Behavioral Risk and HIV-1 Molecular Diversity: Making the Connections

Drug Abuse and Risky Behaviors: The Evolving Dynamics of HIV/AIDS
NIDA, NIH, May 8, 2007

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Outline

- Introduction
- Molecular epidemiology and segregation by risks
- Risk and complexity
- Associations in the Opiate Users Research Cohort
- Breakpoint analyses and networks
- Conclusions

Introduction: Molecular Epidemiology

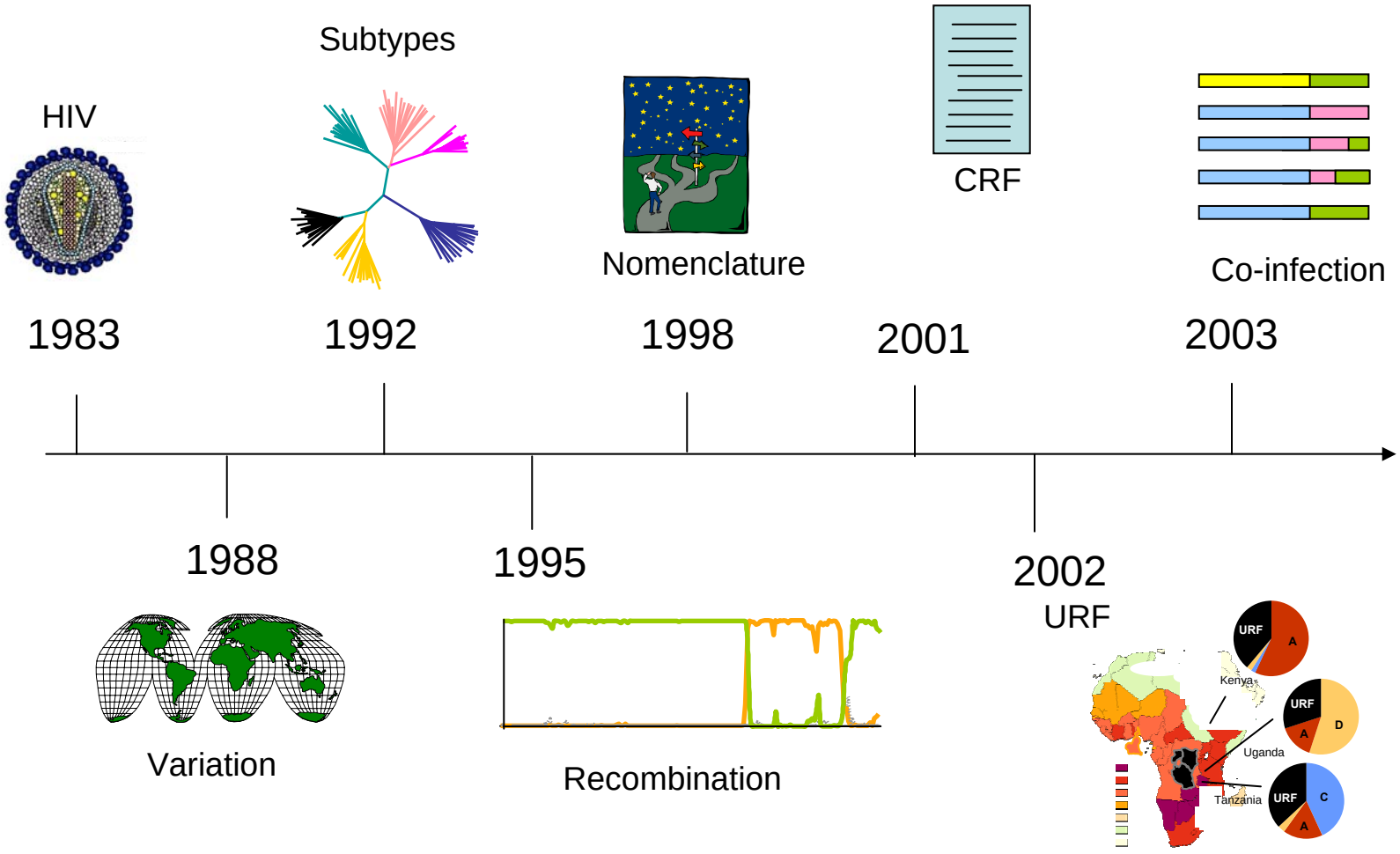
From a public health perspective, the advent of molecular epidemiology, which allows tracking of pathogens based on unique genetic sequences or antigenic properties, has revolutionized how epidemiologists investigate and evaluate epidemics and assess endemic diseases.

Robertson BH, Nicholson JK. New microbiology tools for public health and their implications. Annual Review of Public Health. 2005;26:281-302

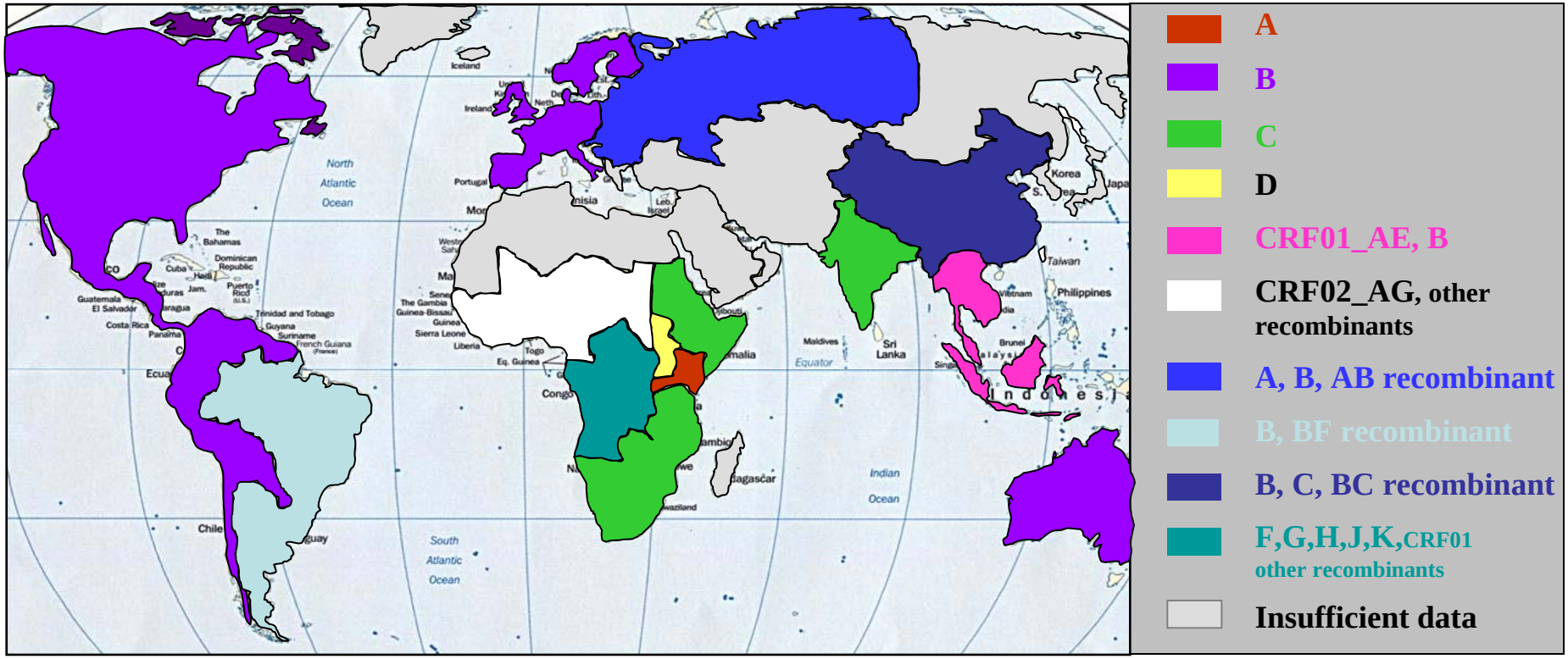
Introduction: HIV Genetic Diversity

- HIV-1 is a genetically diverse virus with high rates of genetic change: mutation, recombination, dual infection, super-infection
- The genetic diversity of HIV challenges the human immune system, vaccine development, measures of anti-viral drug resistance
- HIV-1 genetic diversity allows for epidemiologic investigations

HIV-1 Molecular Epidemiology: Historical Timeline and Key Milestones



Global Distribution of Subtypes and Recombinants



Uses of Molecular Epidemiology in HIV-1

Established

- Powerful tool in understanding epidemic dynamics
- Has regional utility, particularly for **common border epidemics**
- Allows use of the virus to track movements of **people** (truckers, sex workers, migrants, soldiers) and **narcotics**

Novel

- Linking diversity to risks could allow for targeting interventions, identifying “hot spots”
- Potential tool for mapping networks

Molecular Epidemiology and segregation by risks

Early Studies of Risks and Subtypes

South Africa 1990s

MSM with B, African Heterosexuals with C

Thailand 1990s

IDU with B, Heterosexuals with E (CRF01-A/E)

Malaysia 1990s

HIV-1 subtypes in Malaysia among different primary risk categories, 1994-1996.

-

Risk		B	E*	B/E	B/C	Non-typable
All	(N=89)	34 (38%)	48 (54%)	2 (2%)	1 (1%)	4(4.5%)
IDU	(N=53)	29 (55%)	19 (36%)	2 (4%)	1 (2%)	2 (4%)
Hetero	(N=27)	4 (15%)	23 (85%)	-	--	--
SW	(N=9)	1 (11%)	6 (67%)	--	--	2 (22%)

* E now called CRF01_A/E

IDU significantly more likely to have HIV-1 subtype B than those with sexual risks (heterosexuals and SW combined) **OR 5.9 (95% CI 1.9, 18.5) p < .001.**

All of the 3 dually reactive sera were from IDU.

Beyrer, et al. AIDS & Hum Retro, 1998

Risk and Complexity

Multi-region Hybridization Assays
(MHA)

Dual Infection

Dual infection, more common in high-risk groups, is the engine driving recombination and an important source of HIV diversity

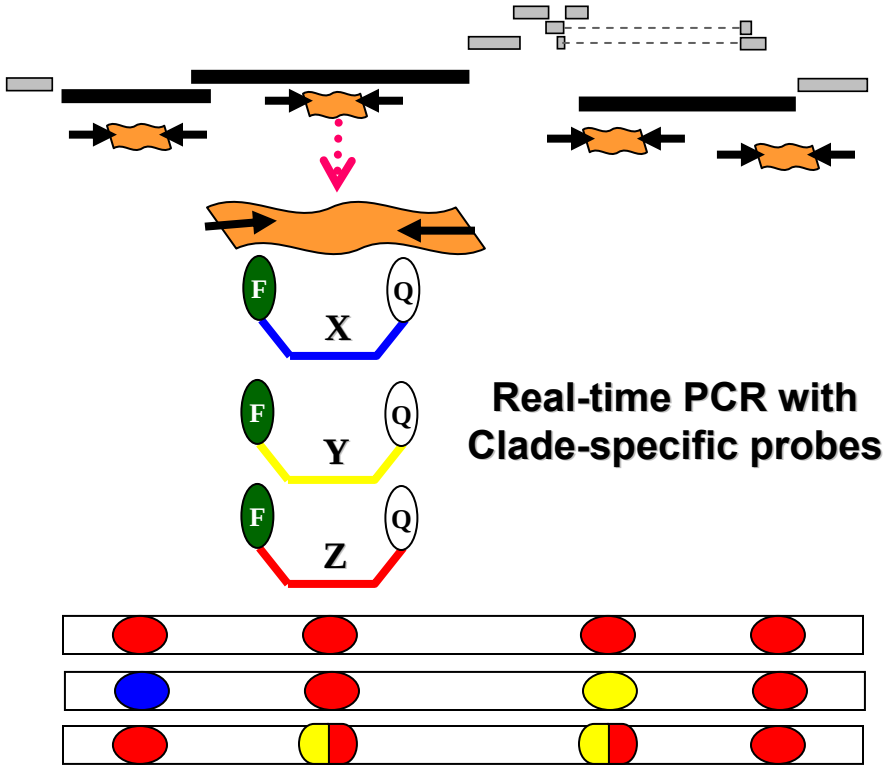
Many different recombinants can emerge in a dual infected individual, who may transmit them to others; dual infection is an accelerator of HIV diversity in populations

Many recombinant strains are generated within high risk social networks, which also have high rates of transmission; this coincidence of factors can accelerate the initial spread of new variants

A clear picture of the evolving HIV-1 epidemics in Asia can only be achieved through the study of large cohorts, using high-throughput and high-resolution subtyping

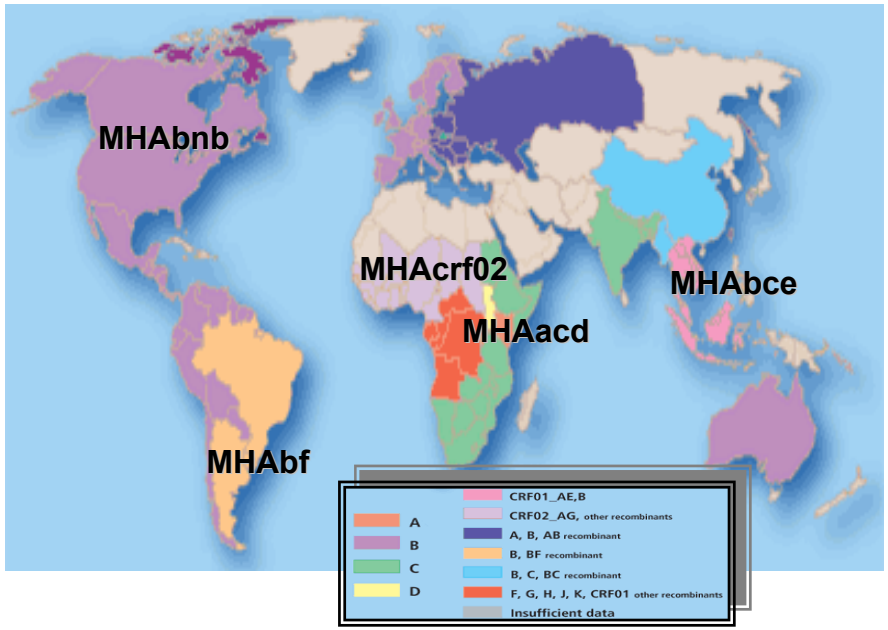
Multi-region Hybridization Assay (MHA) to study HIV-1 genetic diversity in Asia

Principle of MHA



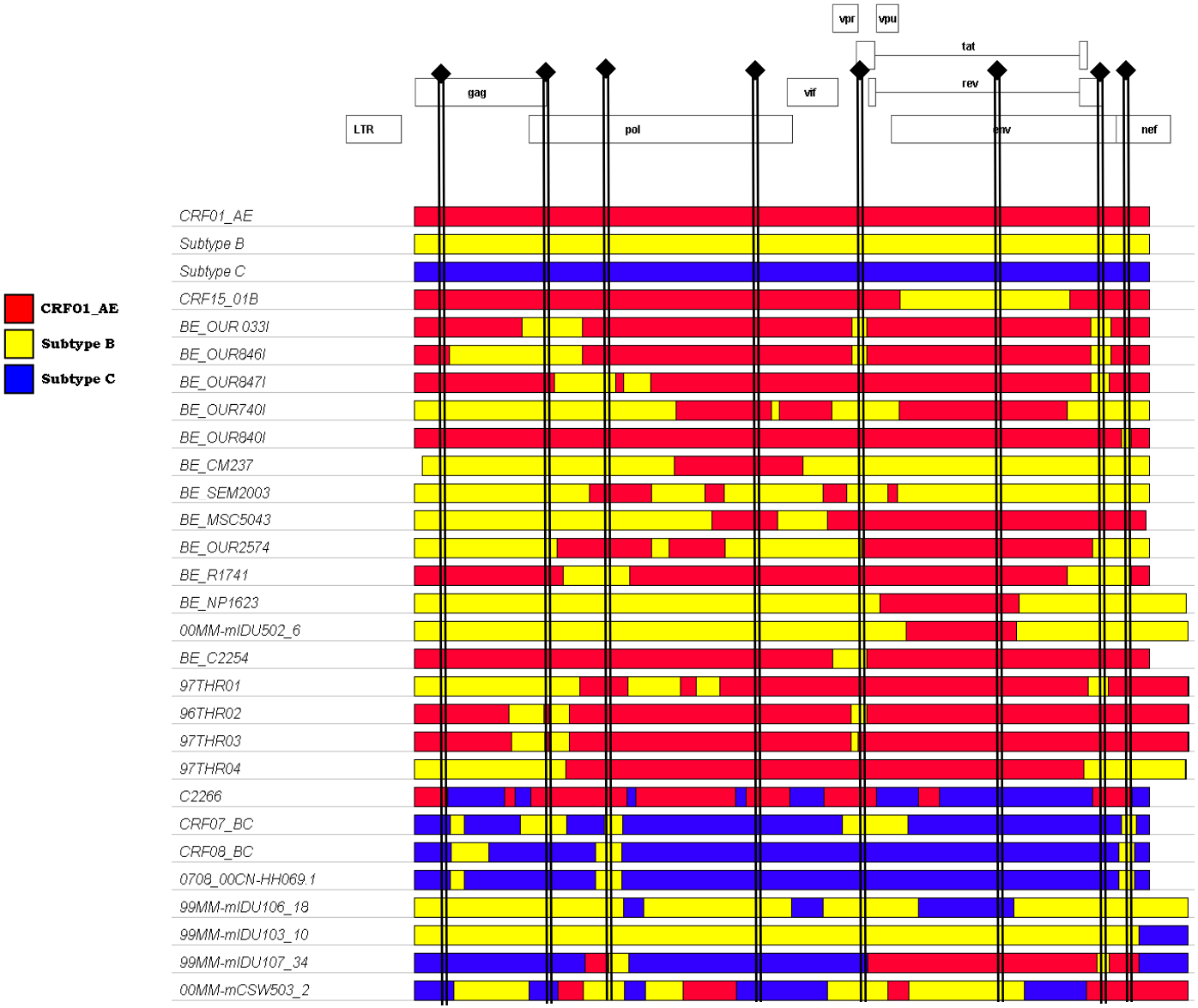
Real-time PCR with Clade-specific probes

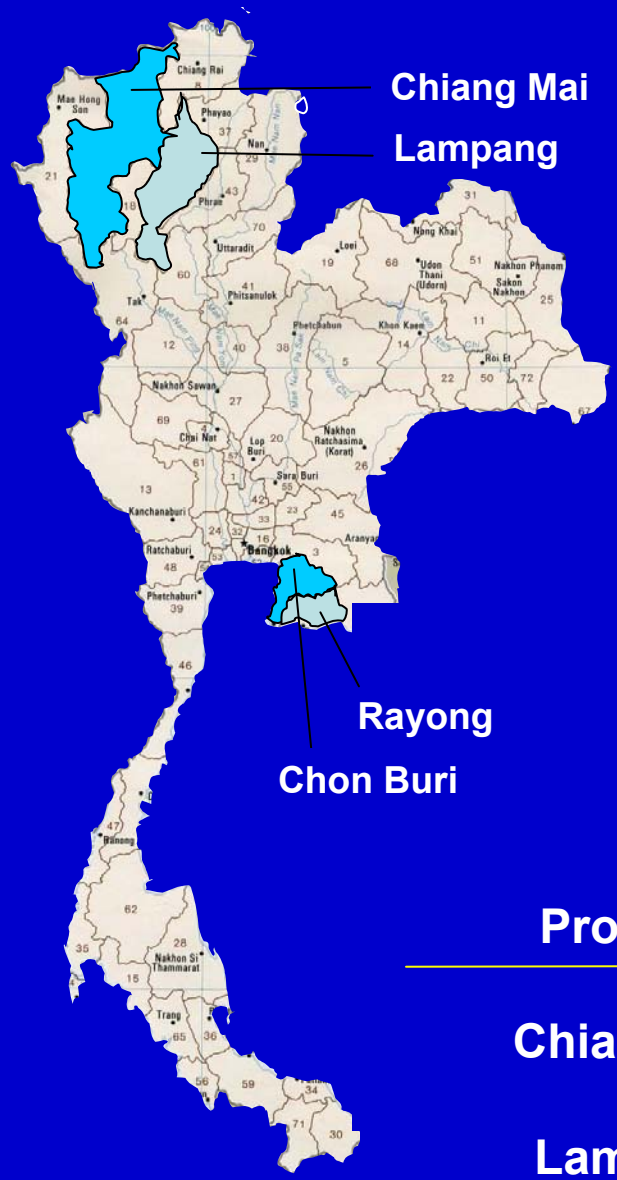
The MHA family



Courtesy Dr. F. McCutchan, USMHRP/HJF

Distinguishing HIV-1 molecular forms in Asia





Comparative Epidemiology in Thailand

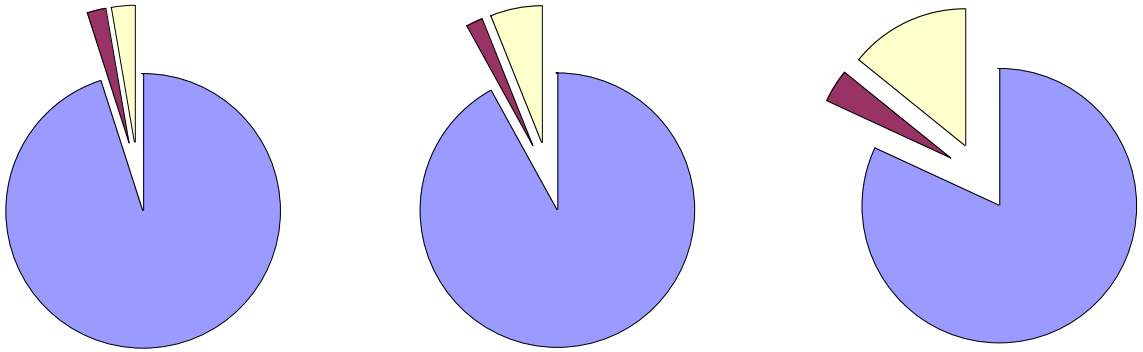
Province	Population	MHA Genotypes (N)
Chiang Mai	IDU (OUR)	336
Lampang	Antenatal Clinic	177
Rayong-Chon Buri	Vaccine Trial Volunteers	293
		806

The cohorts

	RV109	OUR	RV148	
Location	Lampang	Chiang Mai	Rayong Chon Buri	
Year	1996-1998	1999-2000	2004-2006	
Participants	180	2,231	26,675	
Cohort Characteristics	MTCT	Opiate users	Community	
Gender	100% ♀	7% ♀	48% ♀	
Risk factors	heterosexual	IVDU	heterosexual	
HIV sero-prevalence	ca. 3 %	15.6 %	1.6 %	
Genotyped samples	177/180 (98.3%)	336/347 (96.8%)	376/391 (96.2%)	Total 889 / 918 (96.8%)

Proportions of Subtypes, Recombinants, Dual Infections

	Antenatal	Trial Volunteers	IDU
CRF01_AE	94.9%	91.8%	81.8%
Subtype B	2.3%	2.0%	3.9%
Recombinant	2.8%	5.5%	9.2%
Dual	0.0%	0.7%	5.1%



HIV diversity and risks in Thai IDU

336 isolates from Thai IDU in the OUR cohort

81.8%	CRF01_AE
3.9%	B
9.2%	Recombinants: CRF01_AE and B
5.1%	Dual infections

Subtype B:

30 years old or older	OR: 6.9; 95%CI: 1.5-31.7

Dual infection:

lower education level	AOR=5.0 95%CI: 1.4-17.5
initiated injecting ≤ 3 years	AOR=3.4 95%CI: 1.2-9.8

Recombinants and duals:

needle sharing last 3 months	AOR=4.1, 95% CI: 1.41.7

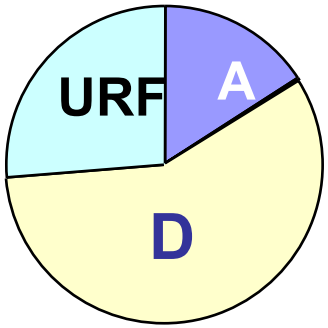
Comparative Epidemiology in East Africa

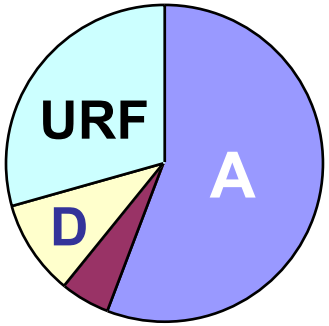
Country	Cohort	Population	MHA Genotypes (N)
Tanzania	HISIS	High risk ♀ (Sex Workers)	238
Tanzania	CODE	Urban and rural communities	487
Uganda	MER	Rural communities	329
Kenya	Kericho	Agricultural Plantation	366
			1420

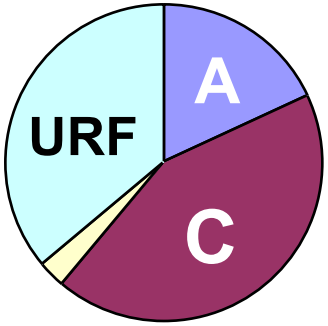
Proportions of Recombinant HIV and Dual Infections in A, D, C Subtype Zone

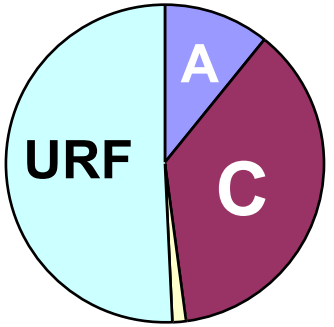
	Agricultural/Rural		Rural/Urban	Urban High Risk
URF	26.4%	29.5%	35.9%	50.8%
Dual	7.0%	7.1%	15.0%	16.4%

MER	KERICHO	CODE	HISIS





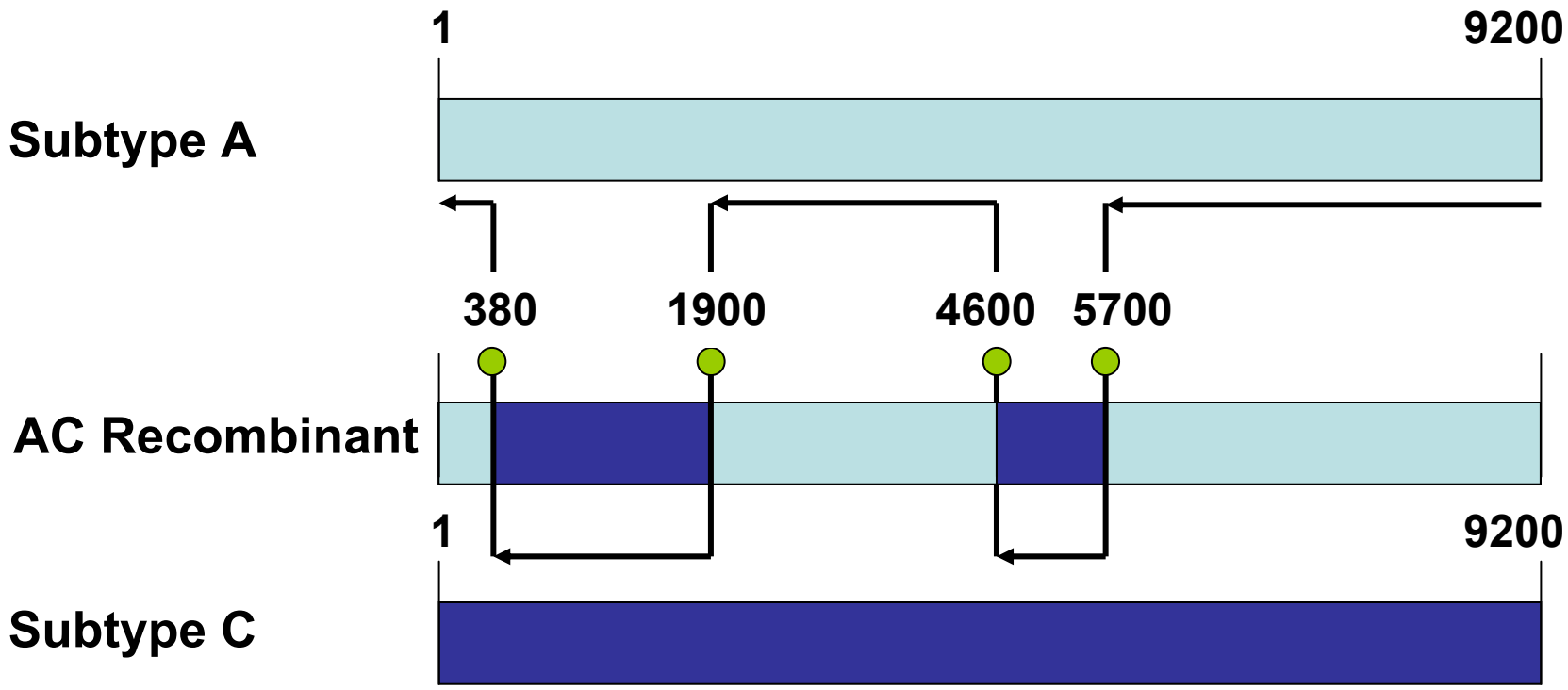




Breakpoint Analyses and Networks

Fine mapping of recombinant breakpoints

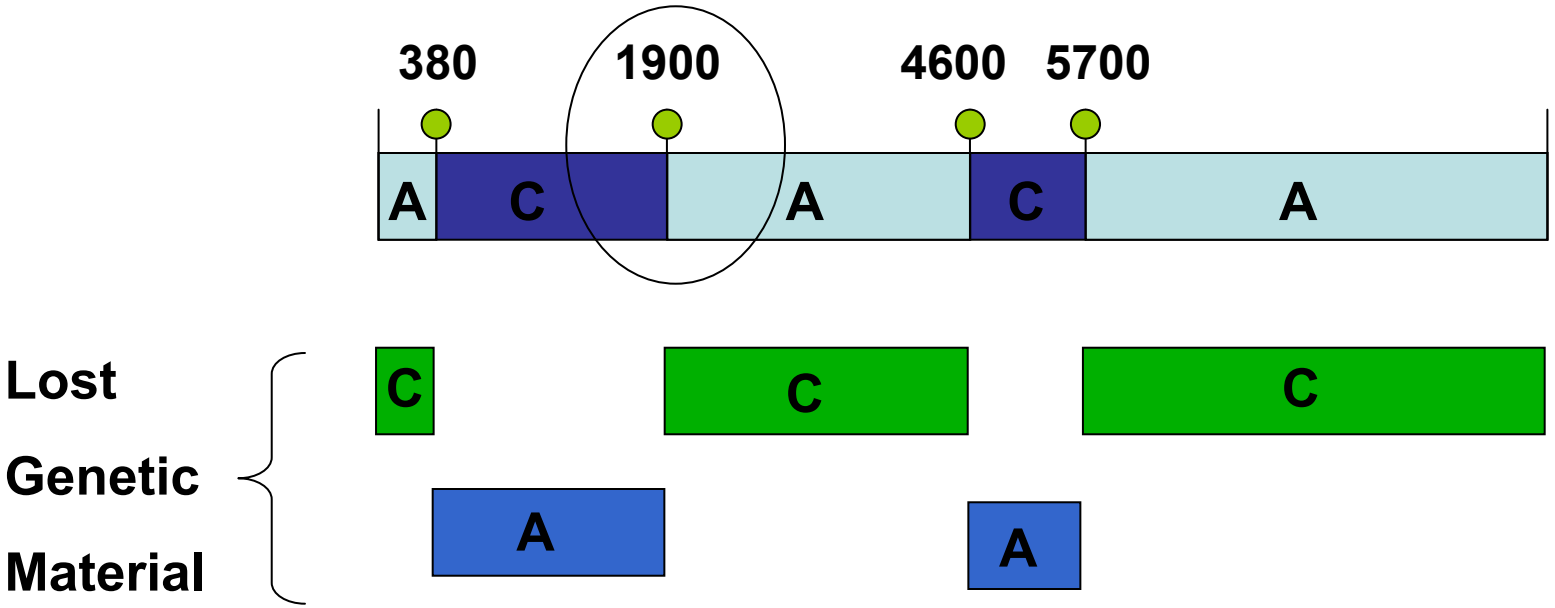
Describing a Recombinant Strain: Subtypes and Breakpoints



Through recombination, parts of the parental strains are lost, and cannot be regained until another dual infection provides opportunity to recombine again

Irreversibility lends stability

Could recombination breakpoints serve as stable markers through many cycles of transmission, permitting mapping of the social networks in which HIV spreads?

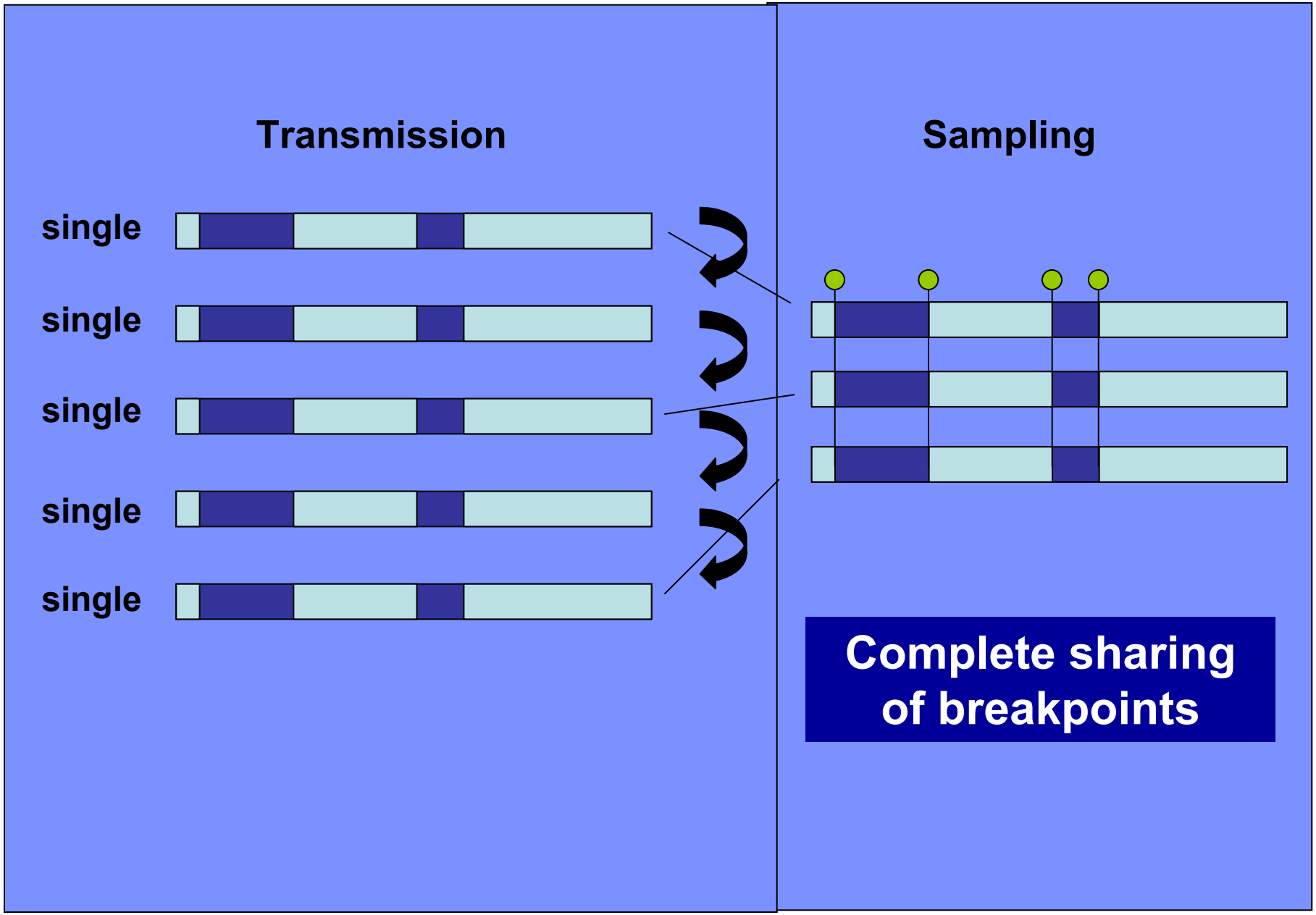


Hypotheses

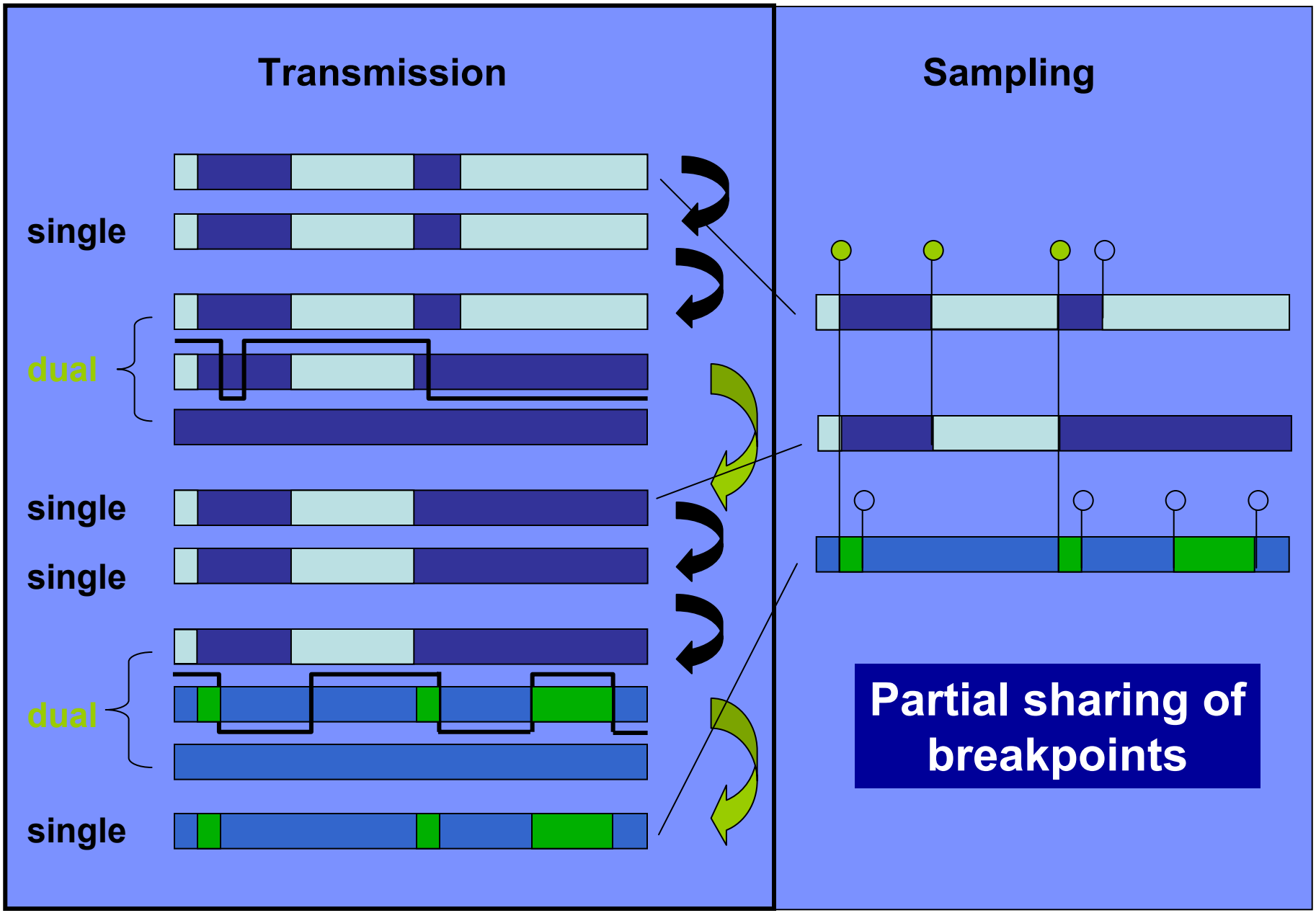
Mapping of shared breakpoints among recombinant strains could provide a new dimension to the molecular epidemiology of HIV-1

The structure and relationships of recombinant strains may provide information about the social networks in which they spread, providing new focus for interventions

Recombinant Strains in “Low Risk” Groups

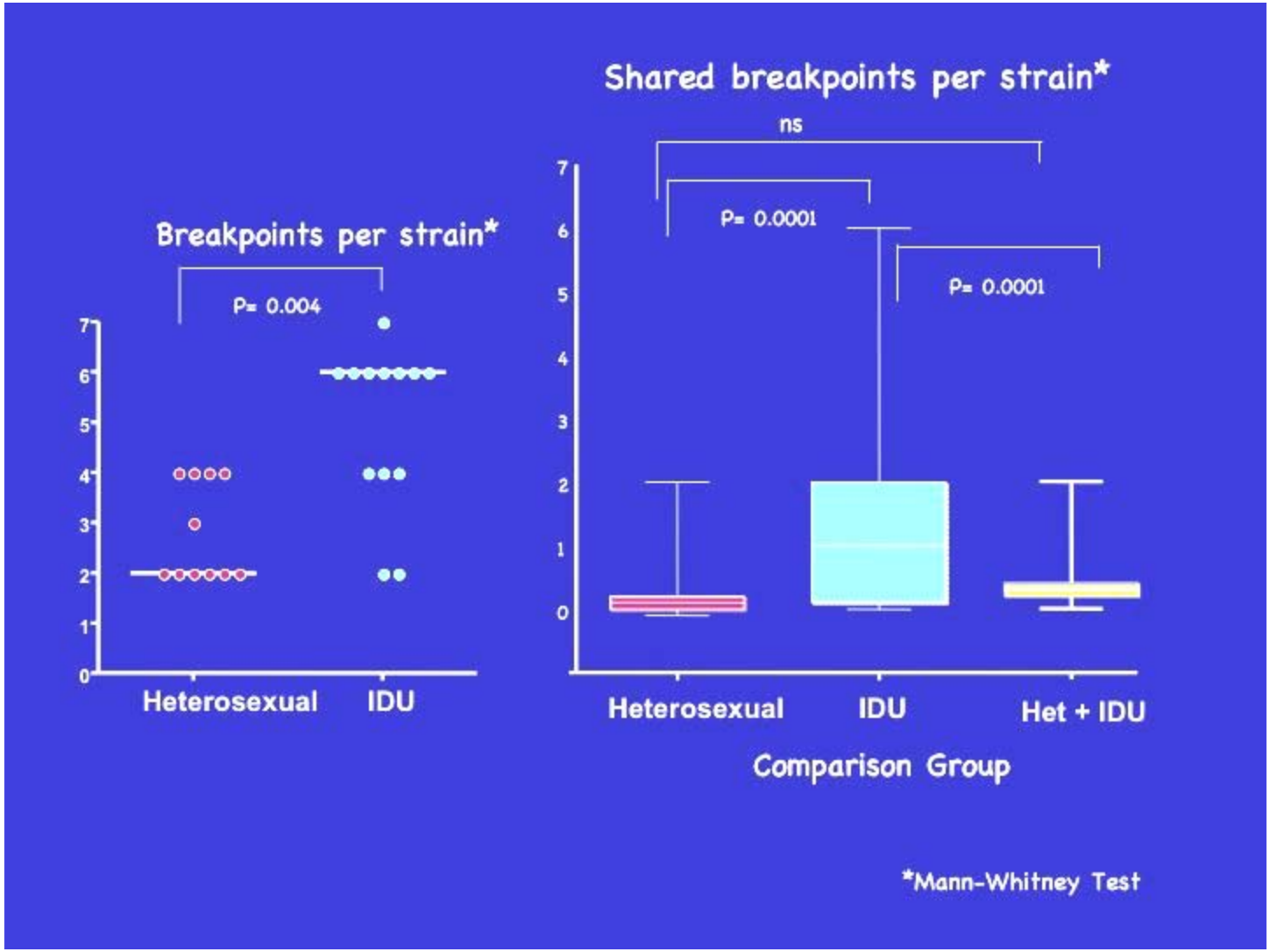


Recombinant Strains in High Risk Groups



Recombinant HIV Networks and Risk Groups in Asia

- 24 CRF01_AE/B recombinants from Thailand and Burma
- 11 from IDU
- 13 from heterosexual transmission


Breakpoints per strain*
P= 0.004
7
6
5
4
3
2
1
0
Heterosexual
IDU
Shared breakpoints per strain*
ns
P= 0.0001
P= 0.0001
7
6
5
4
3
2
1
0
Heterosexual
IDU
Het + IDU
Comparison Group
*Mann-Whitney Test

Network Visualization Software*

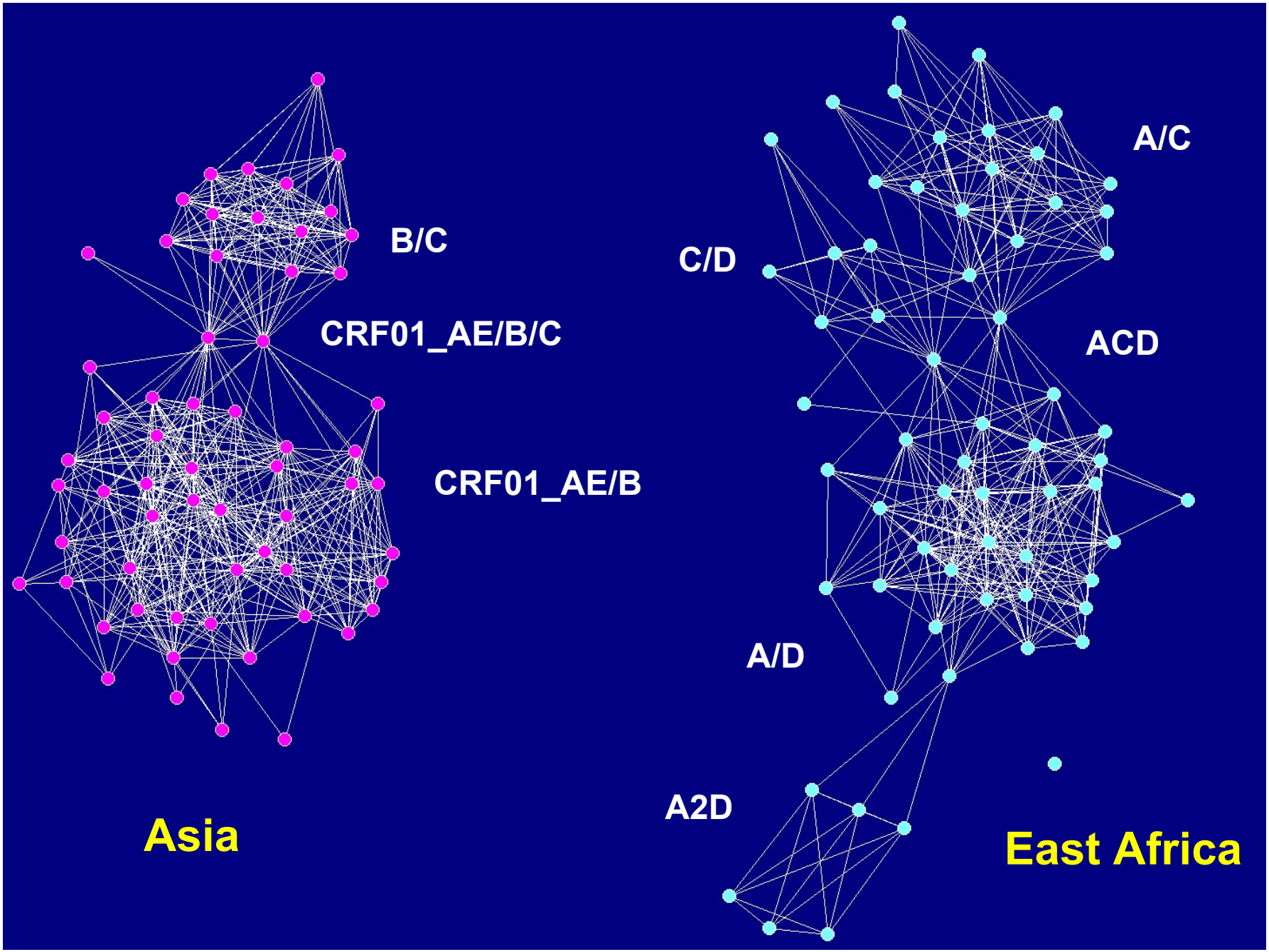
Each strain is a node

Each shared breakpoint is a connection, represented by a line

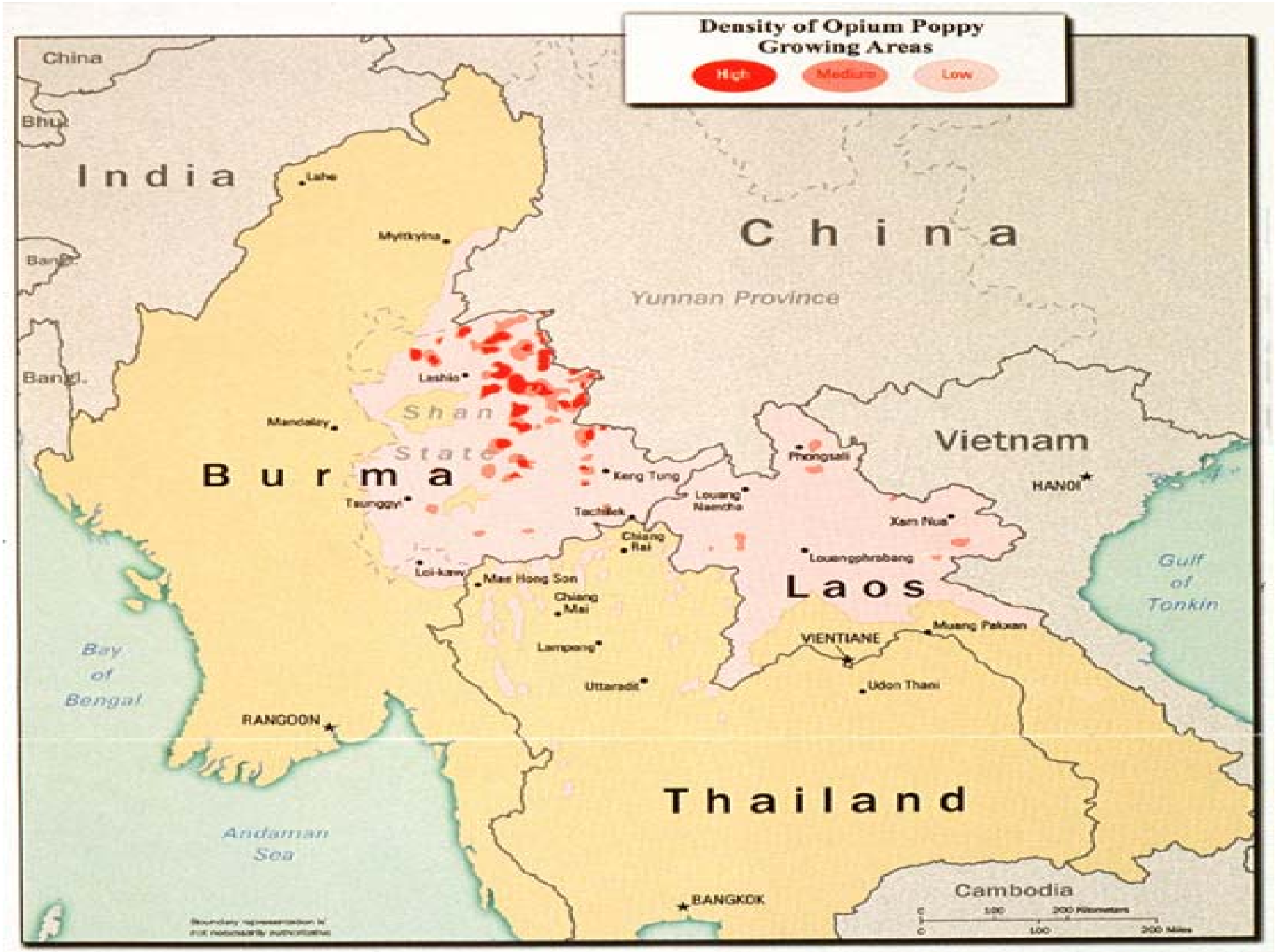
Highly interconnected strains form dense clusters, with less connected strains at the periphery

*UCiNET and NetDraw by S. Borgatti
Boston College/Analytic Technologies

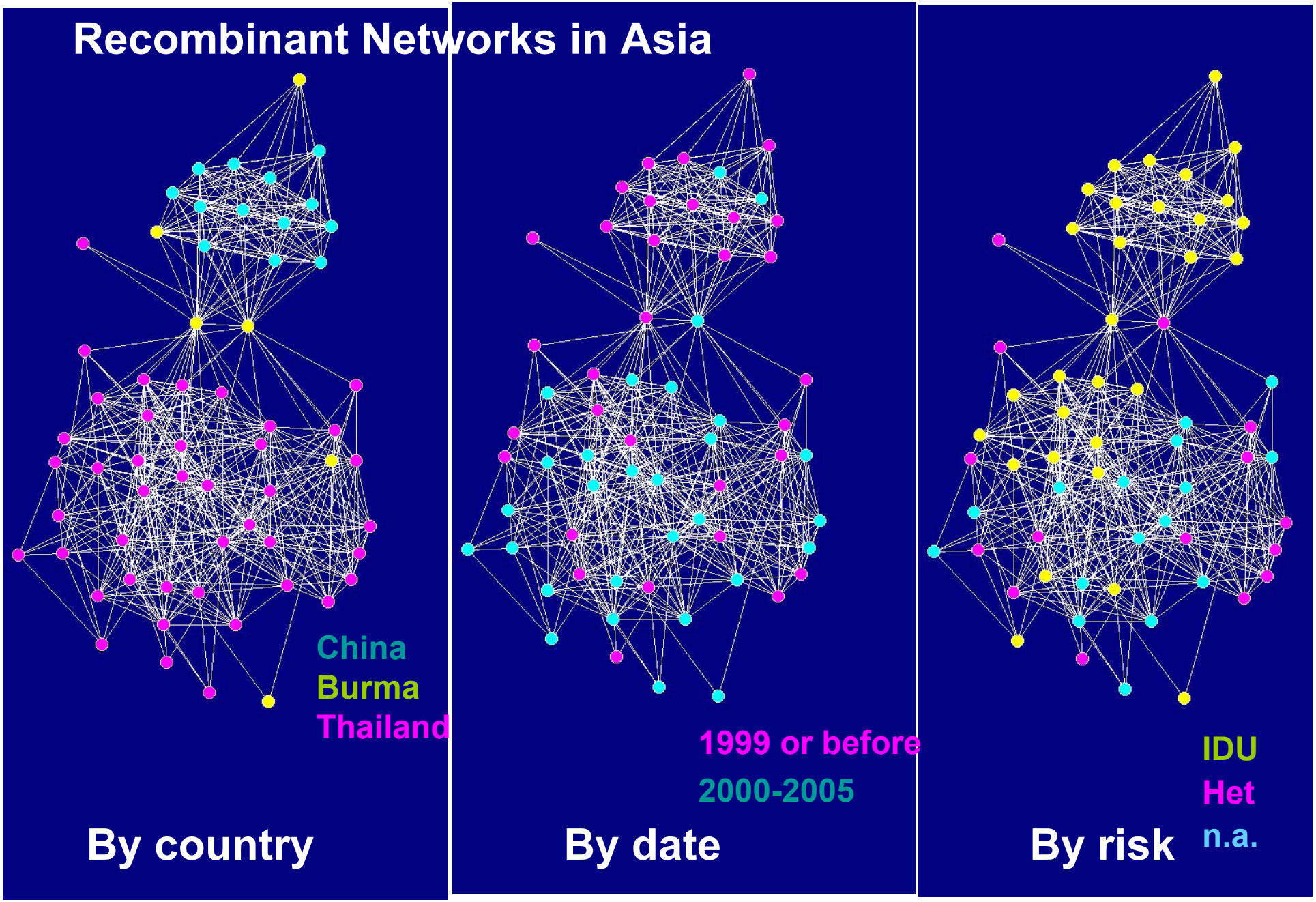
Networks of Shared Breakpoints Among Recombinant Strains



What can be learned about social networks from the relationships among recombinant strains circulating within them?


Density of Opium Poppy Growing Areas
High
Medium
Low
China
India
Myitkyina
Yunnan Province
Lashio
Shan State
Mandalay
Burma
Keng Tung
Tachilek
Loueng Namtha
Chiang Rai
Mae Hong Son
Chiang Mai
Lampang
Uttaradit
Phongsali
Vietnam
HANOI
Xam Nua
Louangphrabang
Laos
Gulf of Tonkin
Muang Pakxan
VIENTIANE
Udon Thani
Bay of Bengal
RANGOON
Thailand
Andaman Sea
BANGKOK
Cambodia

Recombinant Networks in Asia

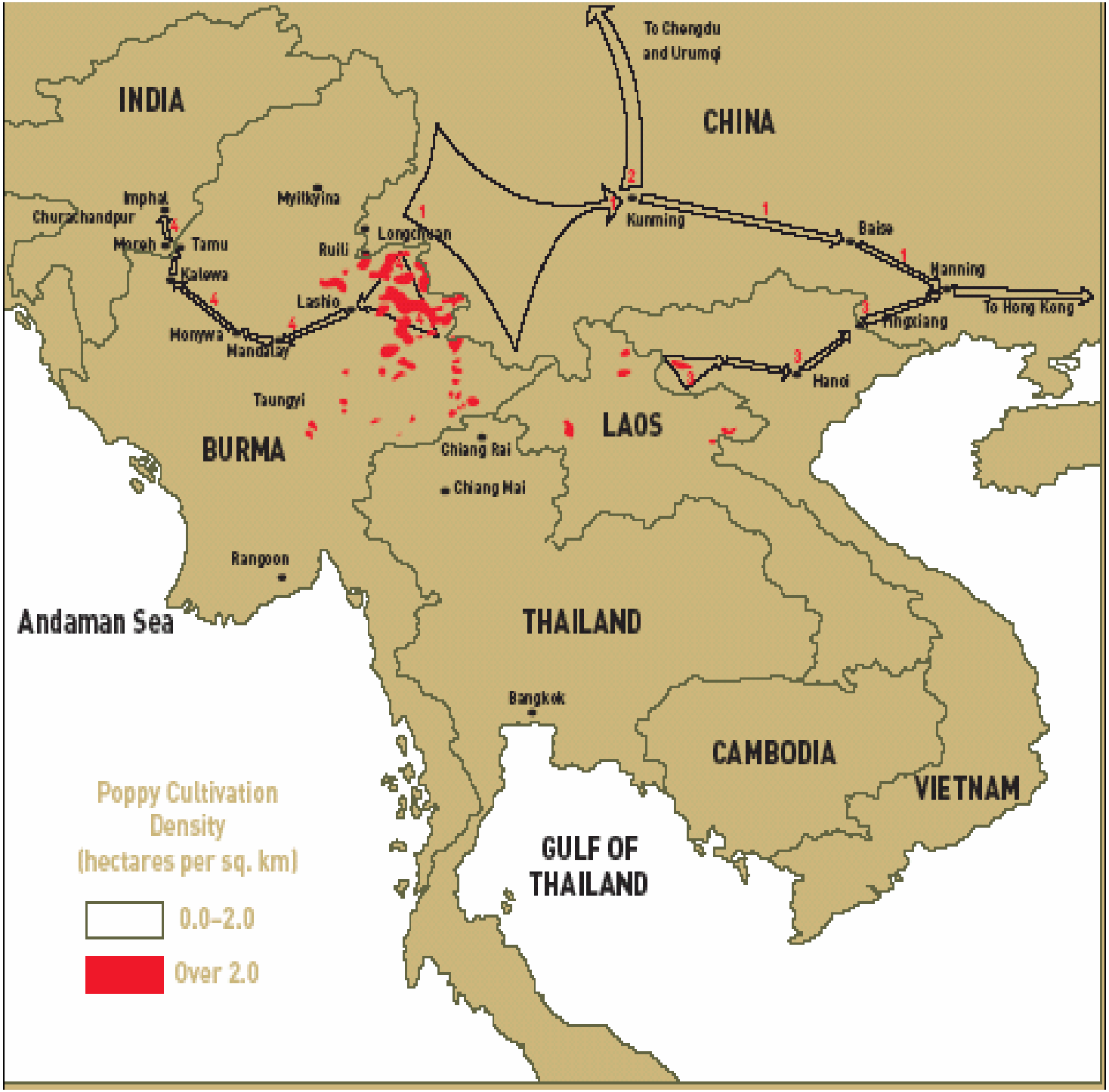


In Asia

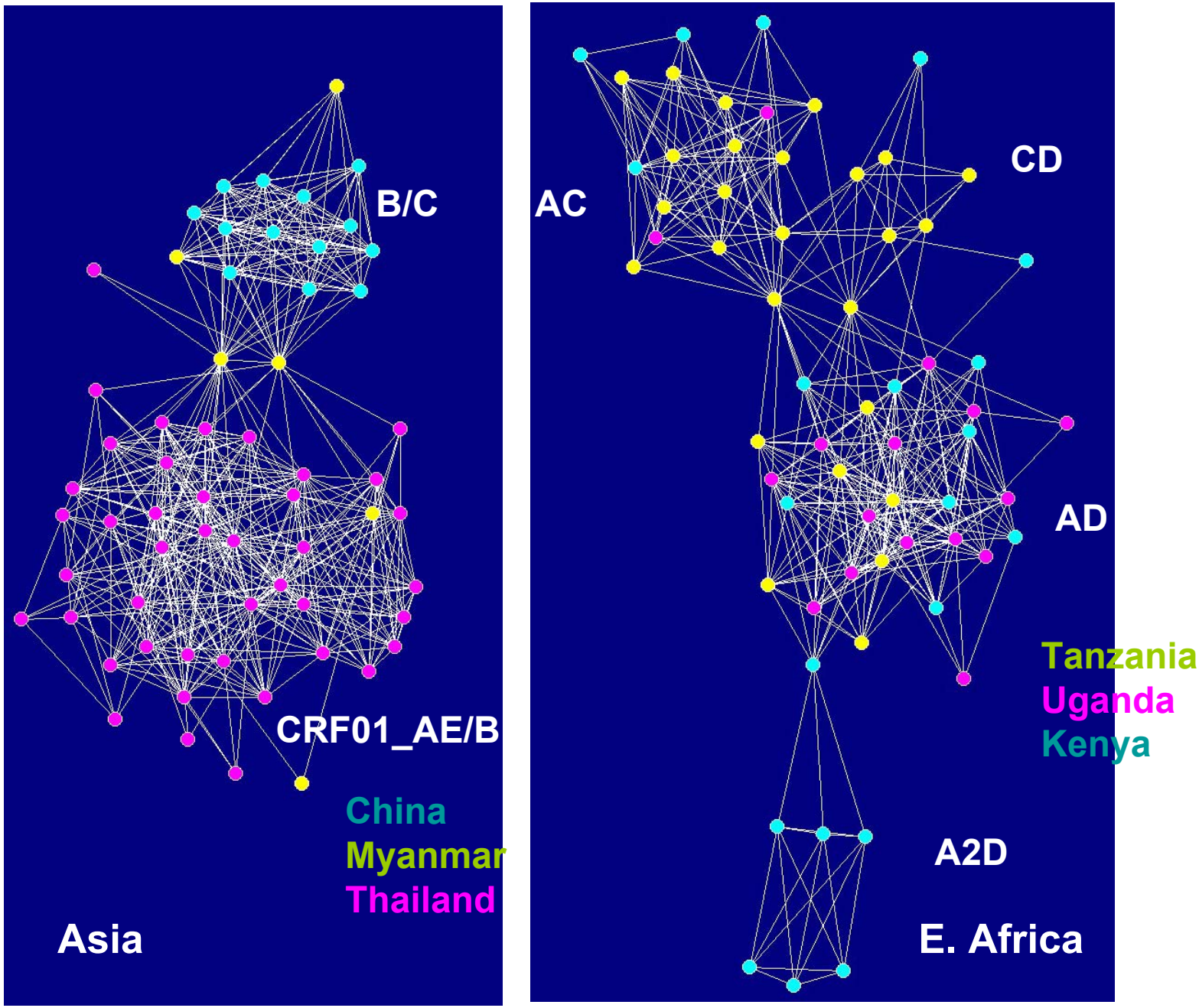
Heterosexual and IDU Networks in Thailand are strongly interconnected and these connections were already established during the first decade of the Thailand epidemic

Fewer connections across national borders

Strains from Burma/Myanmar bridge China and Thailand epidemics


INDIA
CHINA
BURMA
LAOS
THAILAND
CAMBODIA
VIETNAM
Andaman Sea
GULF OF THAILAND
To Chengdu and Urumqi
Kunming
Baise
Hanning
To Hong Kong
Pingxiang
Hanoi
Longchuan
Ruili
Lashio
Myitkyina
Imphal
Churachandpur
Moreh
Tamu
Kalewa
Monywa
Mandalay
Taungyi
Chiang Rai
Chiang Mai
Rangoon
Bangkok
Poppy Cultivation Density (hectares per sq. km)
0.0–2.0
Over 2.0

Connections Across National Borders



Contributors

•Participants in cohort development and other studies in Tanzania, Uganda, Kenya, Thailand, China, Myanmar

•Oliver Hoffmann, Steffan Geis, Leonard Maboko, Donan Mmbando, Eluter Samky, Michael Hoelscher, and other members of the Mbeya Medical Research Programme, Tanzania

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•Special thanks to Jocelyn Chiu and her mentors, Sodsai Tovanabutra, and Eric Sanders-Buell, for inspection and analysis of 1,125,000 nucleotides of sequence alignment

Implications for Prevention

Targeting prevention to highest risk groups may be the most important strategy to limit the genetic complexity of the epidemic, both in Africa and in Asia

Targeting prevention to the most mobile sectors of a given population may also contribute to limiting the overall complexity of strains in an epidemic

Effective size of the social network in which HIV-1 is spreading in E. Africa may be much larger than in Asia, with implications for dissemination of new strains

Heroin trafficking routes appear to predict HIV-1 subtype spread and should be priority zones for prevention

Discussion and Conclusions

Recombinant strains can represent highly informative tools to gain new understanding of the global epidemiology of HIV

Molecular data is more informative when closely linked to demographic data and becomes more useful when closely and systematically analyzed and when epidemiology and narcotics data are included

The structure of social networks, particularly the geographic and social mobility of the highest risk groups, can play key roles in the generation and spread of new HIV diversity generated by recombination