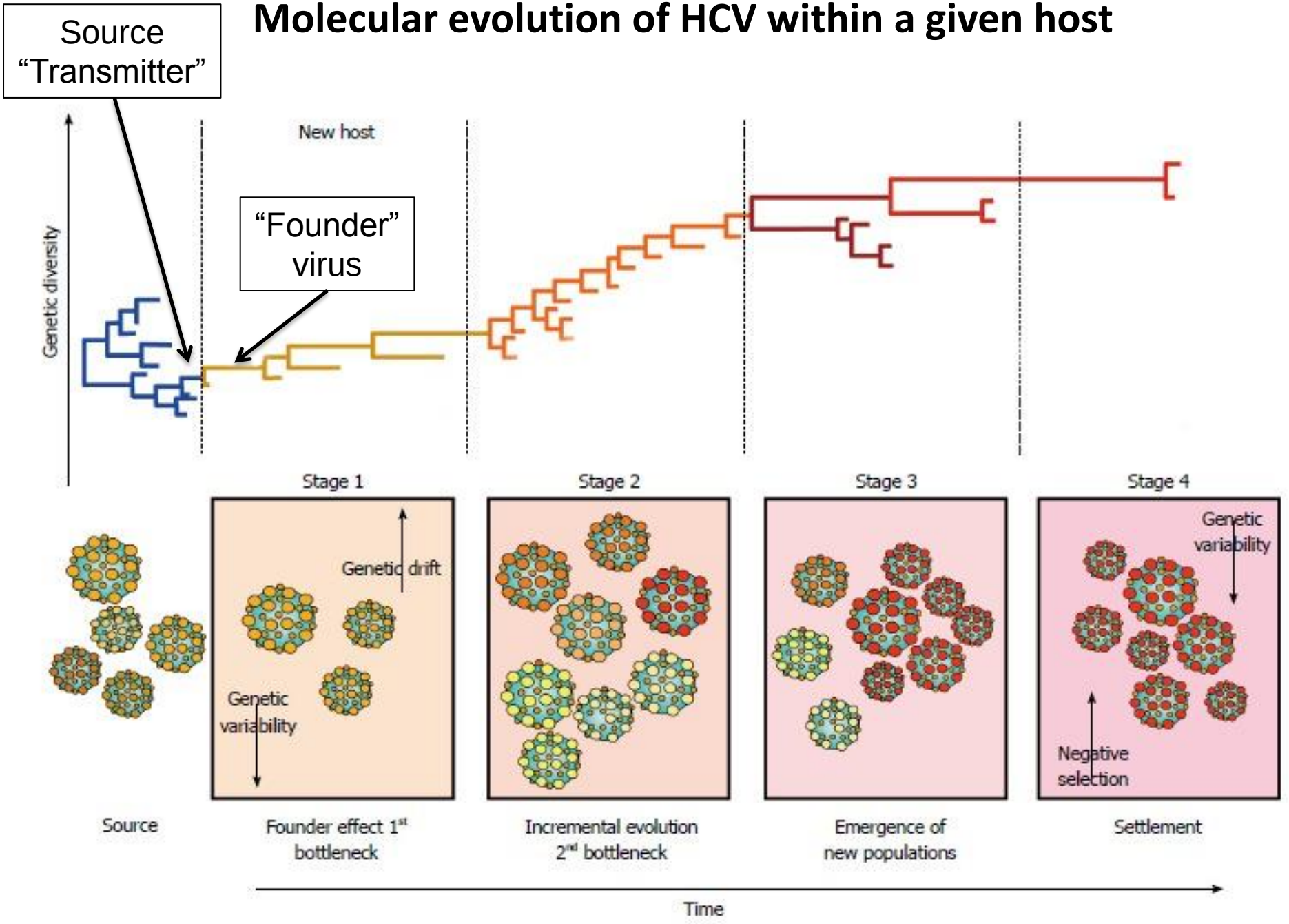
GACTGACT

GACTTTACT

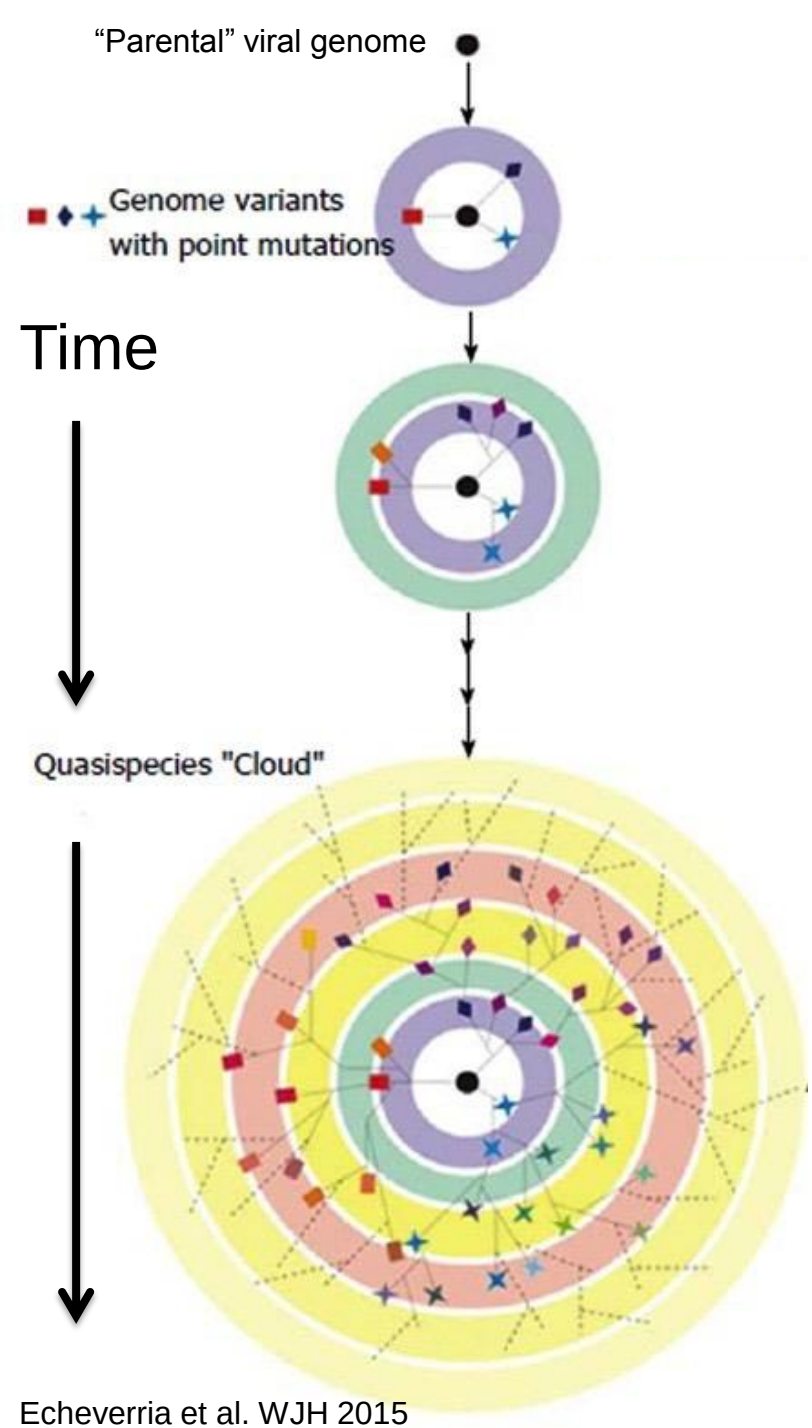


Echeverria et al. WJH 2015

Molecular evolution of HCV within a given host

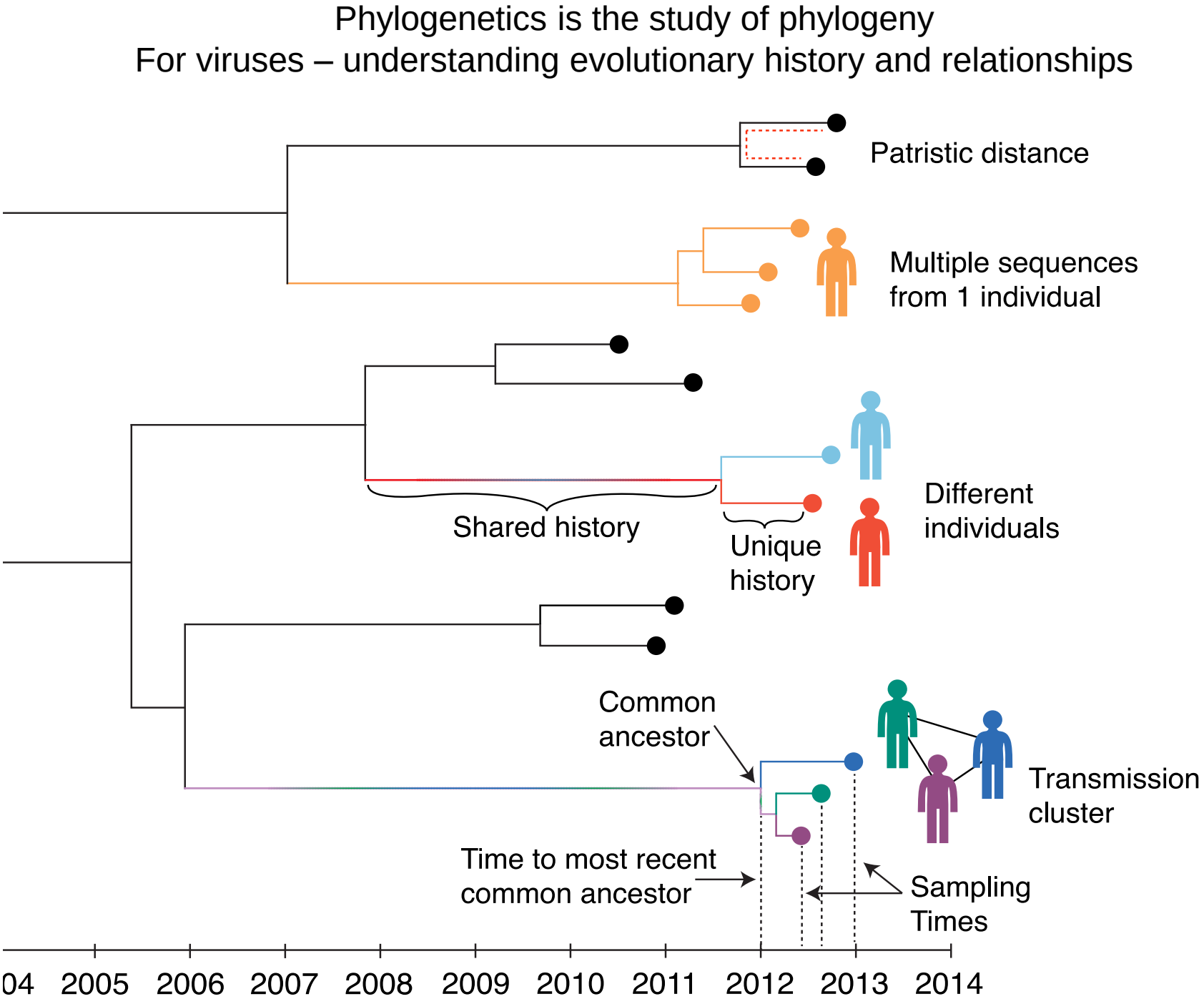


Preciado et al. WJG 2014

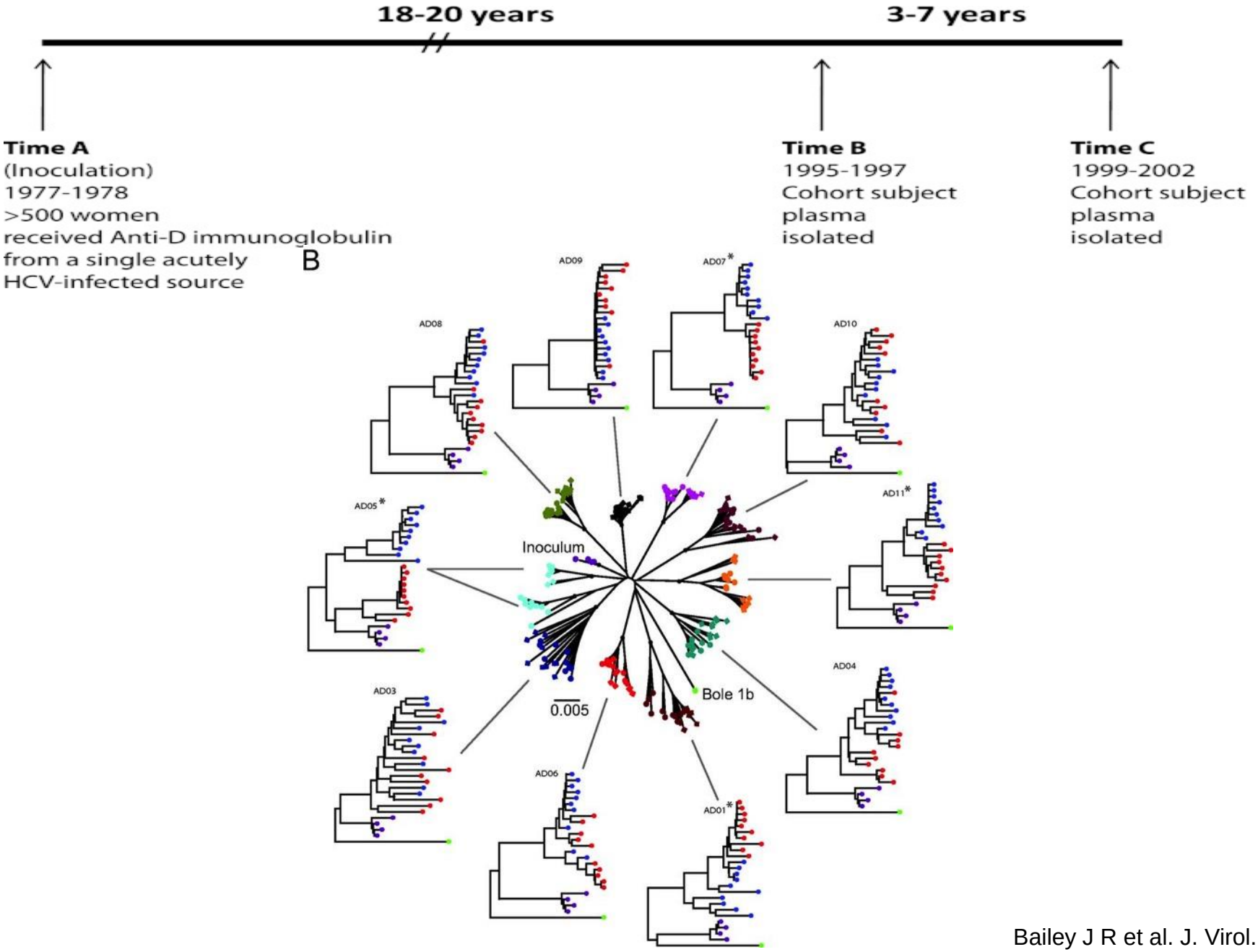


Echeverria et al. WJH 2015

- Molecular epidemiology can characterize the transmission history of an epidemic
- Genetic diversity and transmissions unfold at the same time
- People → similar sequences → transmission cluster

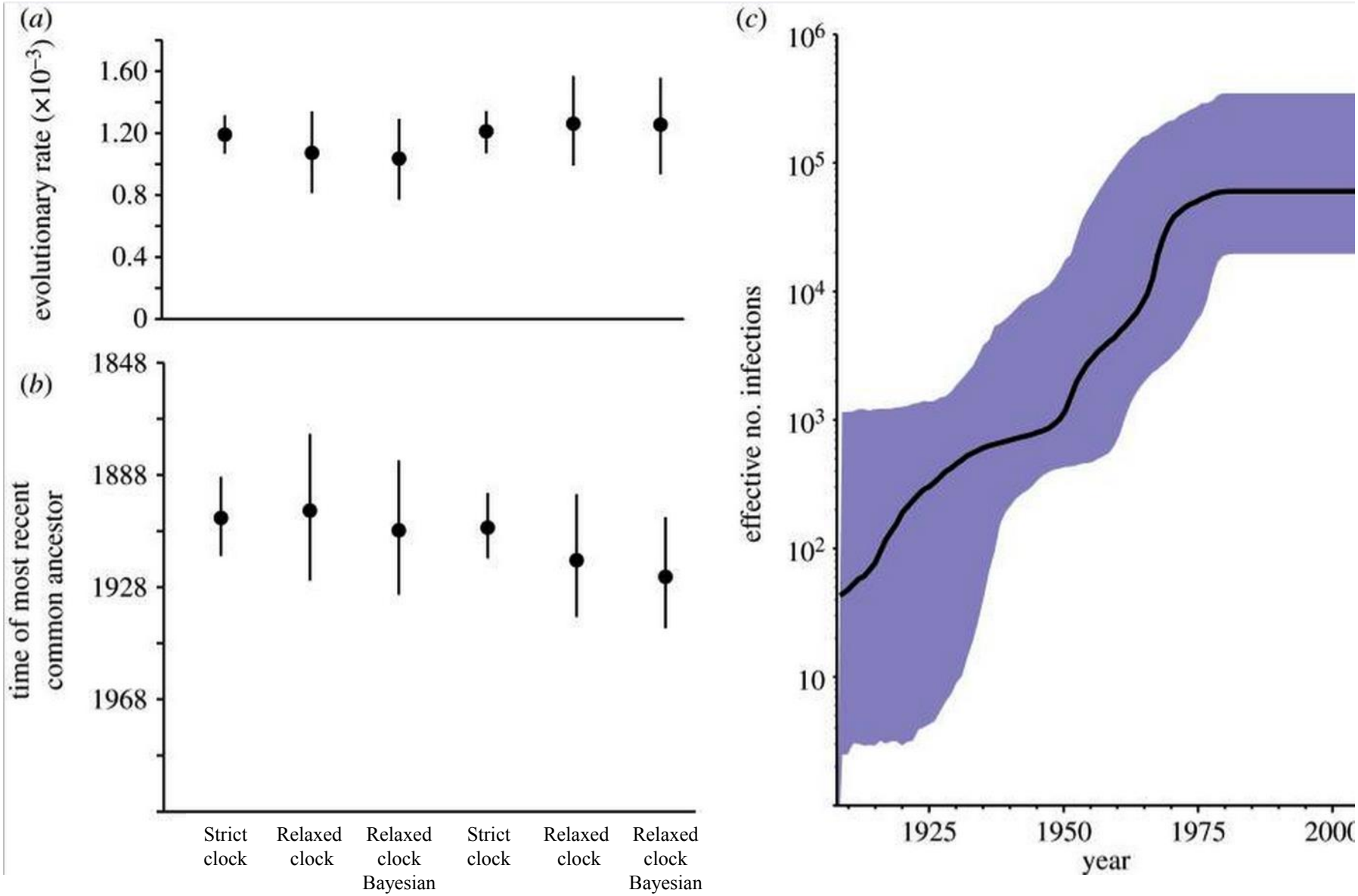

Phylogenetics is the study of phylogeny
For viruses – understanding evolutionary history and relationships
Patristic distance
Multiple sequences
from 1 individual
Shared history
Unique
history
Different
individuals
Common
ancestor
Transmission
cluster
Time to most recent
common ancestor
Sampling
Times
04
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014

Anti-D cohort HCV contaminated Rhogram plasma - Ireland

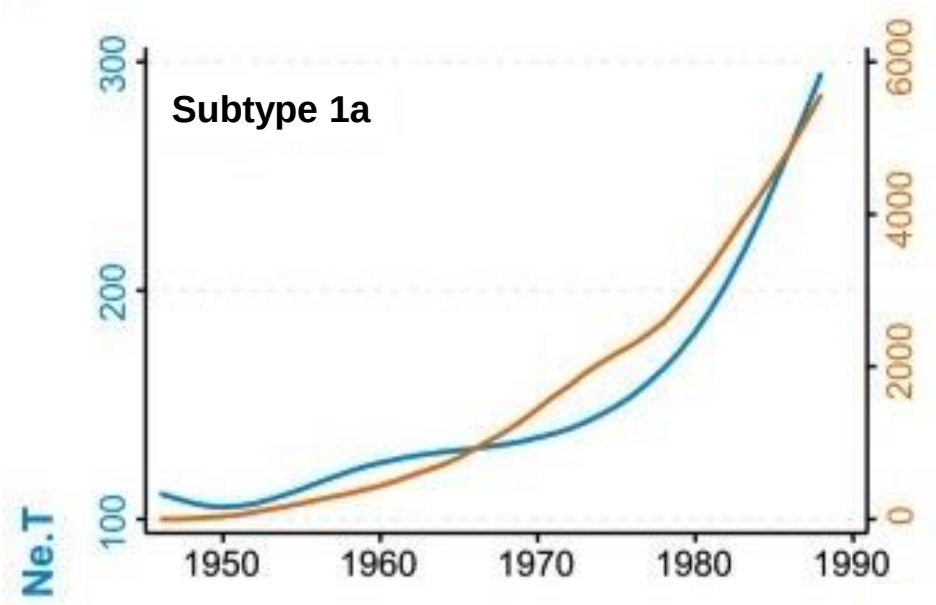


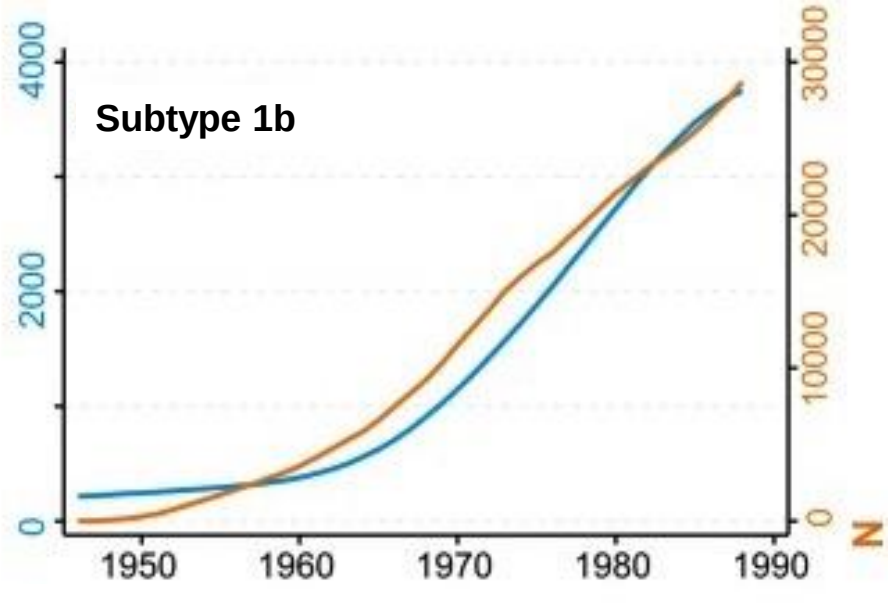
Bailey J R et al. J. Virol. 2012

1. Recreate the transmission dynamics of an outbreak without fully knowing the traditional surveillance epidemiology



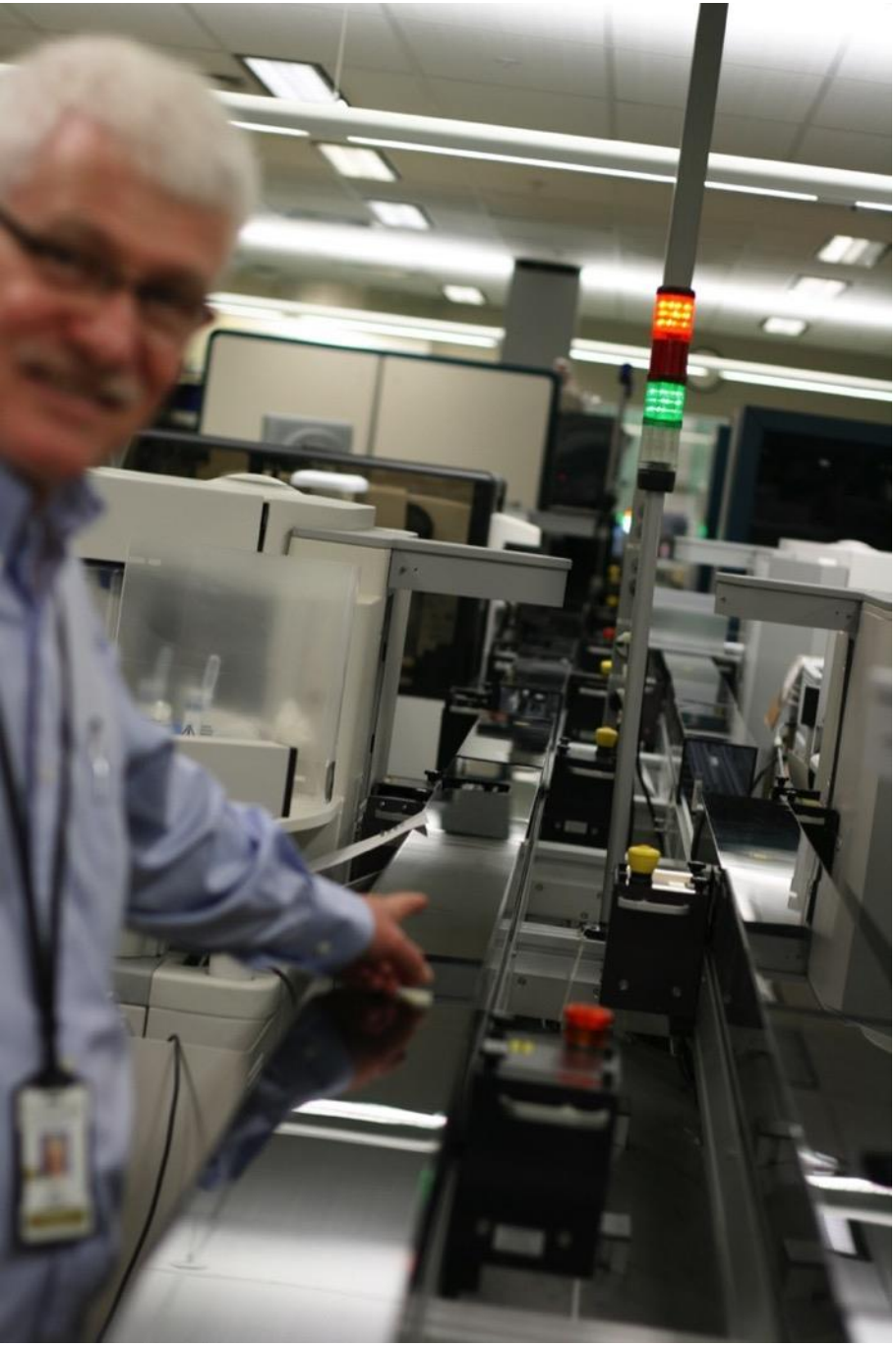
Grey et al. Philos Trans R Soc Lond B Biol Sci. 2013





Magiorkinis et al. (PloS PLoS Comput Biol 2013)

- Molecular phylodynamics can be integrated with traditional epidemiology to estimate transmission dynamics in a HCV viral epidemic
- NeT using genetic data (Bayesian skyline plot) versus N (estimated from surveillance data using back calculation)
- Plots were truncated after 1990 - to characterize HCV transmission prior the virus' discovery in 1989

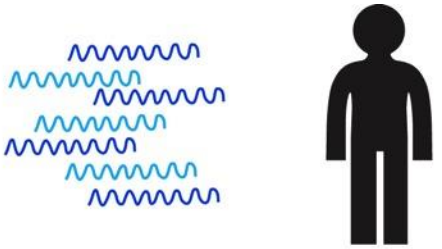


Cohort vs population samples?

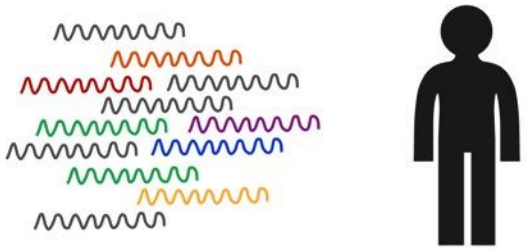
As of August 9, 2015 at the BCCDC, Vancouver, Canada:

- 1,472,830 individuals tested for anti-HCV
- 77,010 anti-HCV+
  - Includes 8,736 seroconverters
  - 4,314 within 24 months

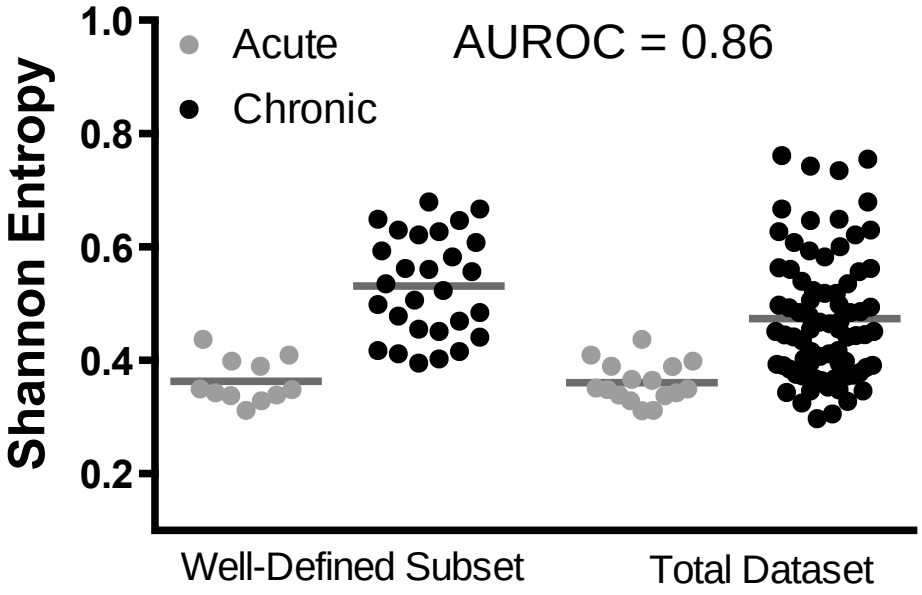
2. Determine if Infection is Incident or Prevalent & Time the Infection - Diversity

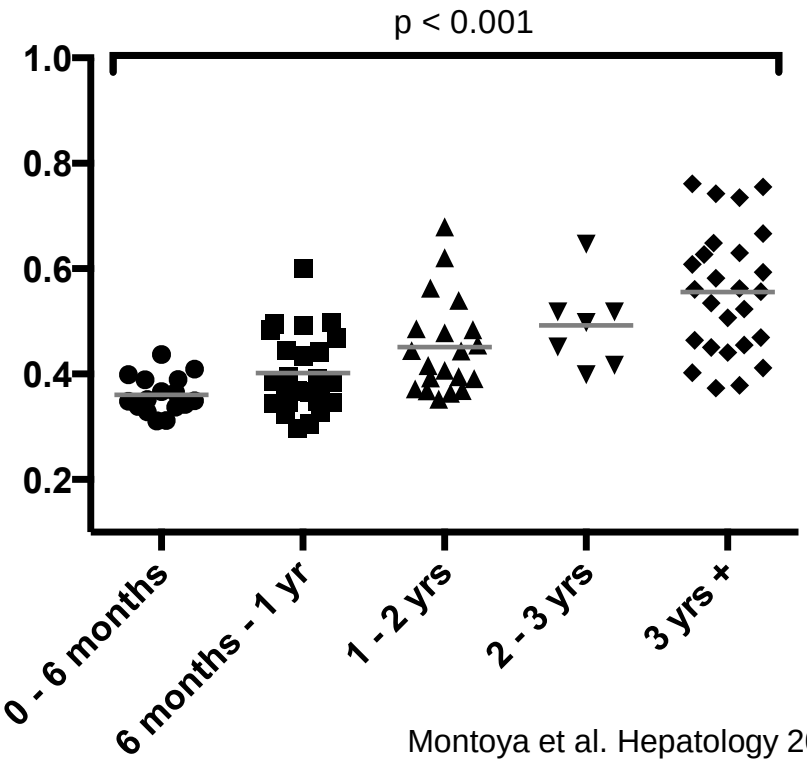


Low diversity ⟶ New Infection



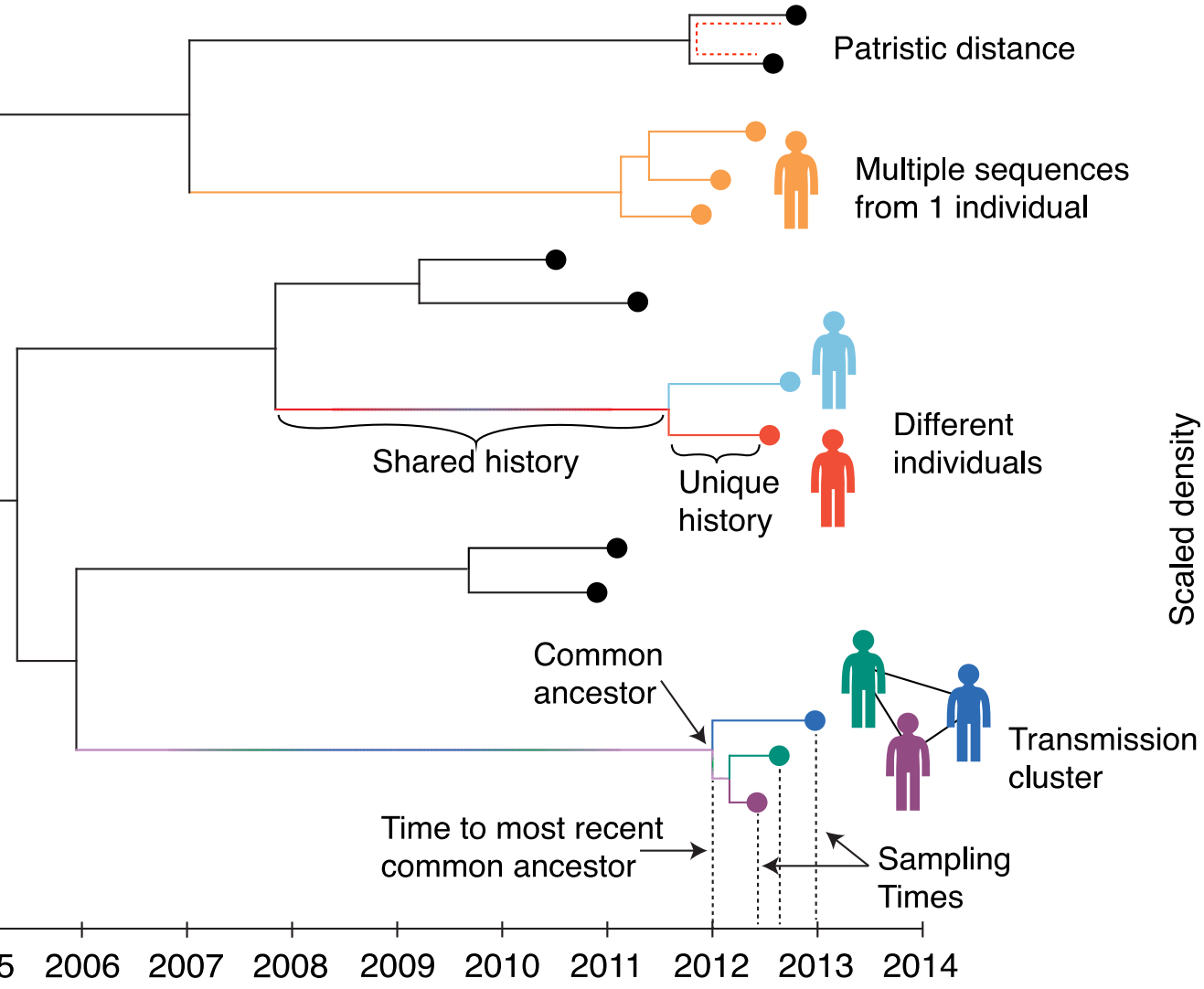
High diversity ⟶ Old infection

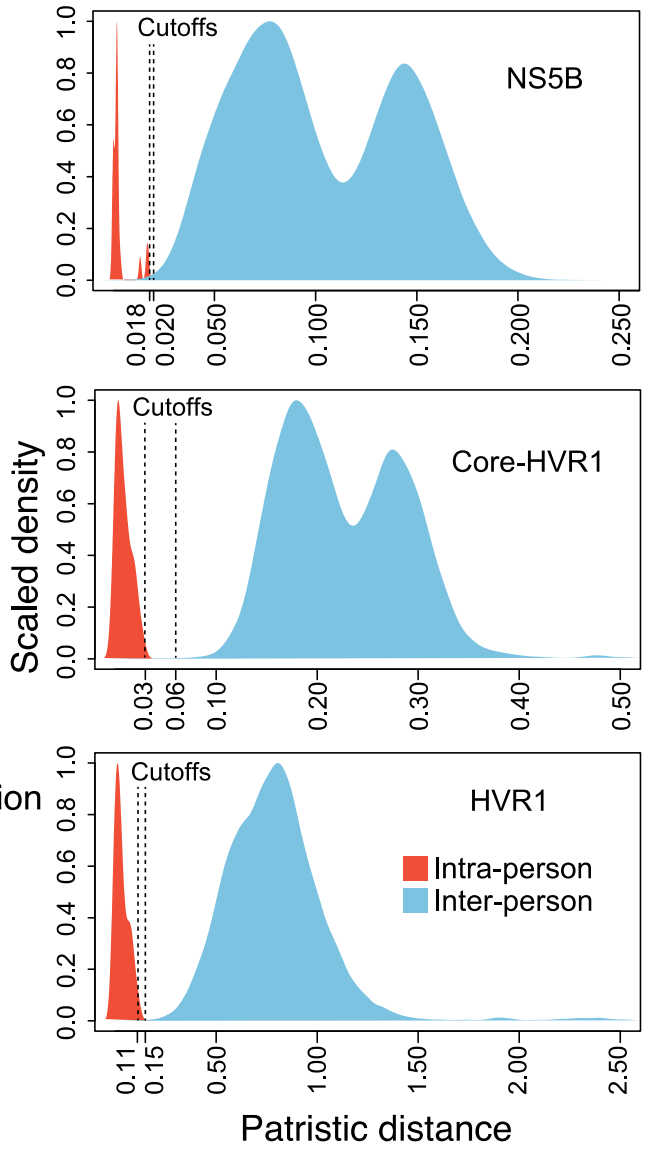




Montoya et al. Hepatology 2015

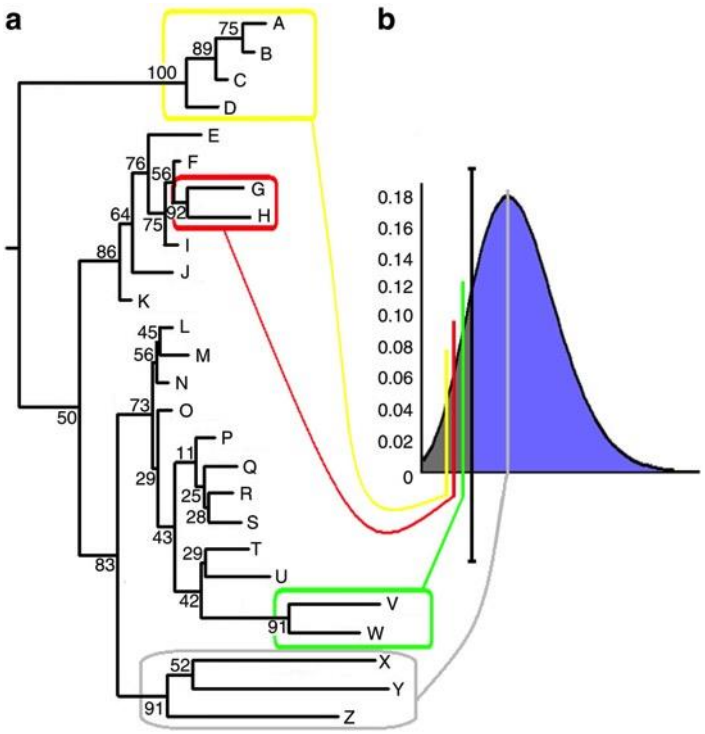
Determine if the Infection is Incident or Prevalent:
Use Temporal Sequence Relatedness to Time the Infection





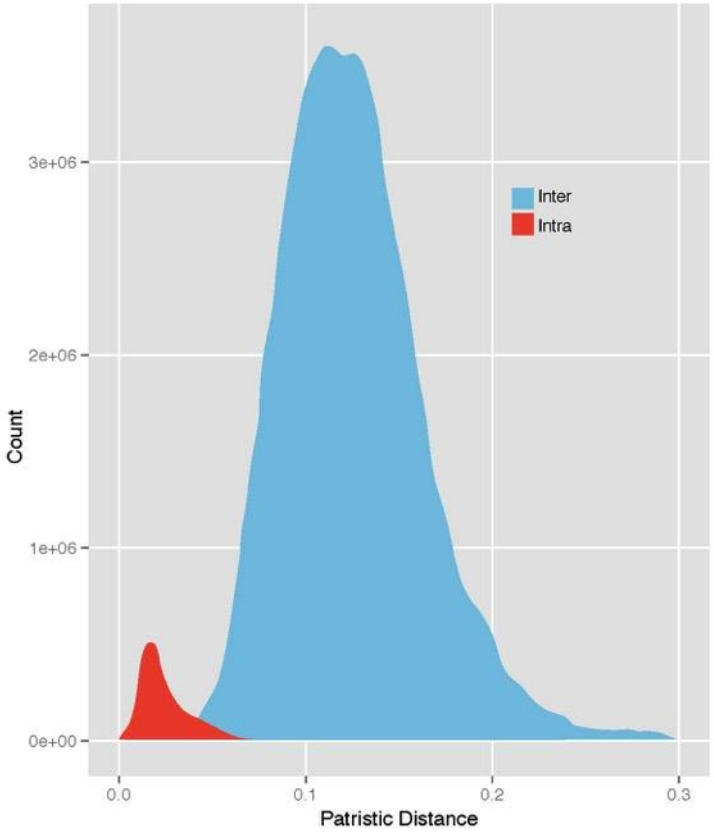
Olmstead et al. Infection Genetics Evolution 2015

Greater the Depth of Sequencing = Better Characterization of Sequence Relatedness Over Time



Looks at a distribution of cluster relatedness - cloning

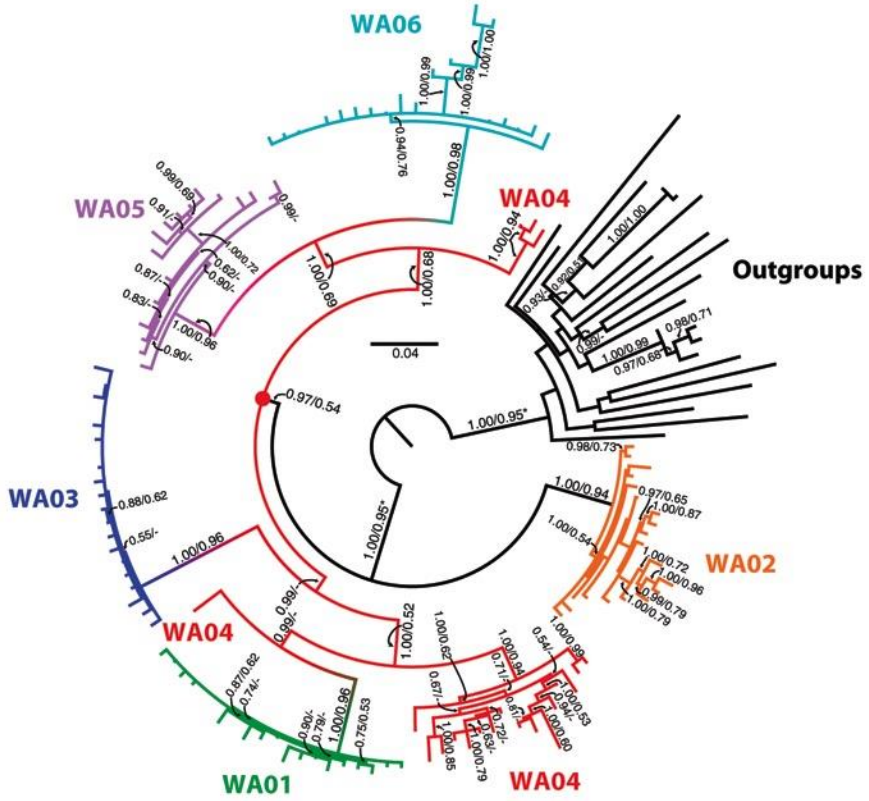
Prosperi et al. Nat Com 2011



Distribution of pairwise patristic distances for PCR product NGS from the NS5b derived from 32,641 reads (n=93) The intra-individual patristic distances are shown in red and the inter-individual patristic distances in blue

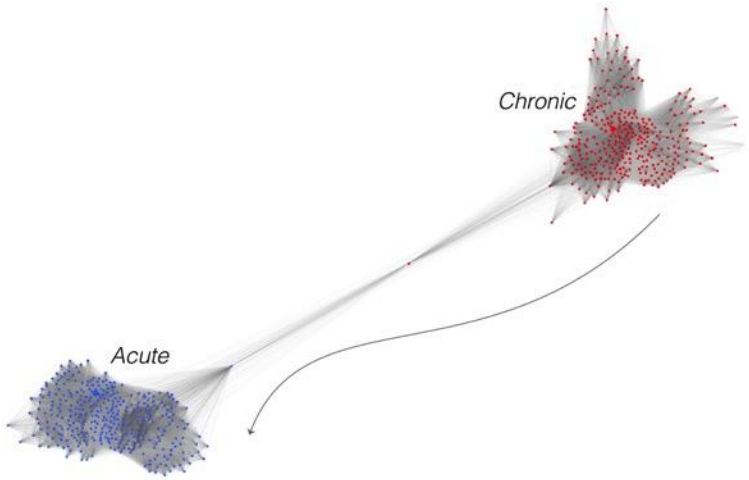
(Montoya – unpublished data)

3. Determine Transmission Directionality: Implications on Privacy?



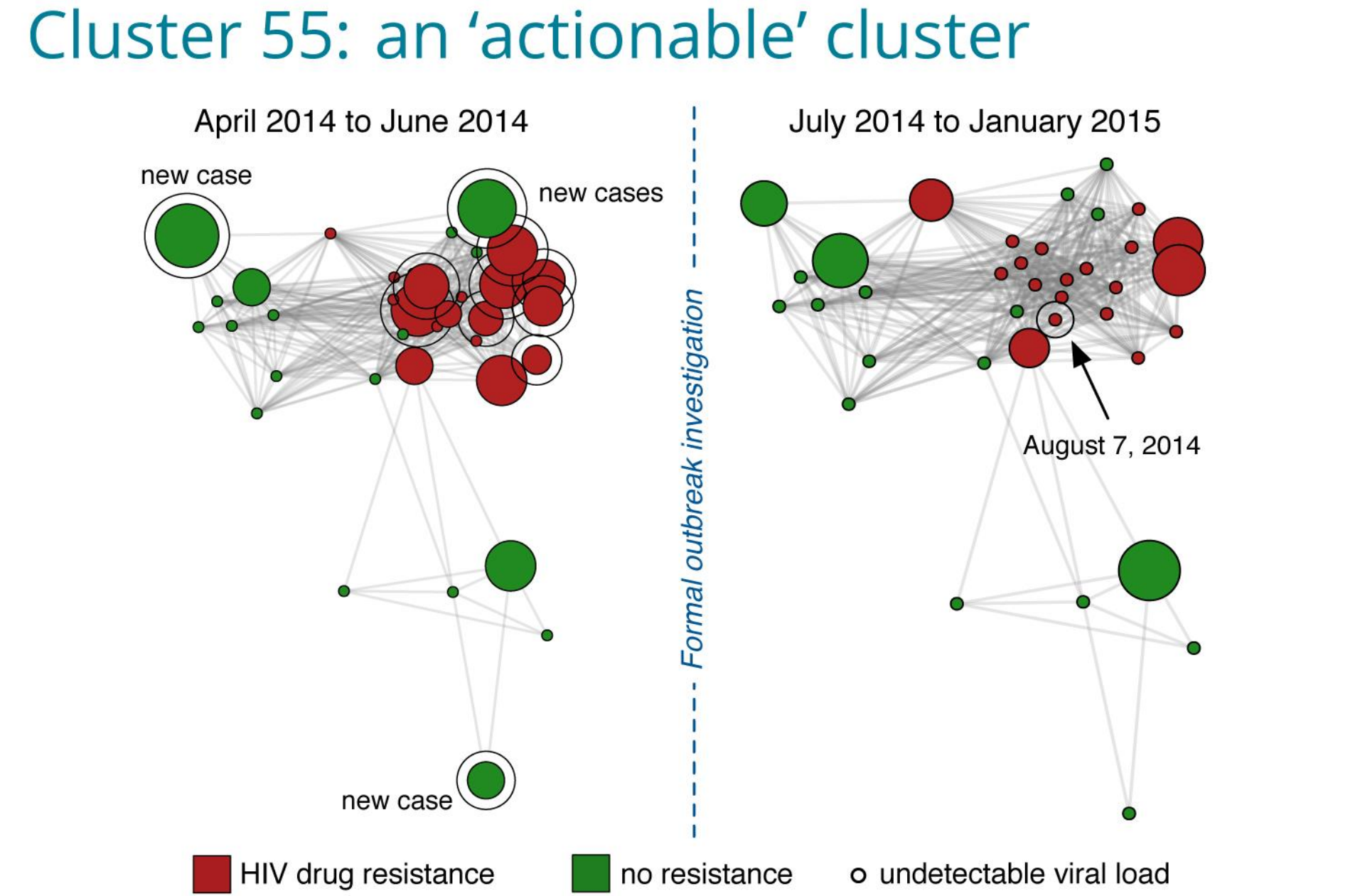
- Superior Court of the State of Washington ruled transmission direction can be established from blinded case samples
- The close paraphyly relationship of viral sequences was used to convict an HIV index case for 17 counts of first degree assault
  - paraphyletic relationships - source viral sequences are more closely related to all recipient sequences than to other source sequences
- Sentenced to 2,137 months

Scadutoa et al., PNAS, 2010



- Use a combination of sequence diversity in the sample to determine the if the infection is acute or chronic
- Combine with sequence relationship to demonstrate directionality (Montoya – unpublished data)

4. Determine if a transmission cluster is expanding or stable



Poon et al. IAS 2015

Sequence Ebola in real time? There's an app for that!

http://ebola.nextflu.org

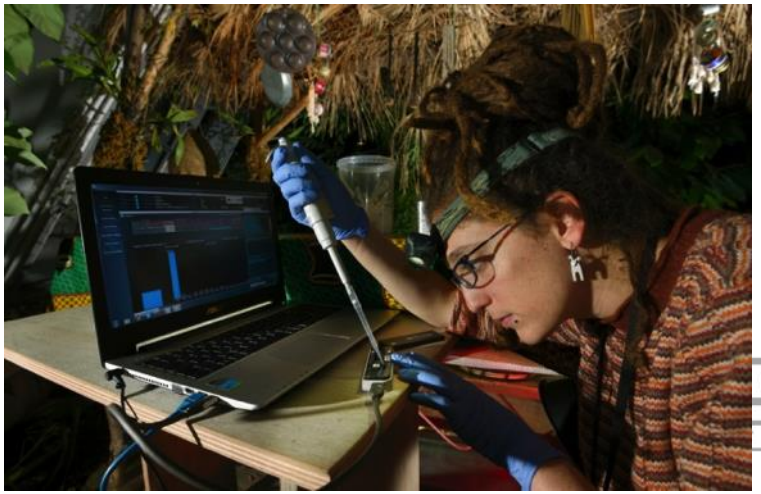
Real-time analysis of Ebola virus evolution

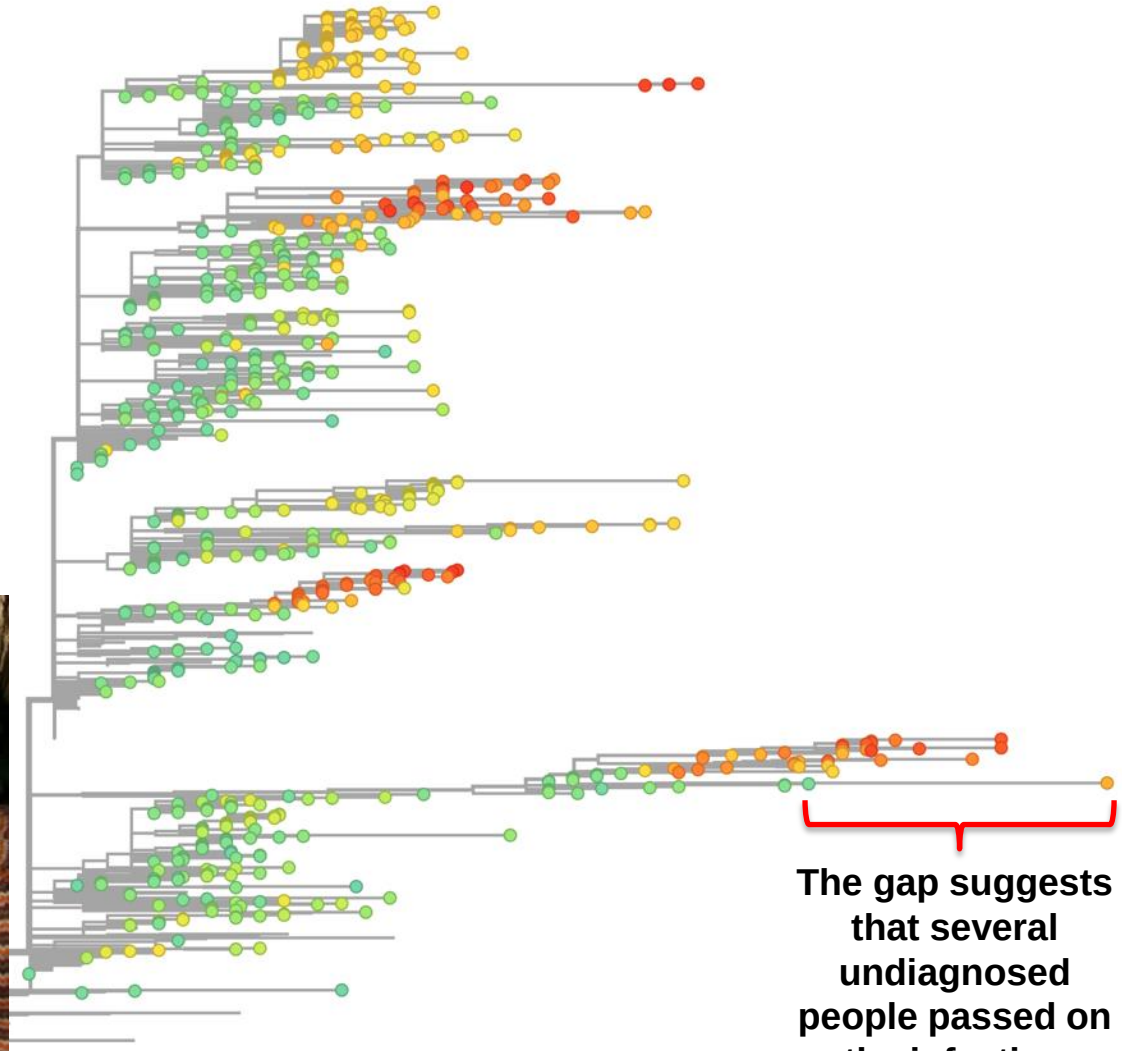
2015 Sep 7

Apr Jul Oct 2015 Apr Jul Oct

Date

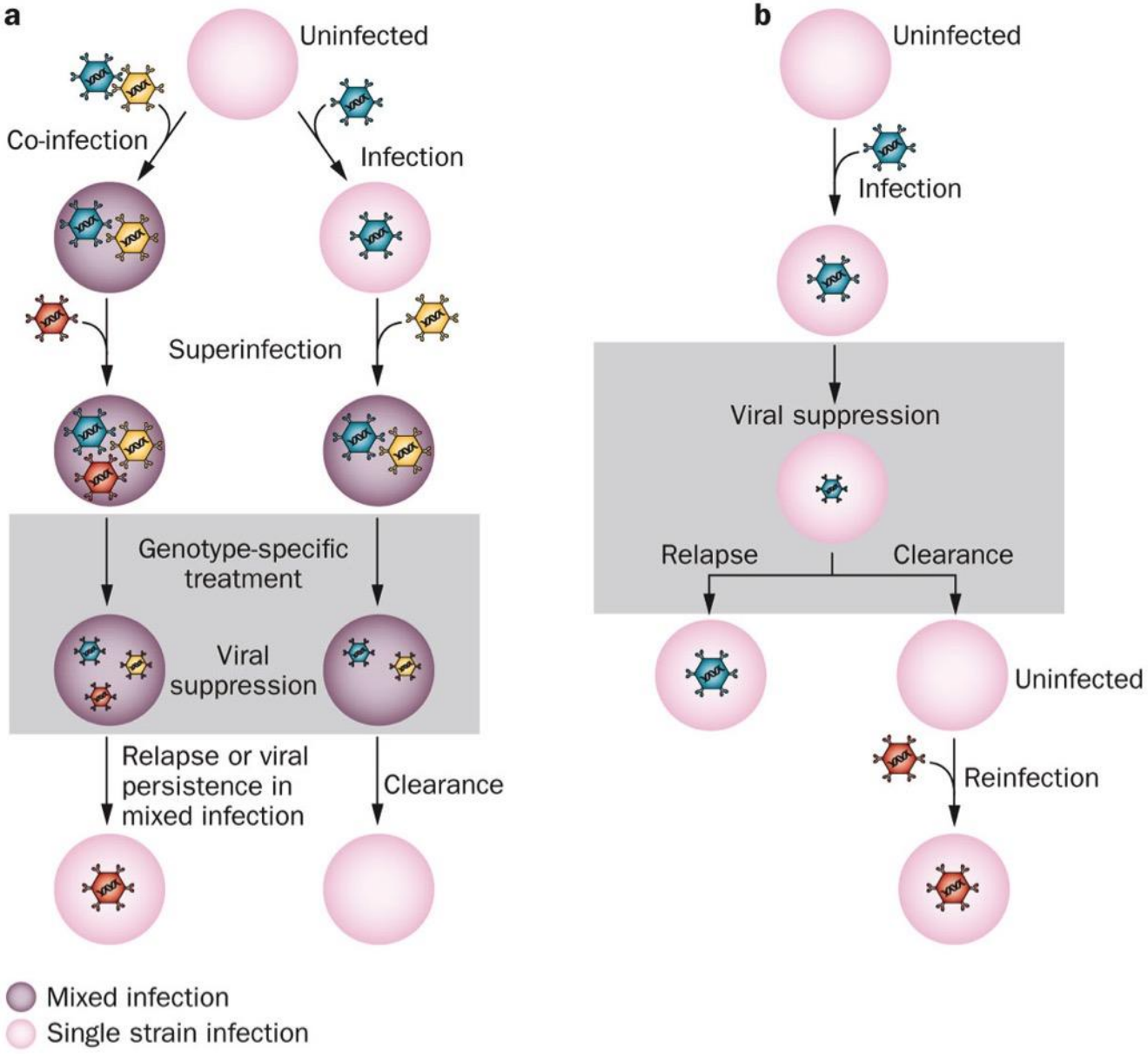
2014.53	2015.09
2014.64	2015.2
2014.76	2015.31
2014.87	2015.42
2014.98	2015.53





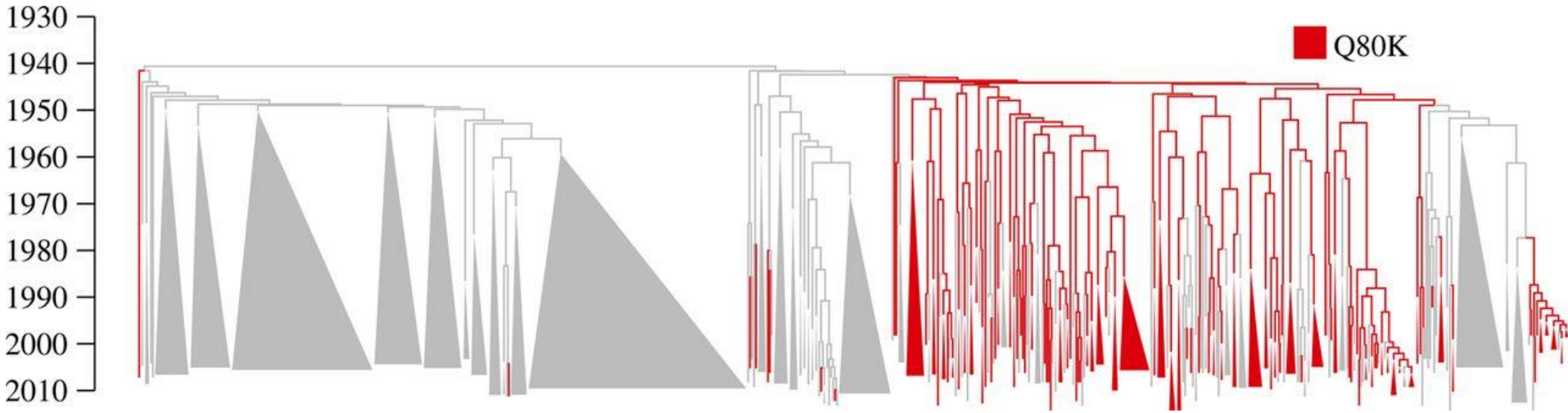
The gap suggests that several undiagnosed people passed on the infection

5. Identify: Mixed Infection; Treatment Relapse vs Reinfection



Cunningham et al. Nat. Rev. 2015; Abdelrahman et al. Hepatology 2015

6. Detect Resistance Associated Variants (RAVs)



- Global phylogeny of hepatitis C virus showing lineages possessing the Q80K polymorphism in nonstructural protein 3 (NS3)
  - The Q80K polymorphism has been associated with reduced susceptibility to the direct acting antiviral inhibitor simeprevir
  - Occurs predominantly in HCV geno 1a, high prevalence in the United States
  - 96% of HCV infections carrying Q80K descend from a single lineage which occurred around the 1940s in the United States, implying that this polymorphism is highly transmissible

McCloskey et al. J Infect Dis. 2015

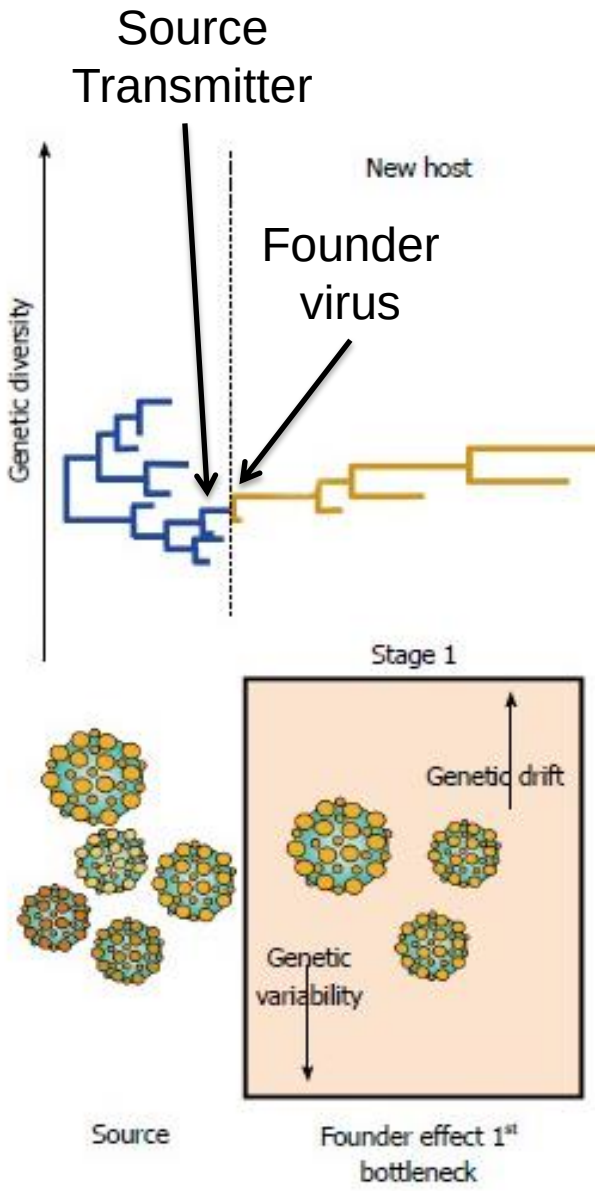
Position	Variant	HCV region	EC50 [fold-change][1] (subtype)	Resistance Level	DAA	References
Q80	K	*NS3*	9.3 (1a), 7.7 (1b)	low	SMV	[31]
			3 (1a), 6.5 (1b)		ASV	[32]
			3 (1a)		PTV	[34]
	R	*NS3*	13 (1a), 6.9 (1b)	low-intermediate	SMV	[31]
			4 (1b)		ASV	[32]
			2 (1a)		PTV	[34]
D168	E	*NS3*	26 (1a), 43 (1b)	low-intermediate	SMV	[31]
			58 (1a), 78 (1b)		ASV	[32]
			14 (1a), 4 (1b)		PTV	[34]
M28	V	*NS5A*	1.3 (1a)	intermediate	DCV	[19]
			n.d.		LDV	n.d.
			58 (1a)		OMV	[18]
Q30	H	*NS5A*	1477 (1a)	low-high	DCV	[19]
			73 (1a)		LDV	[21]
			3 (1a)		OMV	[36]
L31	M	*NS5A*	341 (1a), 3 (1b)	low-high	DCV	[19]
			140 (1a), 2.5–100 (1b)		LDV	[21, 22]
			2 (1a), 0.9 (1b)		OMV	[33]
	F	*NS5A*	5 (1b)	low	DCV	[20]
			n.d.		LDV	n.d.
			10 (1b)		OMV	[18]
Y93	C	*NS5A*	1864 (1a)	high	DCV	[19]
			327 (1a)		LDV	[21]
			1675 (1a)		OMV	[18]
	F	*NS5A*	n.d.	low-intermediate	DCV	n.d.
			2.5–100 (1a)		LDV	[22]
			n.d.		OMV	n.d.
	H	*NS5A*	5432 (1a), 24 (1b)	intermediate-high	DCV	[19]
			3309 (1a), 1319 (1b)		LDV	[21]
			41383 (1a), 77 (1b)		OMV	[36]
	N	*NS5A*	47477 (1a)	high	DCV	[19]
			>100 (1a)		LDV	[22]
			66739-fold (1a)		OMV	[18]
C316	H	*NS5B*	229 (1b)	high	DSV	[33]
	N	*NS5B*	5 (1b)	low	DSV	[35]
	Y	*NS5B*	1472 (1a), 1569 (1b)	high	DSV	[35]
Y448	H	*NS5B*	975 (1a), 46 (1b)	intermediate-high	DSV	[35]
S556	G	*NS5B*	30 (1a), 11 (1b)	intermediate	DSV	[35]
	N	*NS5B*	29 (1a)	intermediate	DSV	[35]
	R	*NS5B*	261 (1a)	high	DSV	[33]
C316N+S556G		*NS5B*	38 (1b)	intermediate	DSV	[33]

Personalized medicine based on pathogen genome

EC50 values of baseline RAVs within NS3, NS5A and NS5B

Dietz J, et al. PLoS ONE, 2015

7) Study Early Infection Events – Vaccine Design?



- Characterize ‘transmitter/founder’ viruses at the earliest infection time points
- Full length molecular clones responsible for initiating hepatocyte infection and eliciting the initial immune responses
- Strategy to identify drivers of the early immune response & potential conserved epitopes for vaccine development

Preciado et al. WJG 2014

Stoddard et al. mBio 2015, Mitchell et al. mBio 2015

Molecular Epidemiology of the Future

1st Generation "Dore-Bot"

Not only what virus you have, but:

1) Determines the transmission dynamics with incomplete surveillance data
2) Determines if infection is incident or prevalent and times the infection
3) Determines infection directionality - serious privacy implications?
4) Identifies transmission clusters needing attention
5) Identifies treatment relapses or a reinfection and mixed infections
6) Enables personalized drug selections for patients harbouring RAVs
7) Determines early infection events and informs vaccine design

BC Centre for Disease Control
An agency of the Provincial Health Services Authority

Acknowledgements

Dr. Andrea Olmstead
Vincent Montoya
Iris Luo
Dr. Art Poon
Dr. Jeffrey Joy
Dr. Jason Grebely
François Lamoury
Brendan Jacka
Dr. Tanya Applegate
Dr. Richard Harrigan

CIHR IRSC
Canadian Institutes of Health Research
Instituts de recherche en santé du Canada

BC Centre for Disease Control
An agency of the Provincial Health Services Authority

UBC

National CIHR Research Training Program in Hepatitis C
Subvention nationale de formation des IRSC sur l'hépatite C

Provincial Health Services Authority
Province-wide solutions. Better health.