



Genetic architecture and evolution  
of emerging artemisinin resistance  
in *Plasmodium falciparum*

Roberto Amato

University of Oxford

**MalariaGEN**  
GENOMIC EPIDEMIOLOGY NETWORK

# Thanks and credits to....



**Olivo Miotto**  
**Jacob Almagro-Garcia**  
Chris Spencer  
Gil McVean  
**Dominic Kwiatkowski**

**All Kwiat Group!**



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Magnus Manske  
Jim Stalker  
Daniel Mead  
Sam Oyola  
Eleanor Drury  
Susana Campino

**All Team 112!**

**All Community Project Investigators**

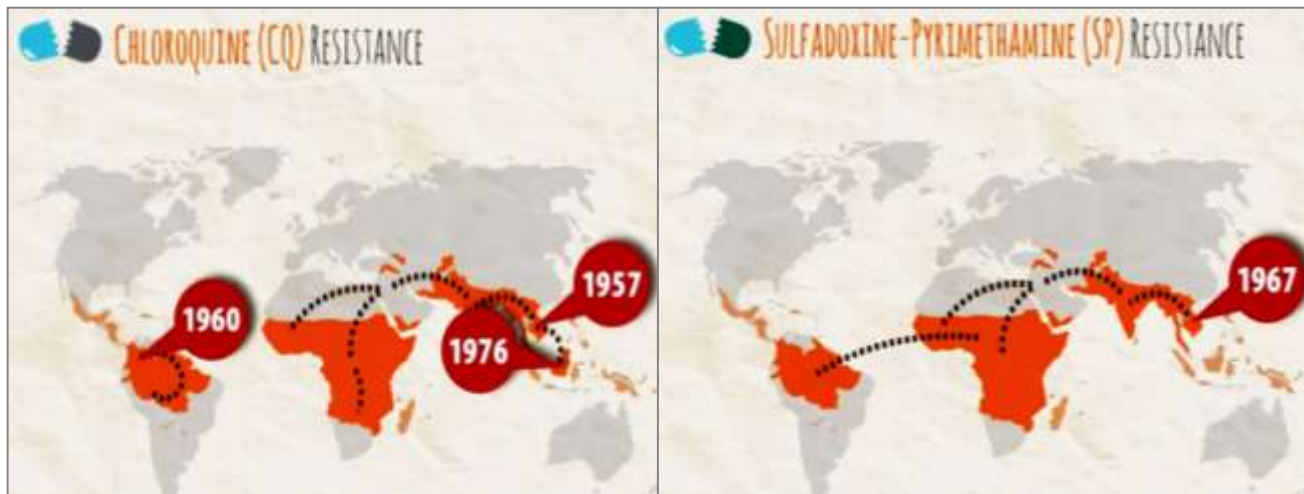
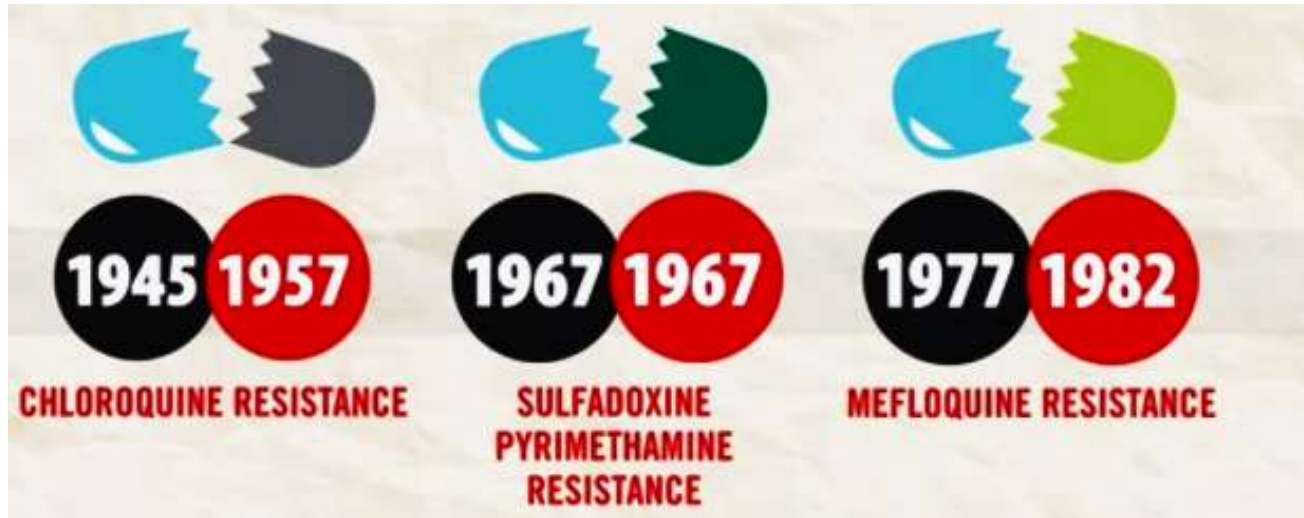


Nick Day  
Nick White  
Arjen Dondorp  
Charlie Woodrow  
Liz Ashley



In the previous episodes...

# Drug Resistance History



Visit the [MalariaGEN](http://MalariaGEN.org) website for the complete animation

# And today...artemisinin

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

## Artemisinin Resistance in *Plasmodium falciparum* Malaria

Arjen M. Dondorp, M.D., François Nosten, M.D., Poravuth Yi, M.D.,  
Debashish Das, M.D., Aung Phae Phy, M.D., Joel Tarning, Ph.D.,

Khin Maung Lwin,  
Sue J. Lee, I  
Mallika Imwong,  
Trent H. Church,  
Pratap Singhasingh,  
Duc

### BACKGROUND

Artemisinin-based  
of falciparum mala  
cerns that the effi  
border, historically

### METHODS

## Emergence of artemisinin-resistant malaria on the western border of Thailand: a longitudinal study

Aung Pyae Phy, Standwell Nkhoma, Kasia Stepniewska, Elizabeth A Ashley, Shalini Nair, Rose McGready, Carit ler Moo, Salma Al-Saai, Arjen M Dondorp, Khin Maung Lwin, Pratap Singhasivanon, Nicholas P J Day, Nicholas J White, Tim J C Anderson, François Nosten



# What about artemisinin resistance?

- “Delayed parasite clearance observed after treatment with an artesunate monotherapy, or after treatment with an artemisinin-based combination therapy (ACT)” [WHO]
- Declining efficiency observed in Southeast Asia
  - From 2.5h to >5h
  - Complete treatment failure observed in western Cambodia due to resistance to partner drug
- Urgent priority for global health
  - Hard to measure clinical phenotype
  - Genetic marker would enable large-scale surveillance
  - Hopefully marker leads to causal mutations



# The *kelch13* gene

- A molecular marker of artemisinin resistance has been identified in vitro [Ariey et al. Dec 2013].
- Different mutations in the *kelch13* propeller domain were shown to be associated with delayed parasite clearance.

ARTICLE

nature

doi:10.1038/nature12876

## A molecular marker of artemisinin-resistant *Plasmodium falciparum* malaria

Frédéric Ariey<sup>1,2†</sup>, Benoit Witkowski<sup>3</sup>, Chanaki Amaratunga<sup>4</sup>, Johann Beghain<sup>1,2†</sup>, Anne-Claire Langlois<sup>1,2</sup>, Nimol Khim<sup>3</sup>, Saorin Kim<sup>3</sup>, Valentine Duru<sup>3</sup>, Christiane Bouchier<sup>5</sup>, Laurence Ma<sup>5</sup>, Pharath Lim<sup>3,4,6</sup>, Rithea Leang<sup>6</sup>, Socheat Duong<sup>6</sup>, Sokunthea Sreng<sup>6</sup>, Seila Suon<sup>6</sup>, Char Meng Chuor<sup>6</sup>, Denis Mey Bout<sup>7</sup>, Sandie Ménard<sup>8†</sup>, William O. Rogers<sup>9</sup>, Blaise Genton<sup>10</sup>, Thierry Fandeur<sup>1,3</sup>, Olivo Miotto<sup>11,12,13</sup>, Pascal Ringwald<sup>14</sup>, Jacques Le Bras<sup>15</sup>, Antoine Berry<sup>8†</sup>, Jean-Christophe Barale<sup>1,2†</sup>, Rick M. Fairhurst<sup>4\*</sup>, Françoise Benoit-Vical<sup>16,17\*</sup>, Odile Mercereau-Puijalon<sup>1,2\*</sup> & Didier Ménard<sup>3\*</sup>

# Open questions

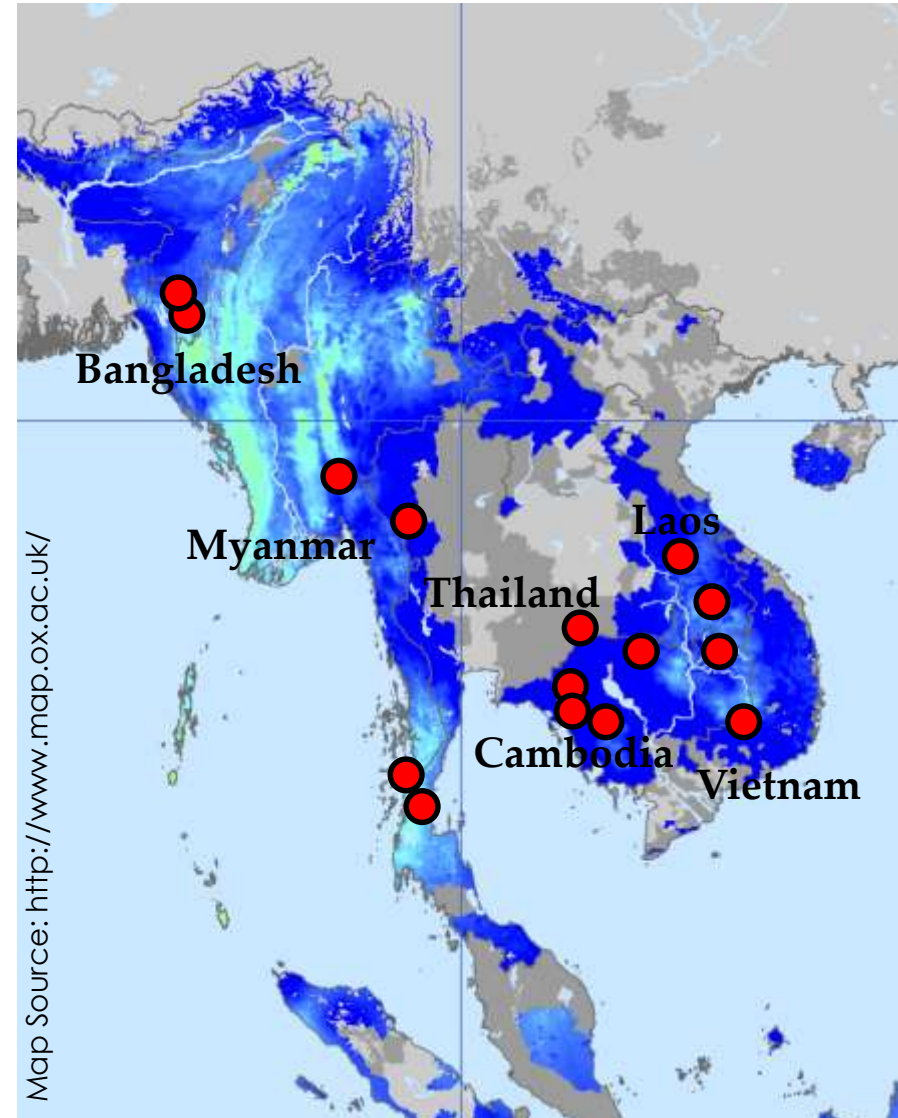
- How many genes are involved?
- Are all parasites equally likely to acquire a resistance-causing mutation?
- What is the geographical distribution of the mutations that cause resistance and of the genetic predisposing factors?
- Is it spreading due to migration of resistant parasites, or does it have multiple origins in different locations?



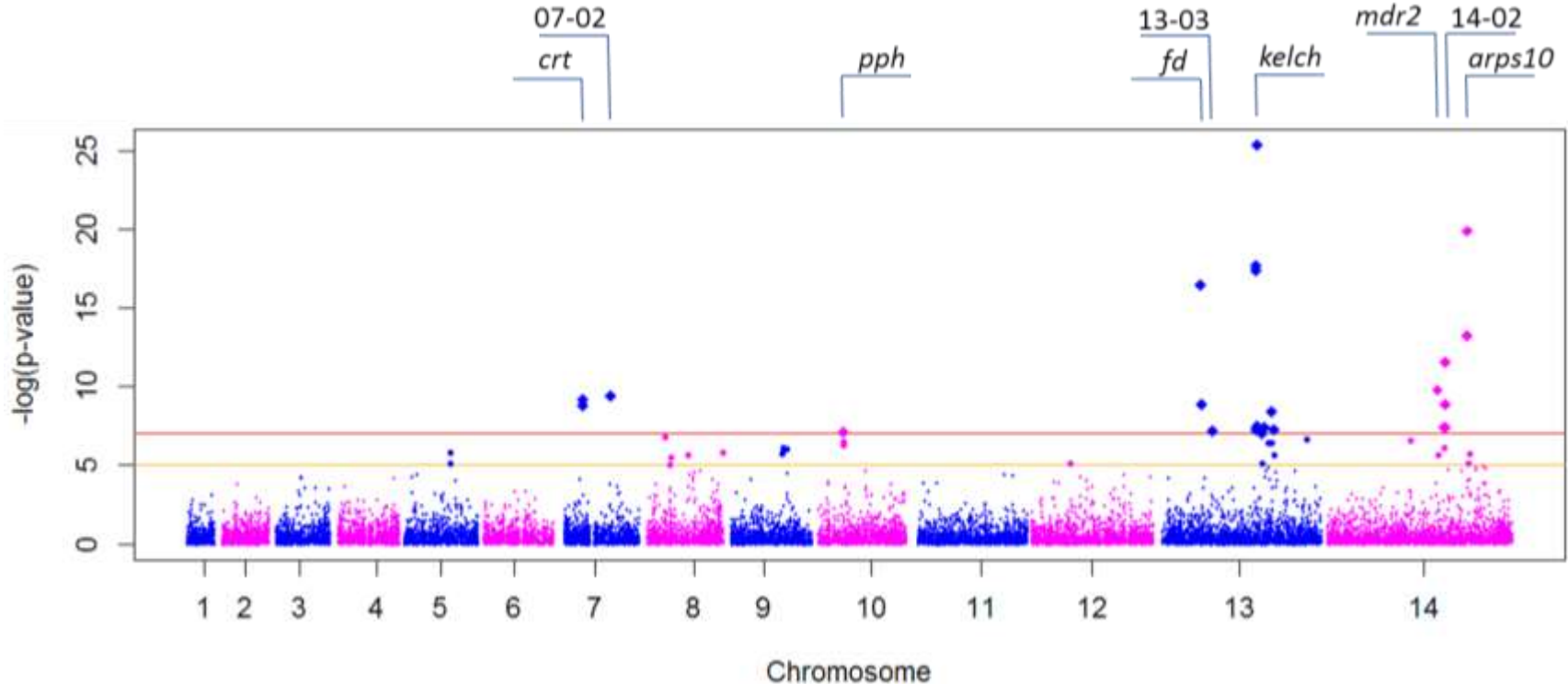
# TRAC/NIH GWAS

- **1,612 clinical samples**
  - Full genome sequence
  - 1,063 with phenotypes
- **15 locations** (+2 in Africa)
  - Cambodia, Vietnam, Laos, Thailand, Myanmar and Bangladesh

**High genetic and geographical resolution**



# GWAS result

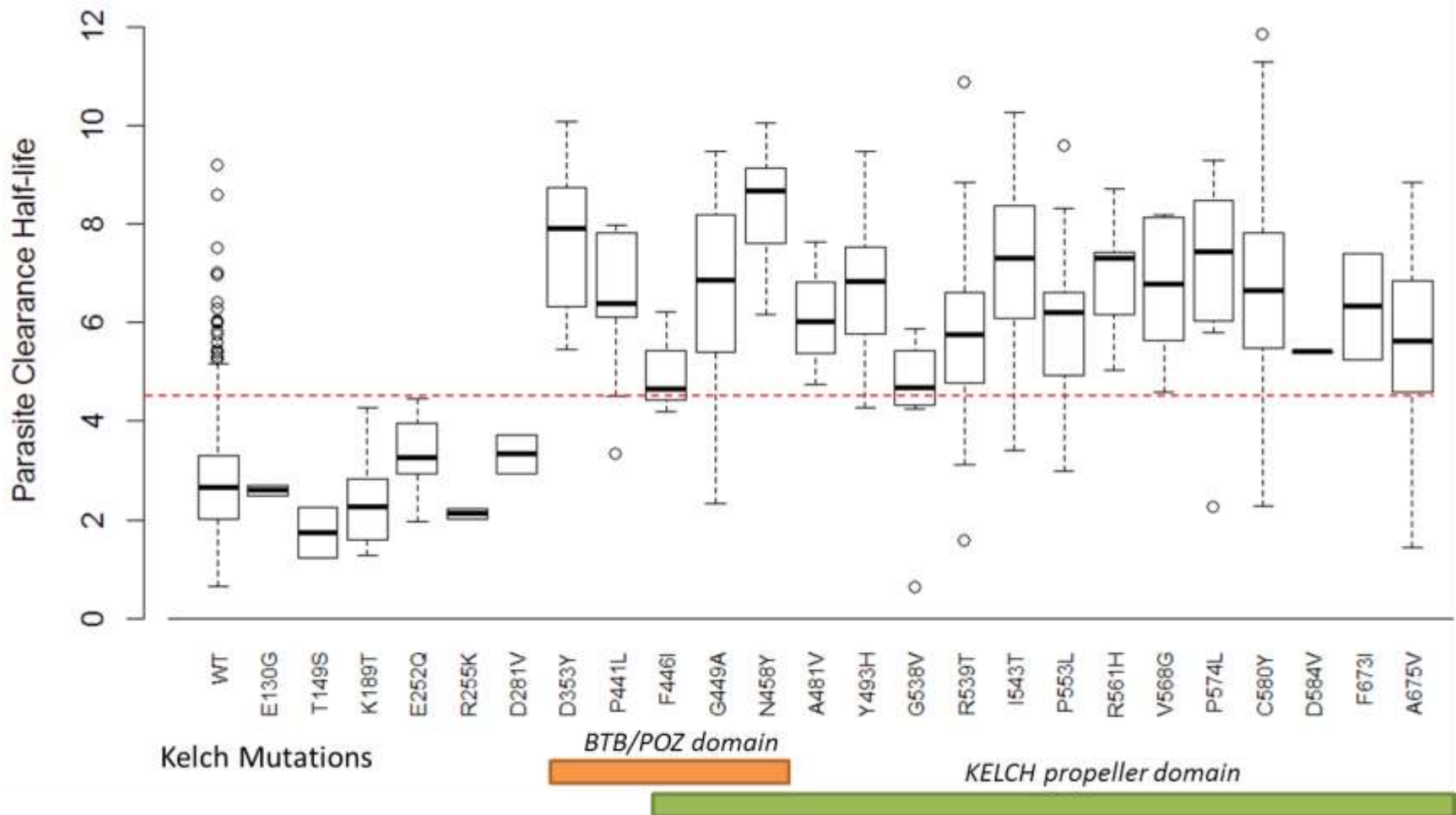


- 18K SNPs with MAF>0.01
- Resistance phenotype expressed as parasite clearance half-life
  - number of hours taken for artemisinin to reduce parasite density by half during the steady-state clearance phase of the treatment
- Linear mixed model (Fast-LMM) to account for population structure

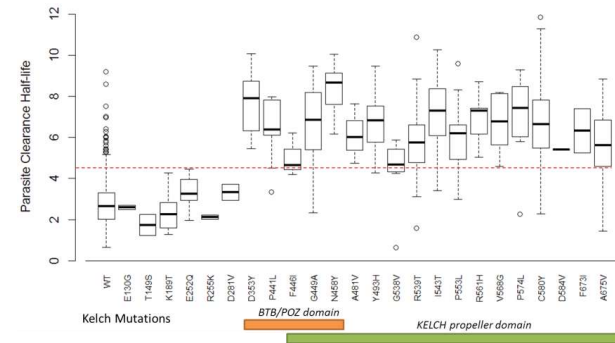
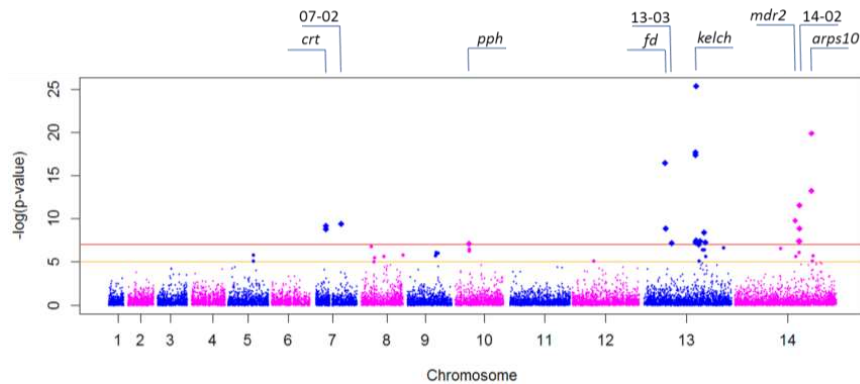
***kelch13* C580Y  $p=10^{-26}$**

**At least 7 distinct loci with  $p<10^{-7}$**

# Extreme allelic heterogeneity



# Observations

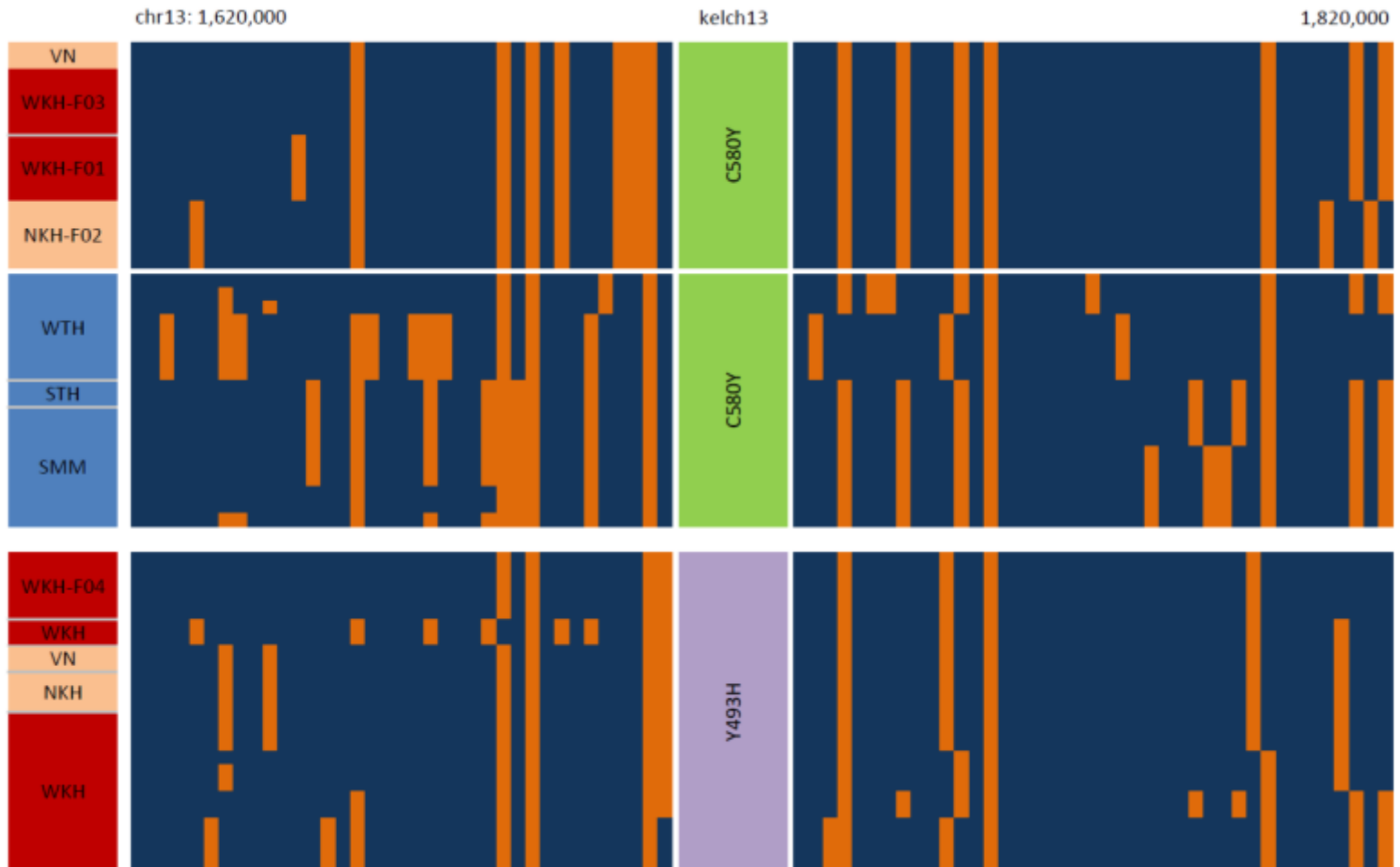


- ❑ C580Y mutations in *kelch13* has a p-value of 1E-26
  - ❑ Why the other mutations are not there or why is this mutations there?
- ❑ Other loci have significant p-values
- ❑ At least 20 non-synonymous mutations in the propeller domain of *kelch13* have a phenotypic effect
  - ❑ How does this compare to the rest of the world?
  - ❑ How does this compare to the rest of the genome?
  - ❑ Are the mutations in *kelch13* all born equal?

# Emergence vs spreading

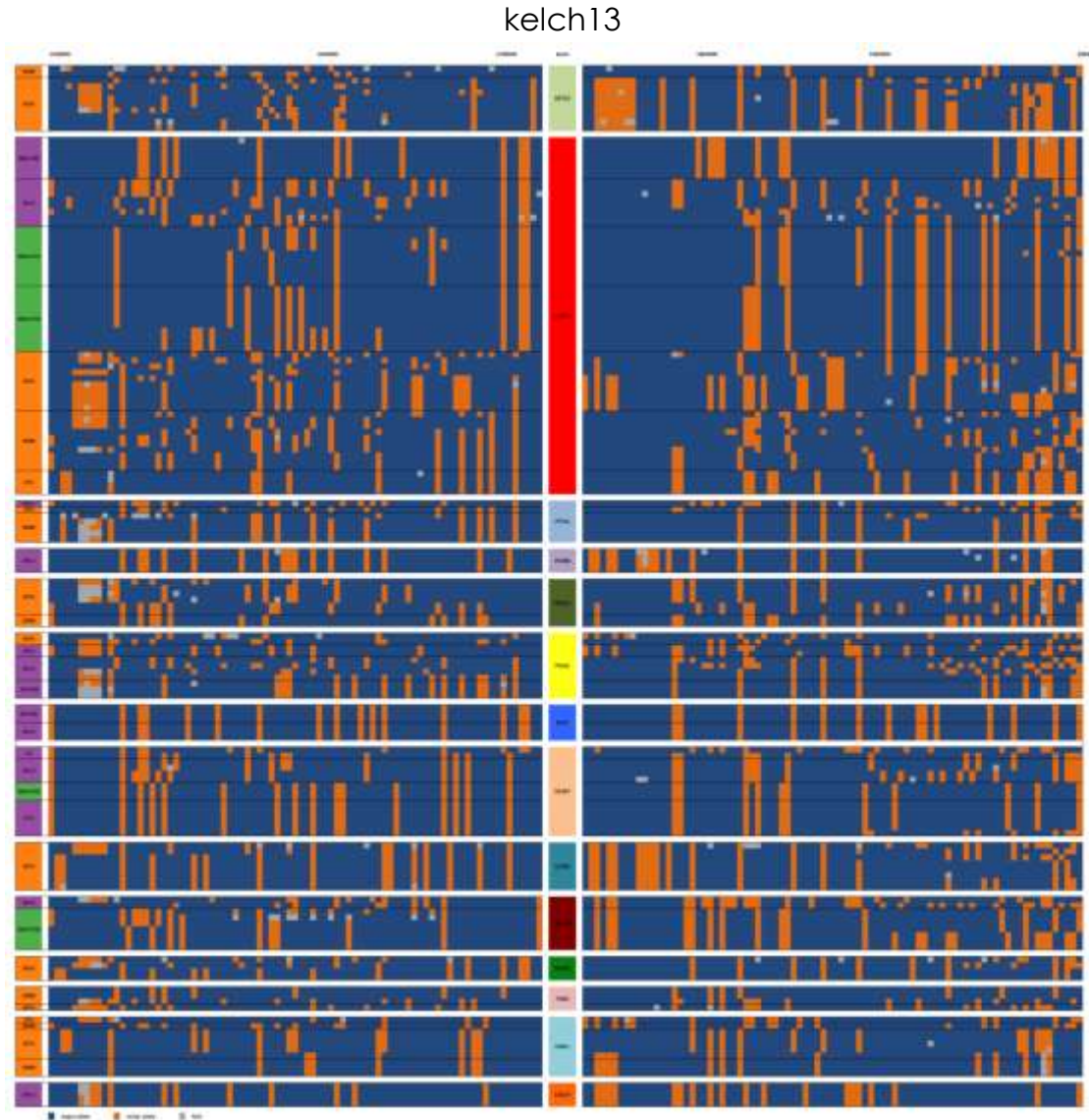
# Resistance is emerging

- WSEA
- ESEA
- WKH

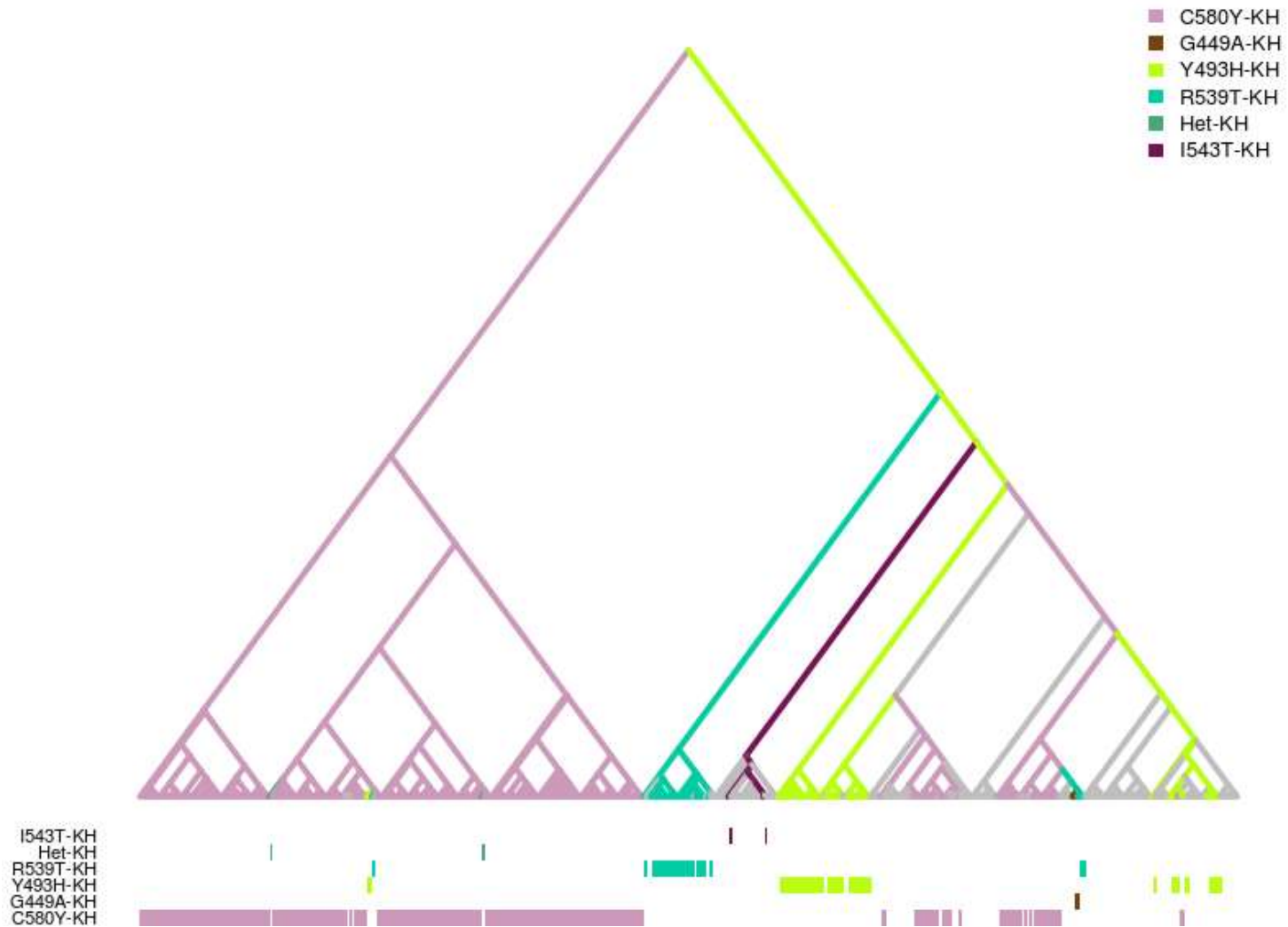


# Resistance is emerging

- WSEA
- ESEA
- WKH



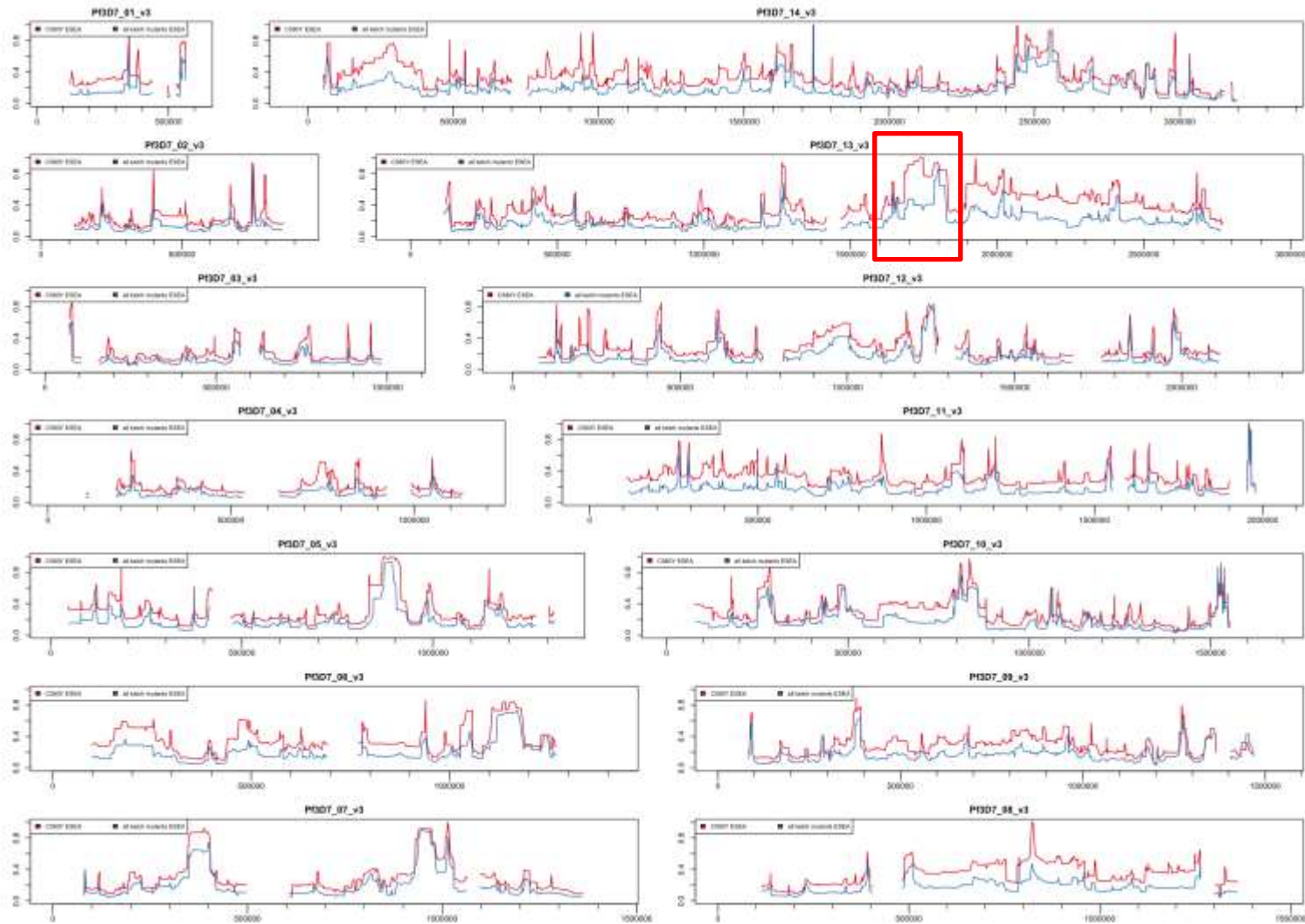
# Compelling evidences of different origins



J. Almagro-Garcia

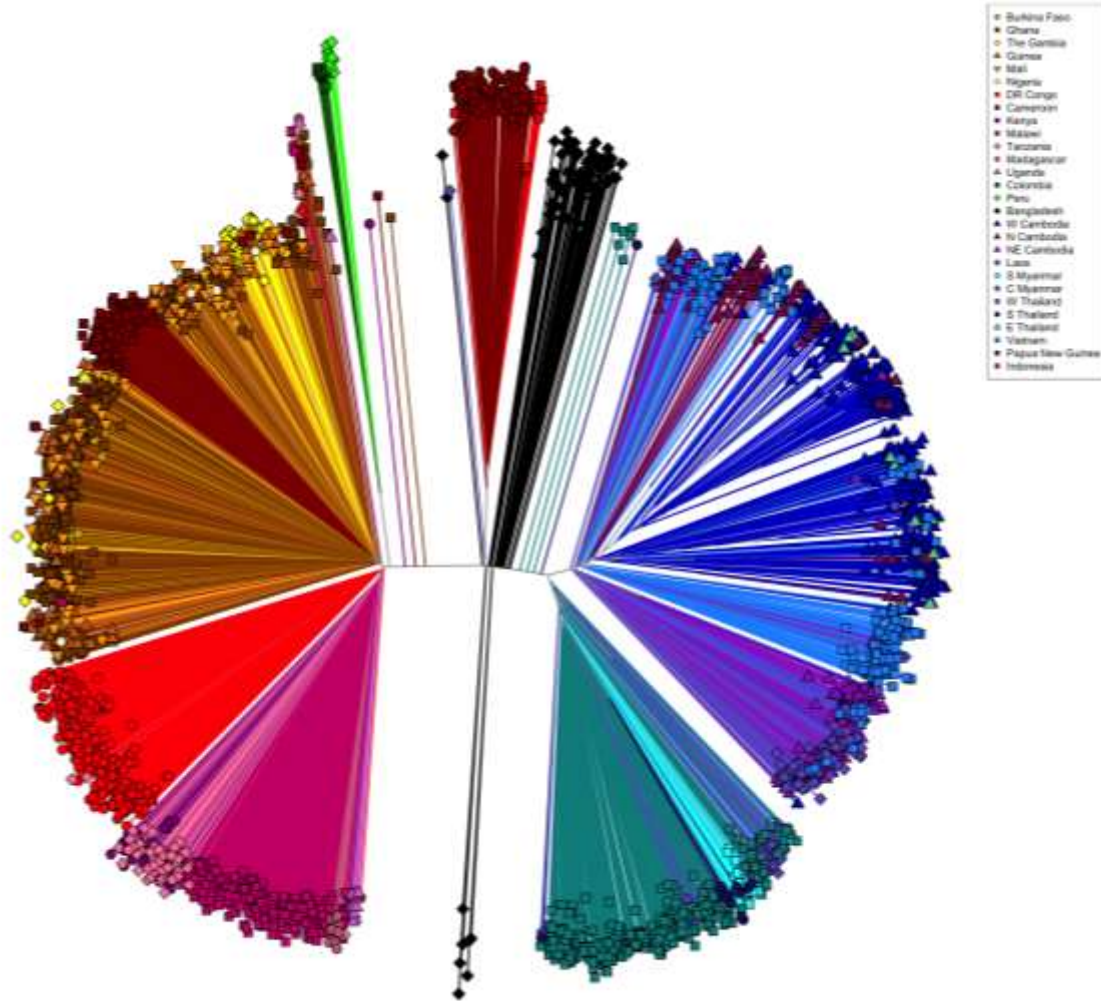


# Haplotype homozygosity



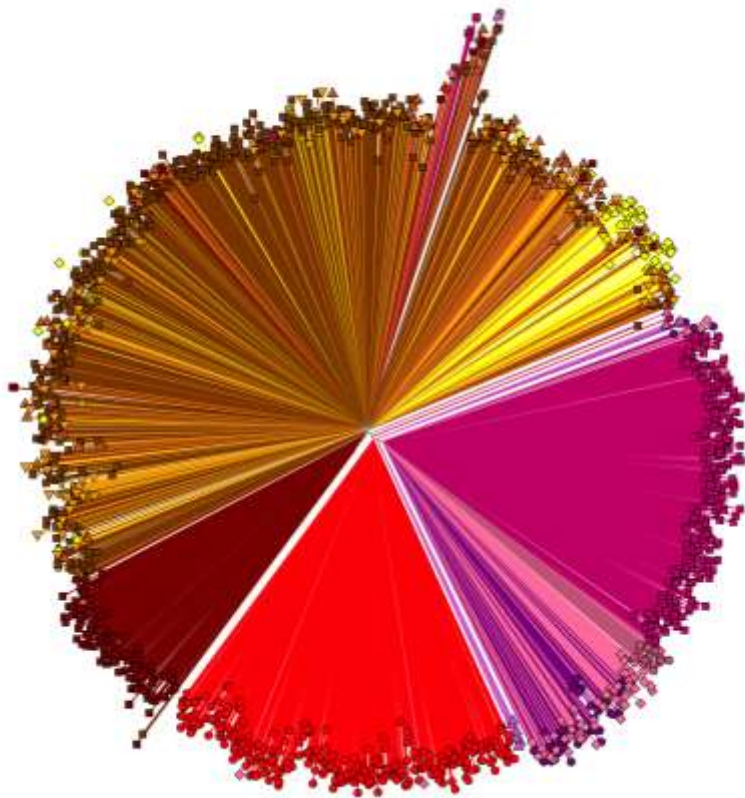
Predisposing genetic  
background

# 3,500 samples



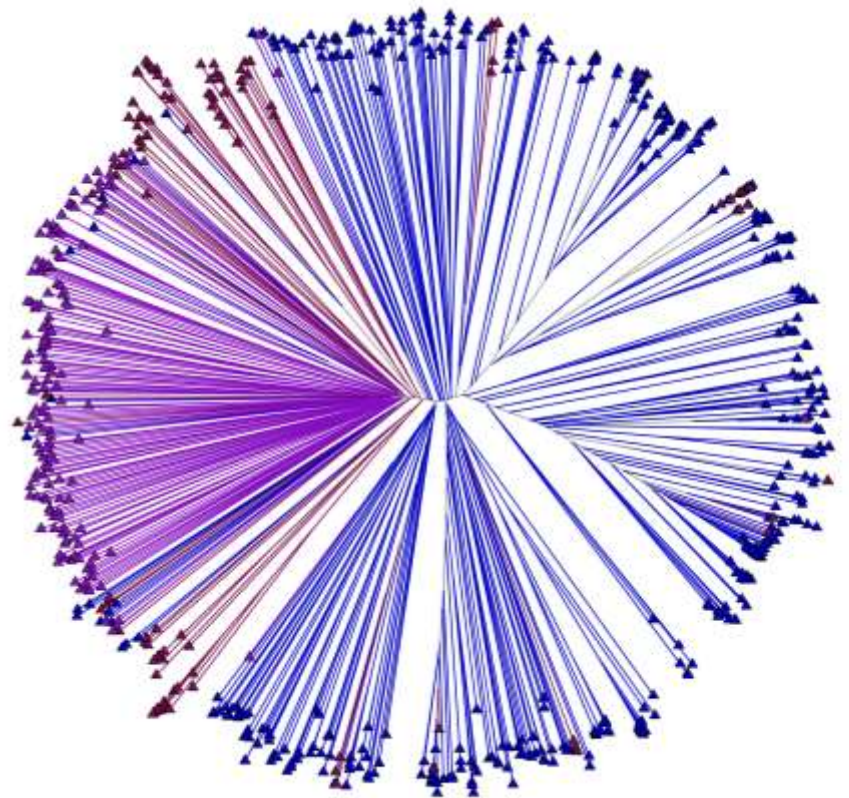
# Different topologies

Africa



- Burkina Faso
- Ghana
- The Gambia
- Guinea
- Mali
- Nigeria
- ZM College
- Cameroon
- Ivory
- Malawi
- Tanzania
- Madagascar
- Uganda

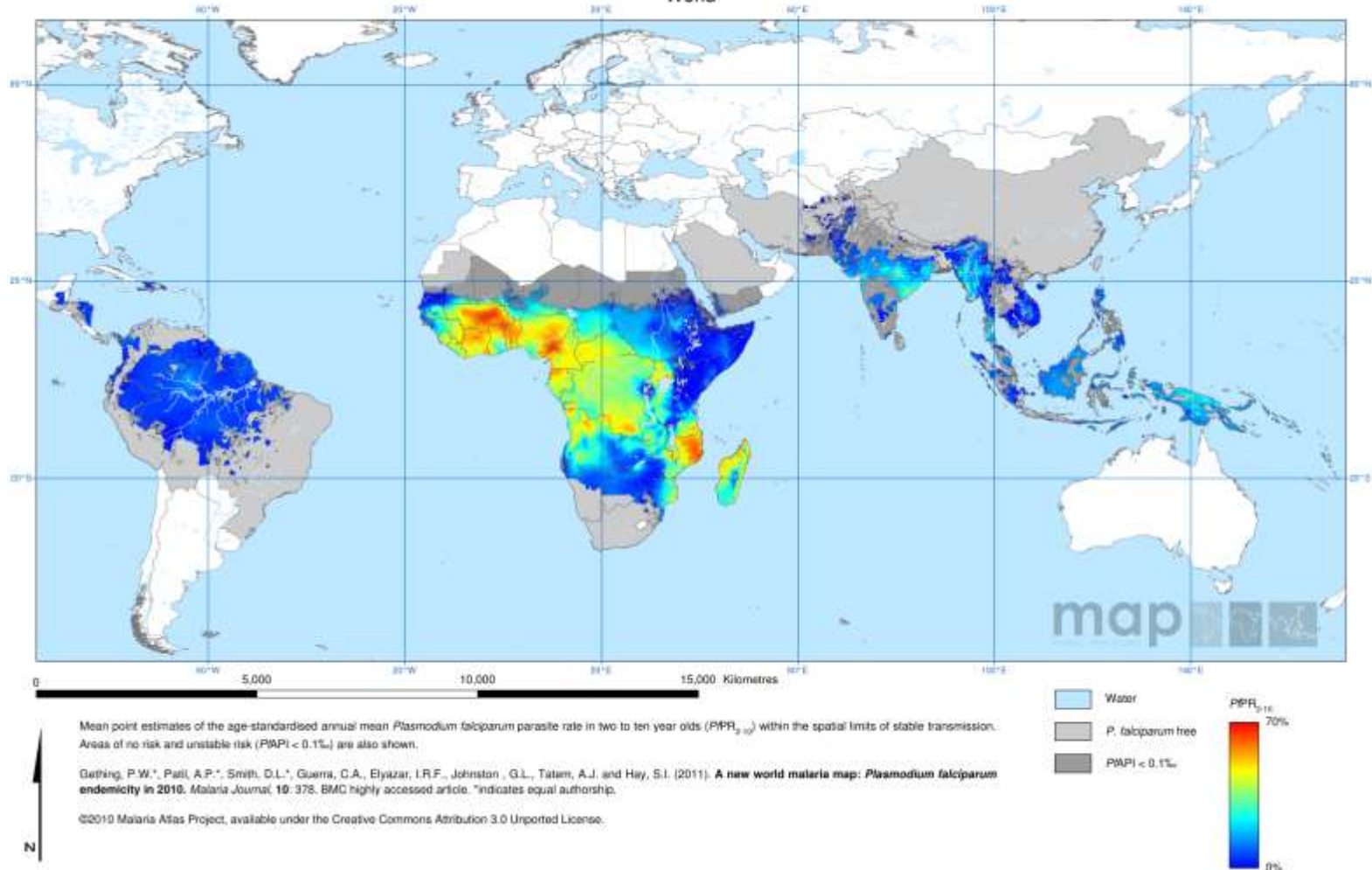
Cambodia



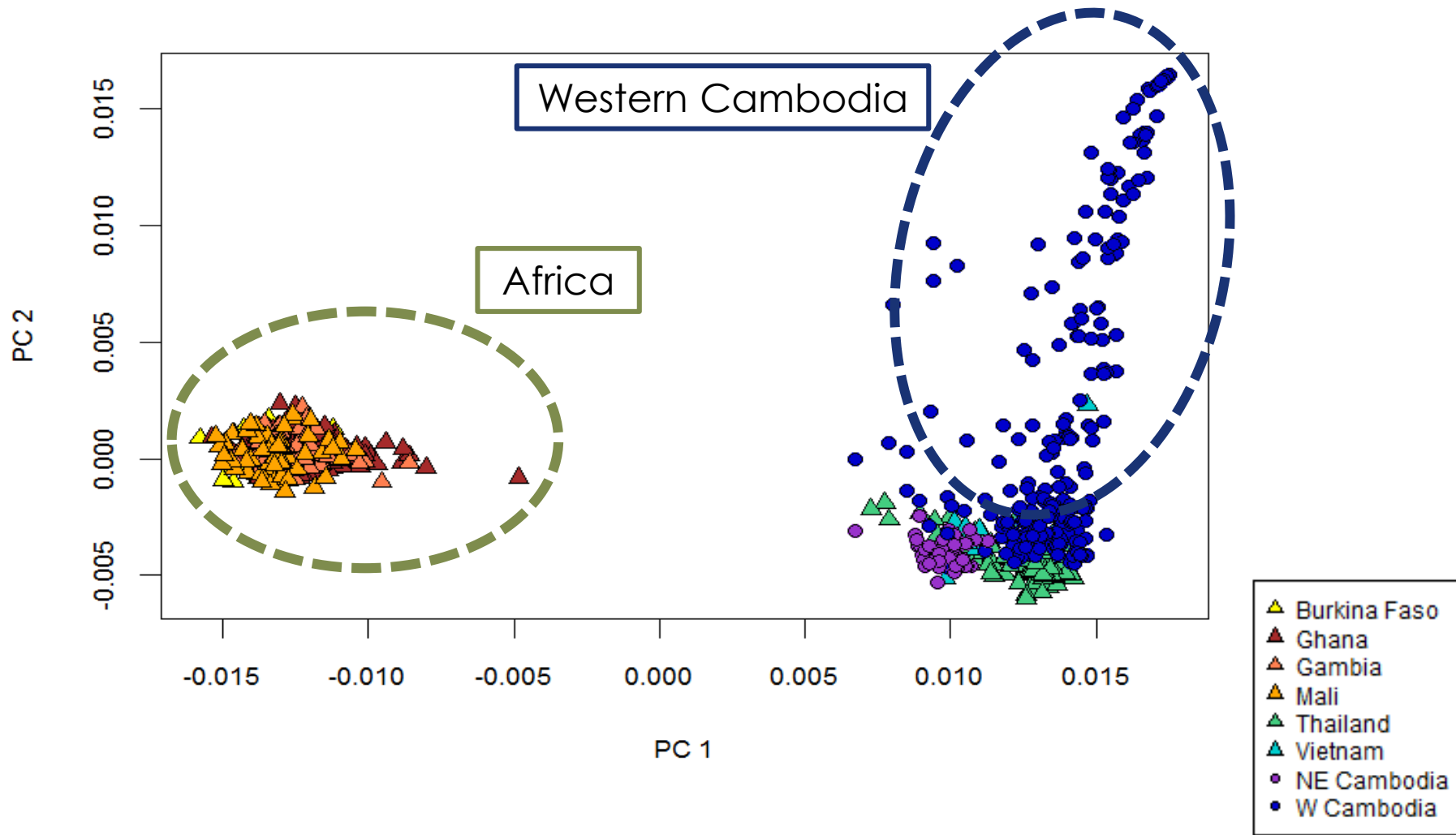
- W Cambodia
- N Cambodia
- No Cambodia

# (E)SEA has low endemicity

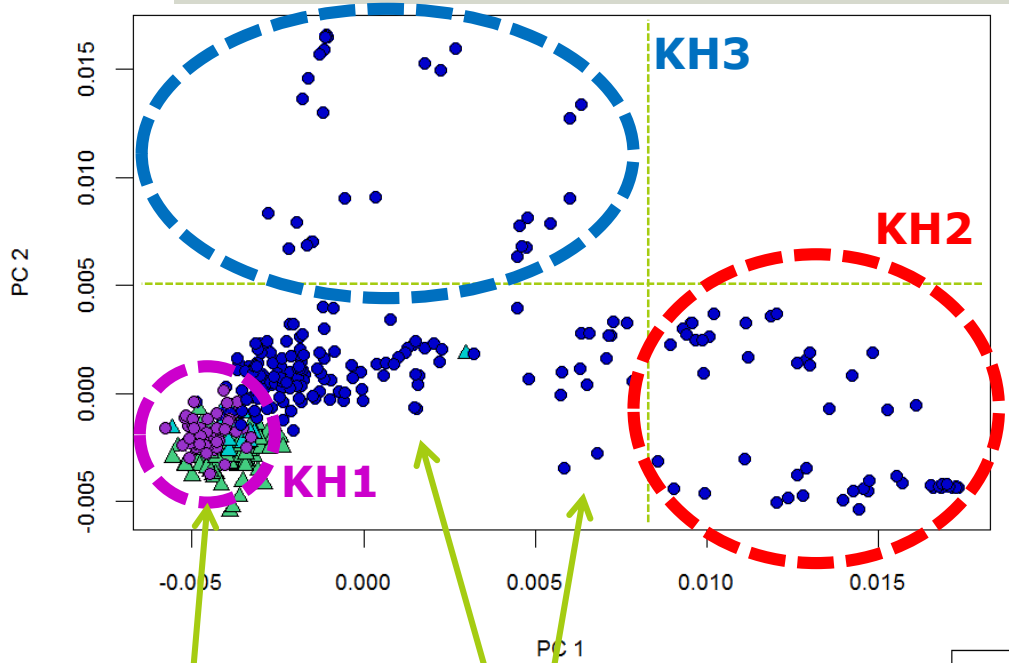
The spatial distribution of *Plasmodium falciparum* malaria endemicity in 2010  
World



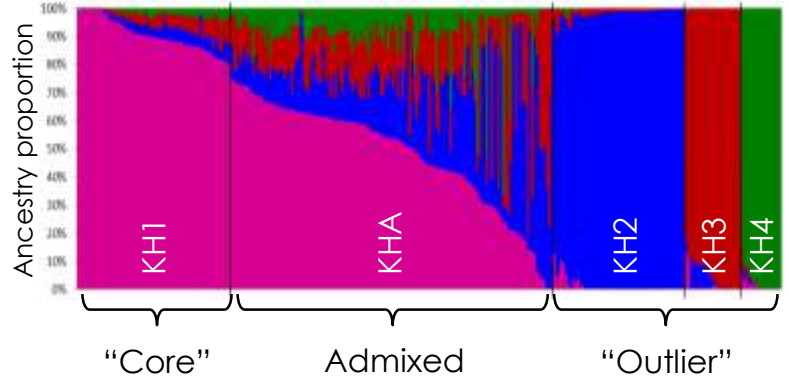
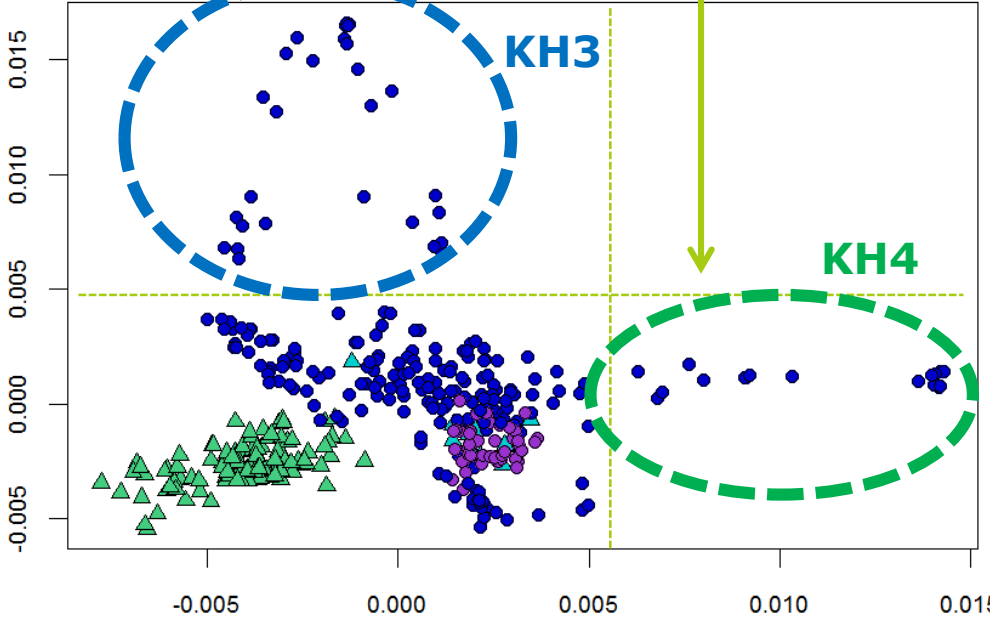
# Population structure: Principal Component Analysis



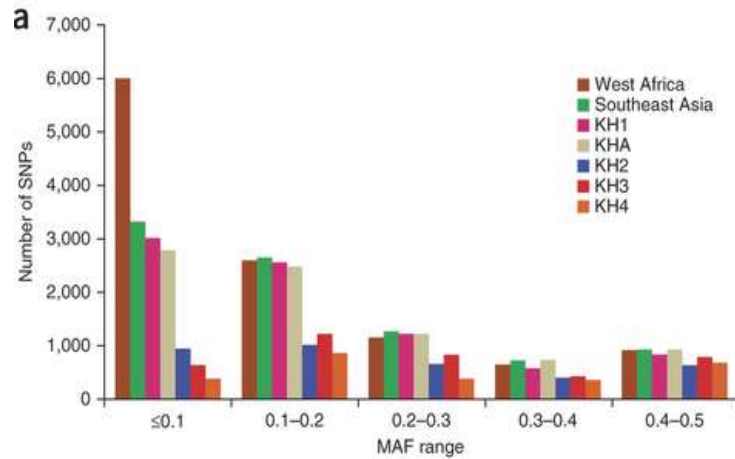
# PCA in SE Asia



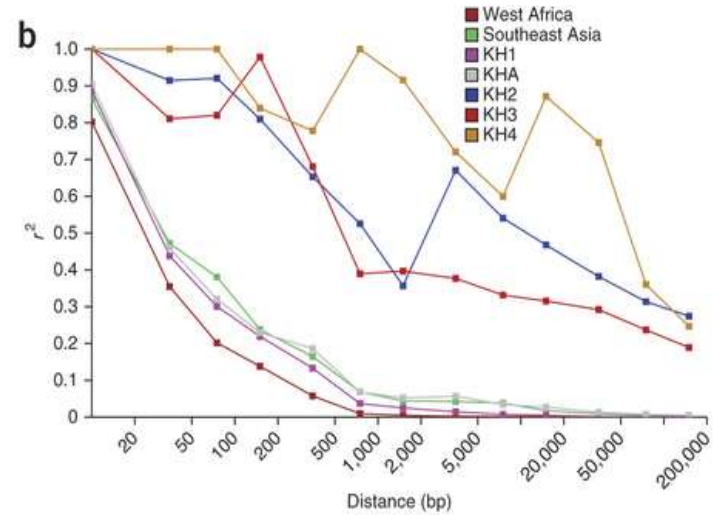
“outlier” clusters



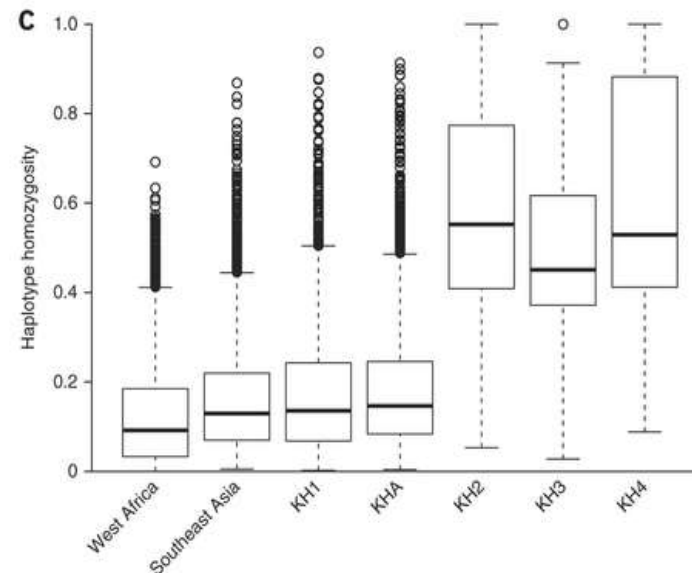
# Evidence for multiple founder effects



Even MAF spectrum



Long haplotypes and low haplotype diversity

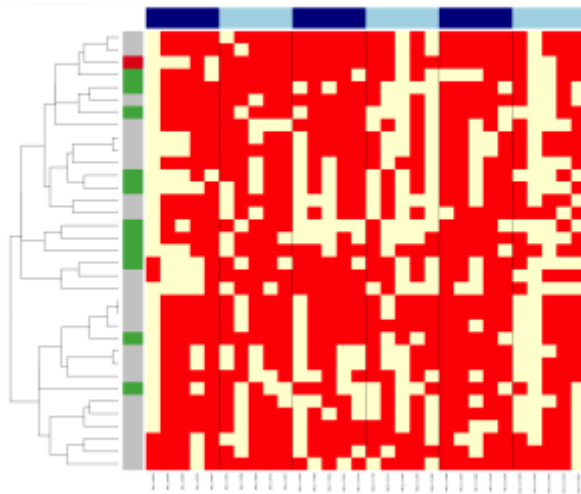




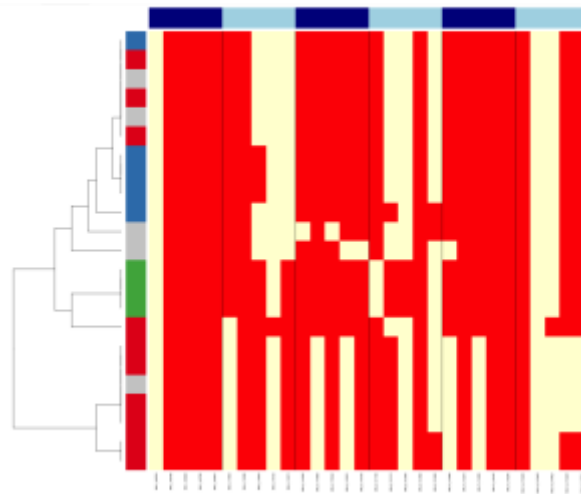
# Surface-associated interspersed genes

Region	Samples	Haplotypes (Hs)	Unique Hs	Hs shared by < 5	Hs shared by $\geq 5$
WAF	247	234	224	10	0
EMF	65	62	59	3	0
SAM	26	22	20	2	0
SAS	13	13	13	0	0
WSEA	129	113	102	11	0
ESEA	443	270	213	46	11
PNG	34	31	28	3	0

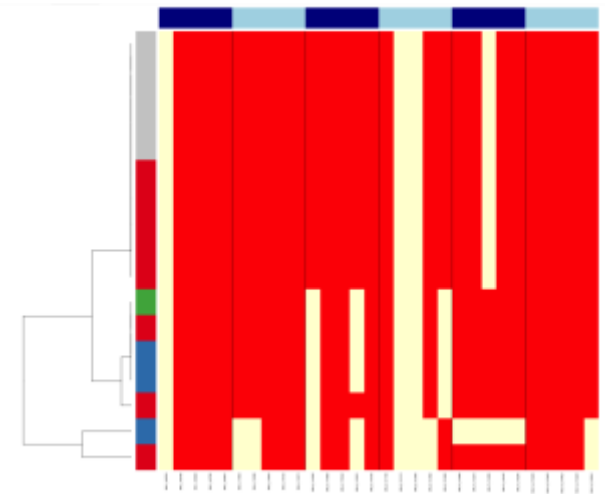
Wild-type



Founder 1

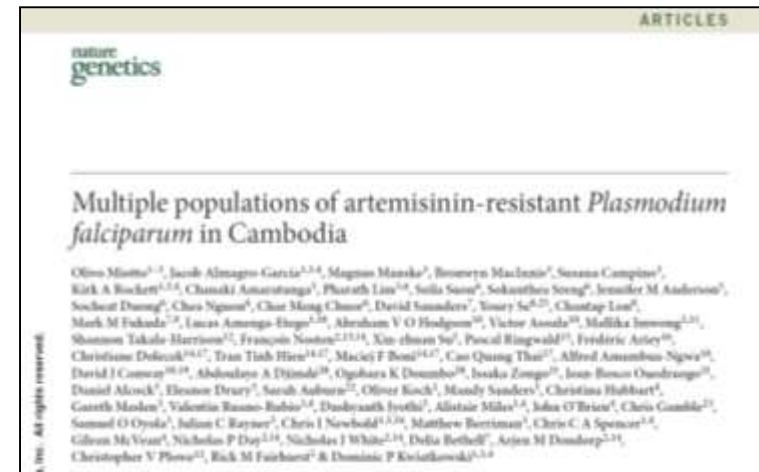
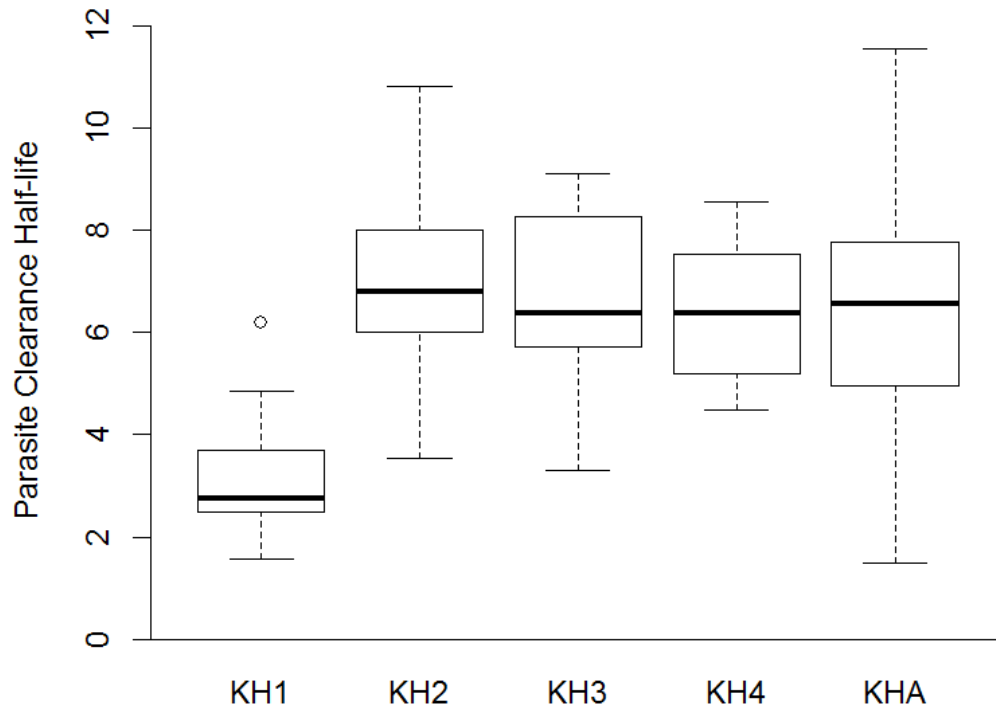


Founder 2

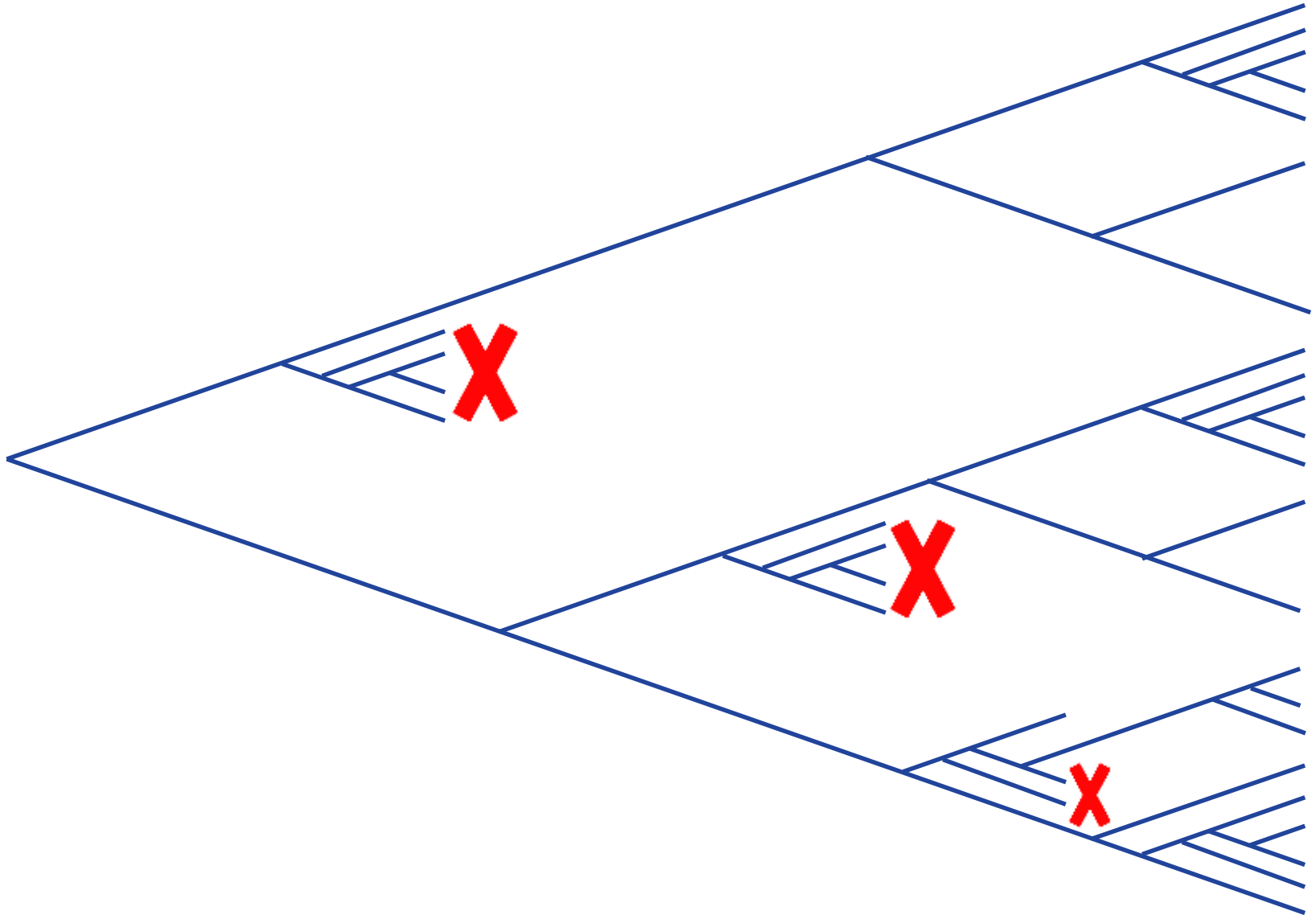


**Extremely rapid clonal expansion**

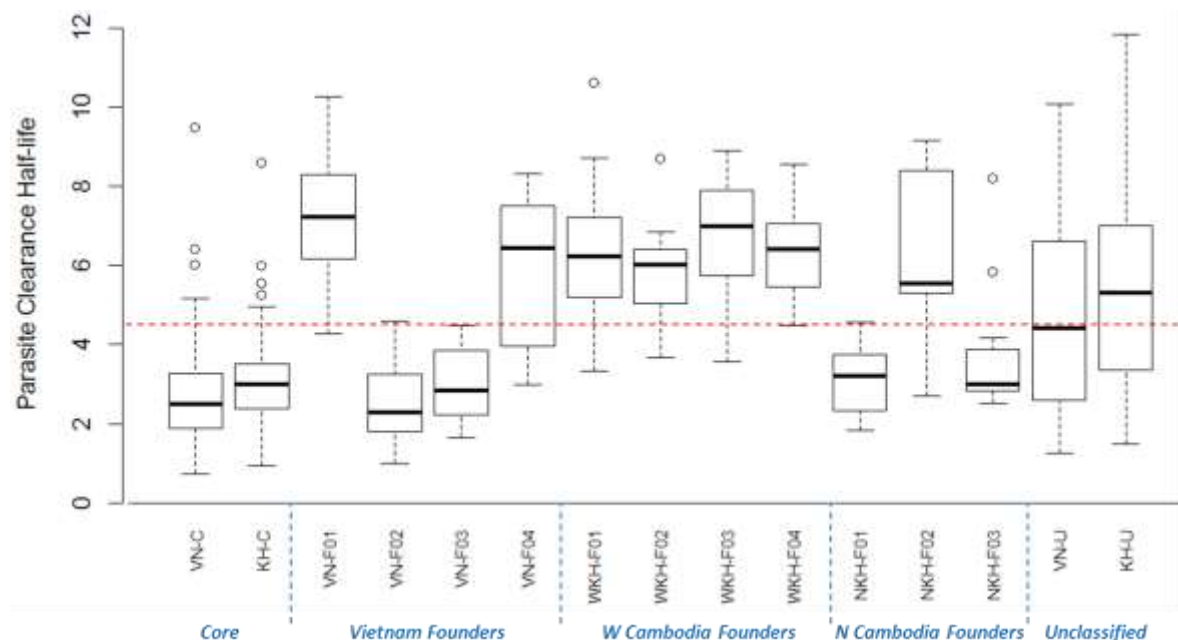
# Subpopulations are ART-R



# “Founder drift”



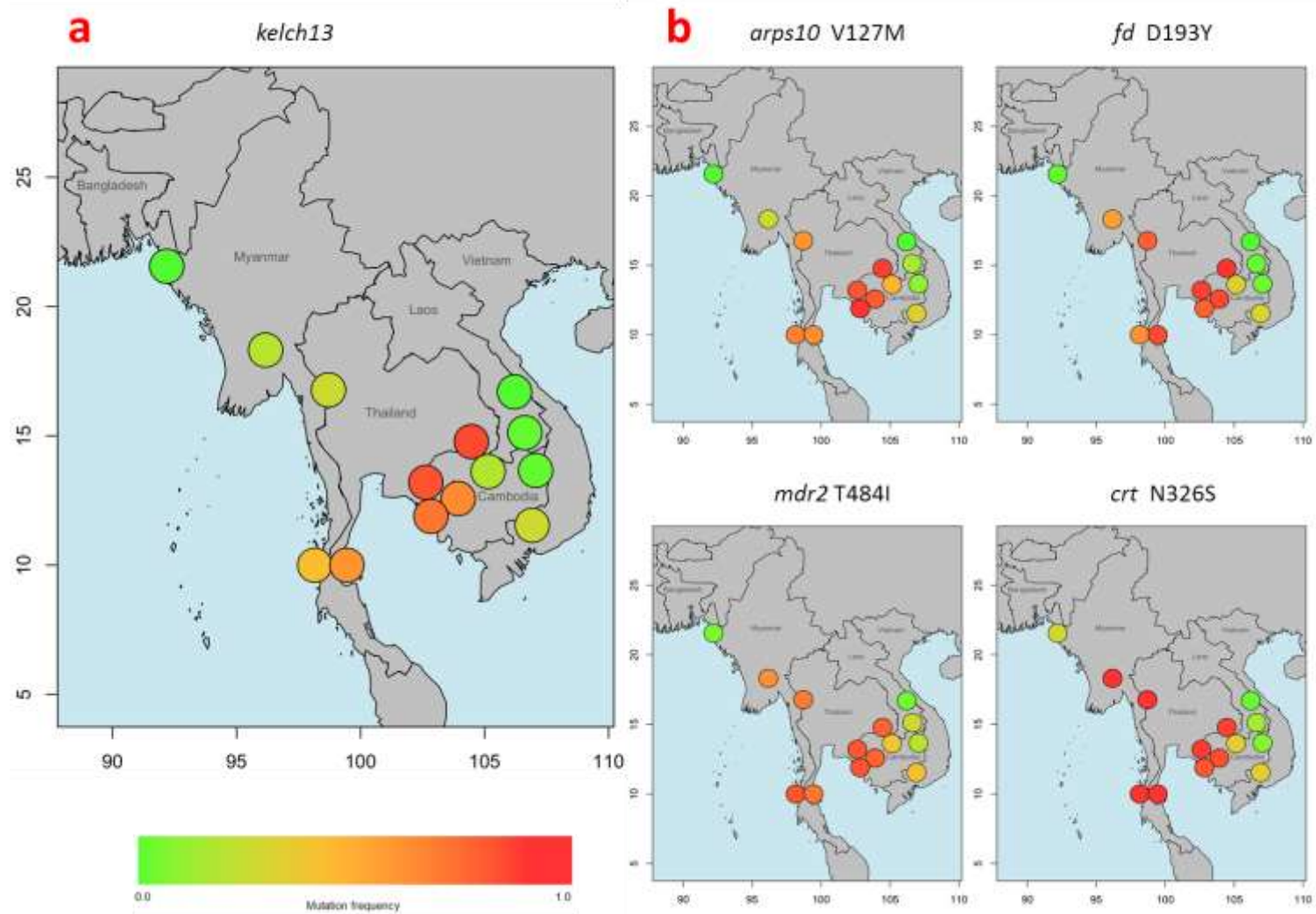
# 7 founder populations strongly associated with artemisinin resistance



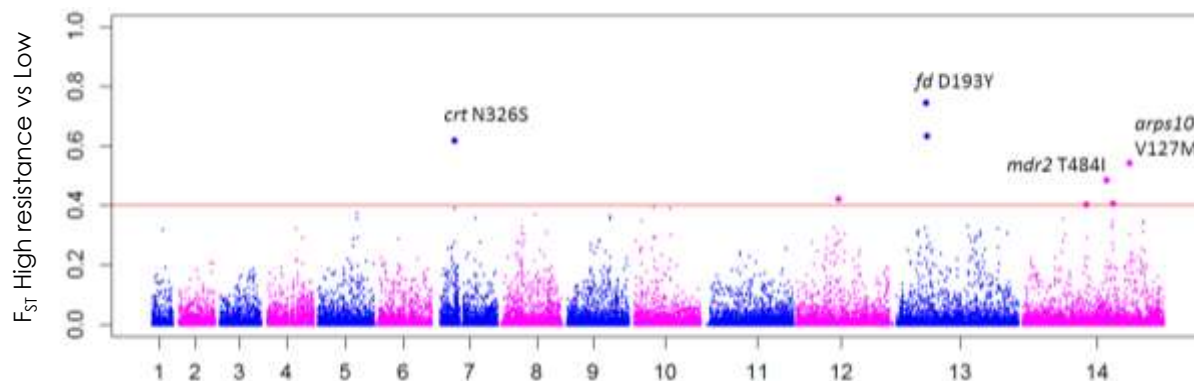
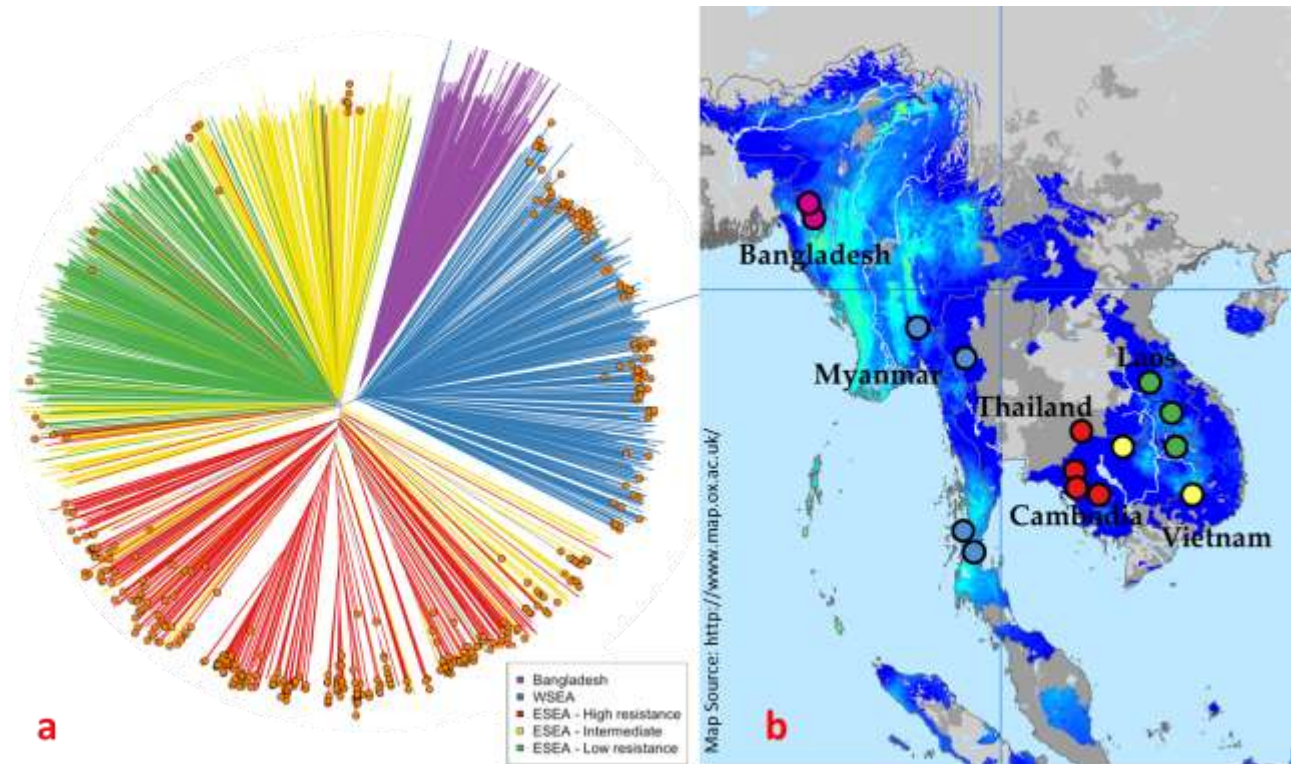
- Each artemisinin-resistant founder populations strongly associated with a specific *kelch13* resistance allele
- But the non-*kelch13* significant SNPs (**background mutations**) are all in there!

Population	WT	Y493H	R539T	I543T	P553L	C580Y	Het	Total
VN-C	69	1			2		4	76
KH-C	122						2	124
VN-F01				20			1	21
VN-F04	1				4		3	8
WKH-F01	2					49	3	54
WKH-F02	1		15					16
WKH-F03						32	2	34
WKH-F04		15						15
NKH-F02						8	1	9
<b>Total</b>	<b>195</b>	<b>16</b>	<b>15</b>	<b>20</b>	<b>6</b>	<b>89</b>	<b>16</b>	<b>357</b>

# *kelch13* and the background alleles have similar geographical distributions



# The genetic background is extremely differentiated even on a short geographic distance

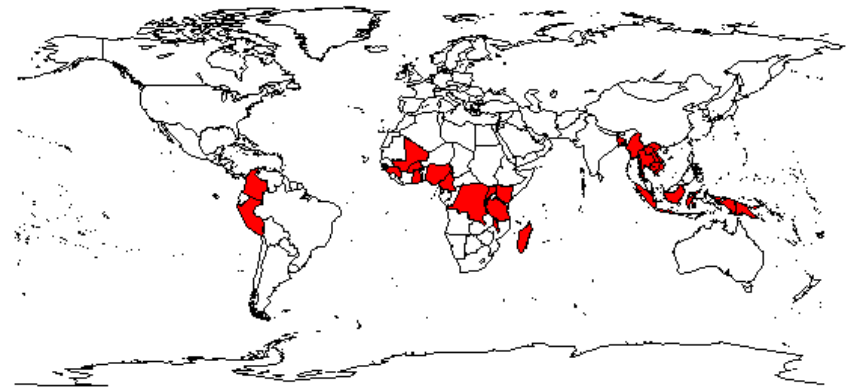


# Contextualizing *kelch13*

Within the genome and across countries

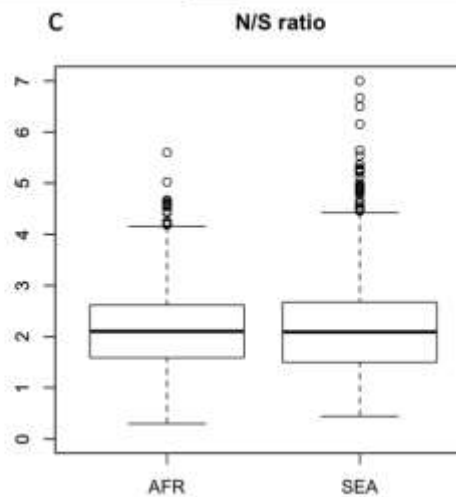
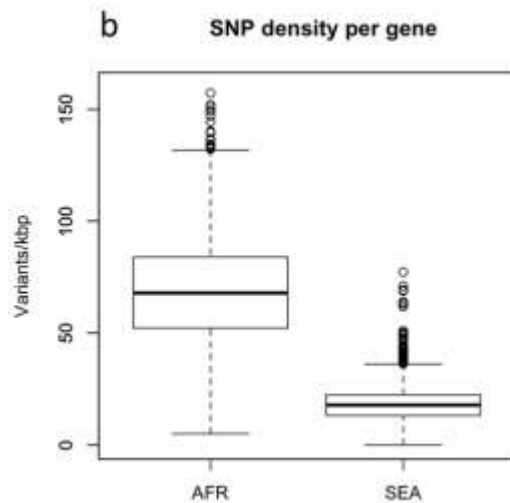
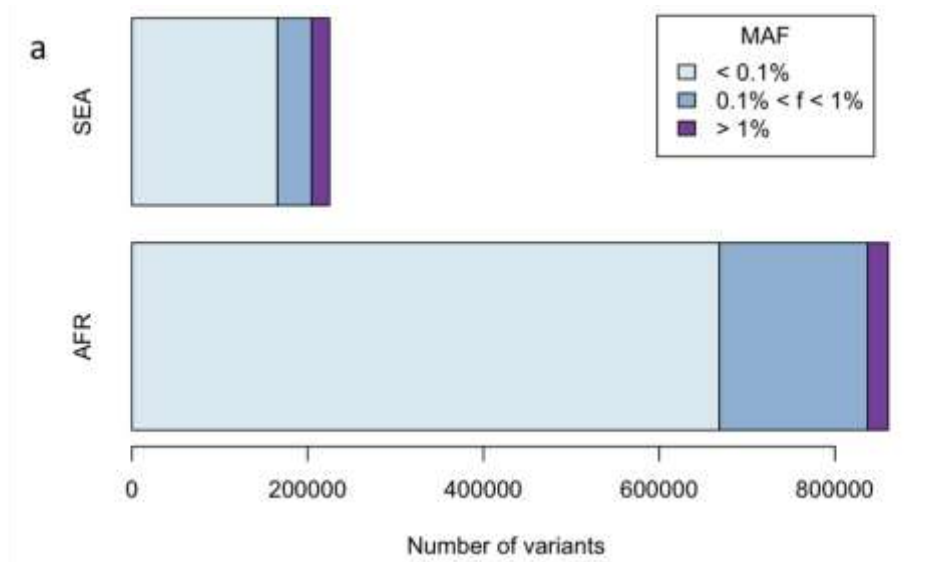
# Geographical distribution of the samples

Region	Sample counts	Country	Sample counts
West Africa	957	Burkina Faso	56
		Cameroon	134
		Ghana	478
		Gambia, The	73
		Guinea	124
		Mali	87
		Nigeria	5
East Africa	410	Kenya	52
		Madagascar	18
		Malawi	260
		Tanzania	68
		Uganda	12
Central Africa	279	D. R. Congo	279
South America	27	Colombia	16
		Peru	11
South Asia	75	Bangladesh	75
West Southeast Asia	494	Myanmar	109
		Thailand	385
East Southeast Asia	1154	Cambodia	815
		Laos	120
		Vietnam	219
Oceania	139	Indonesia (Papua)	17
		Papua New Guinea	122

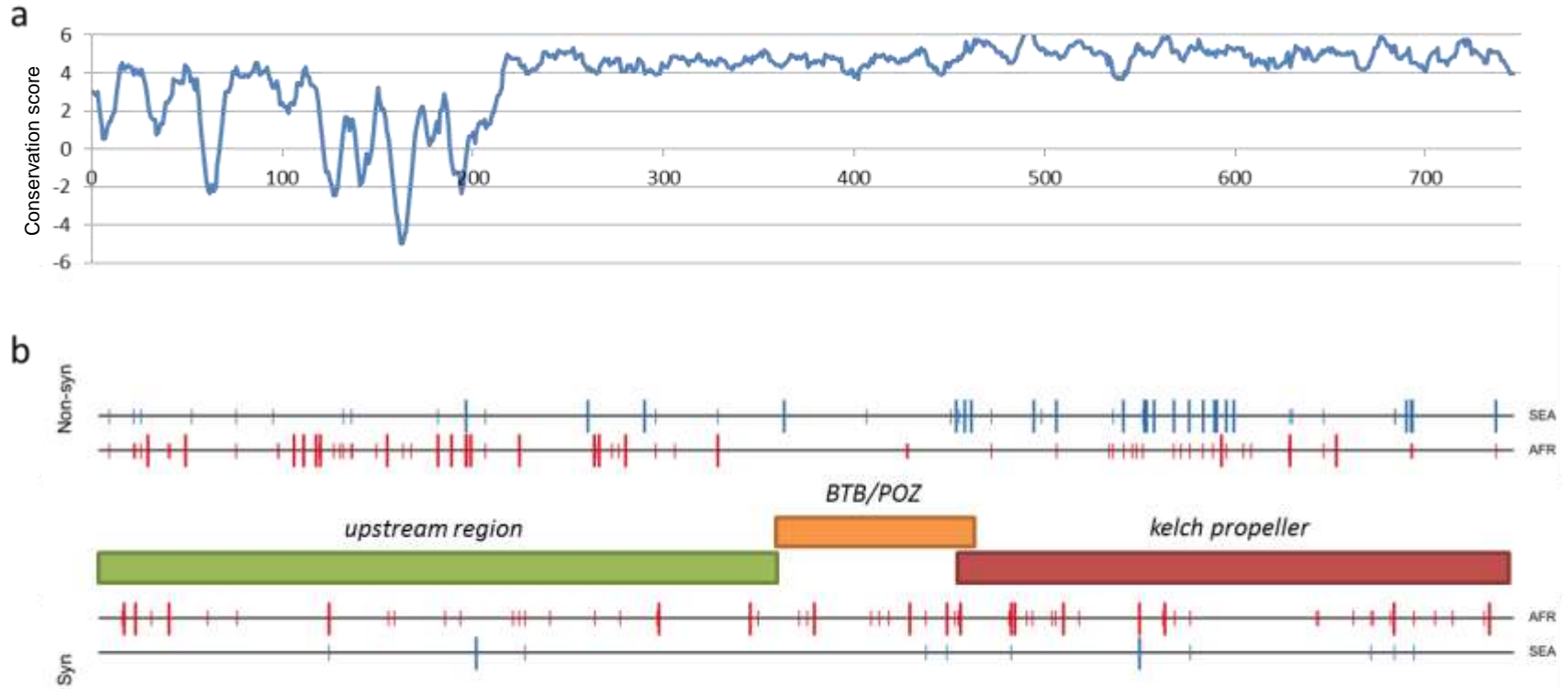




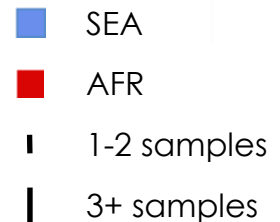
# Density of the variants in AFR and SEA



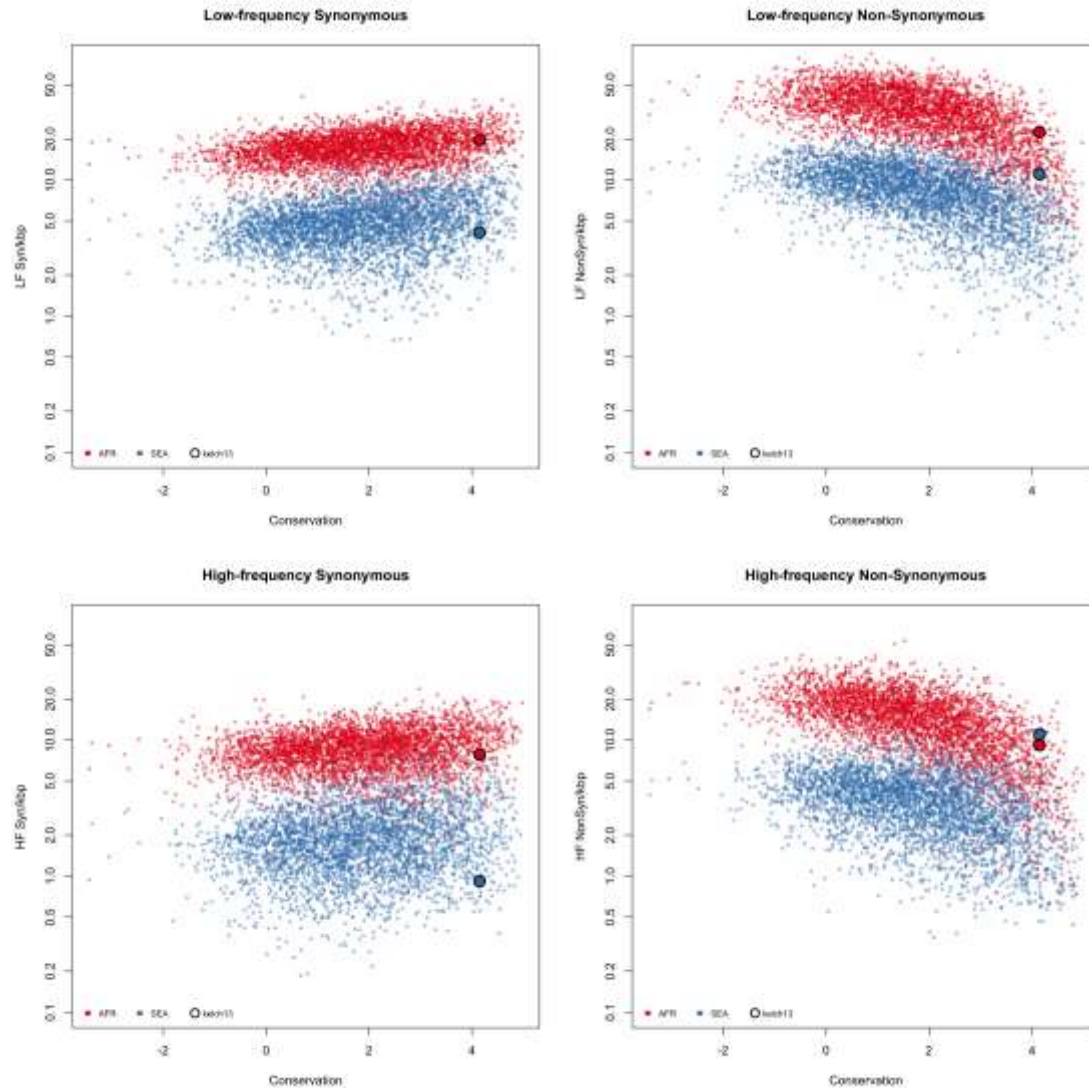
# Distribution of the mutations within *kelch13*



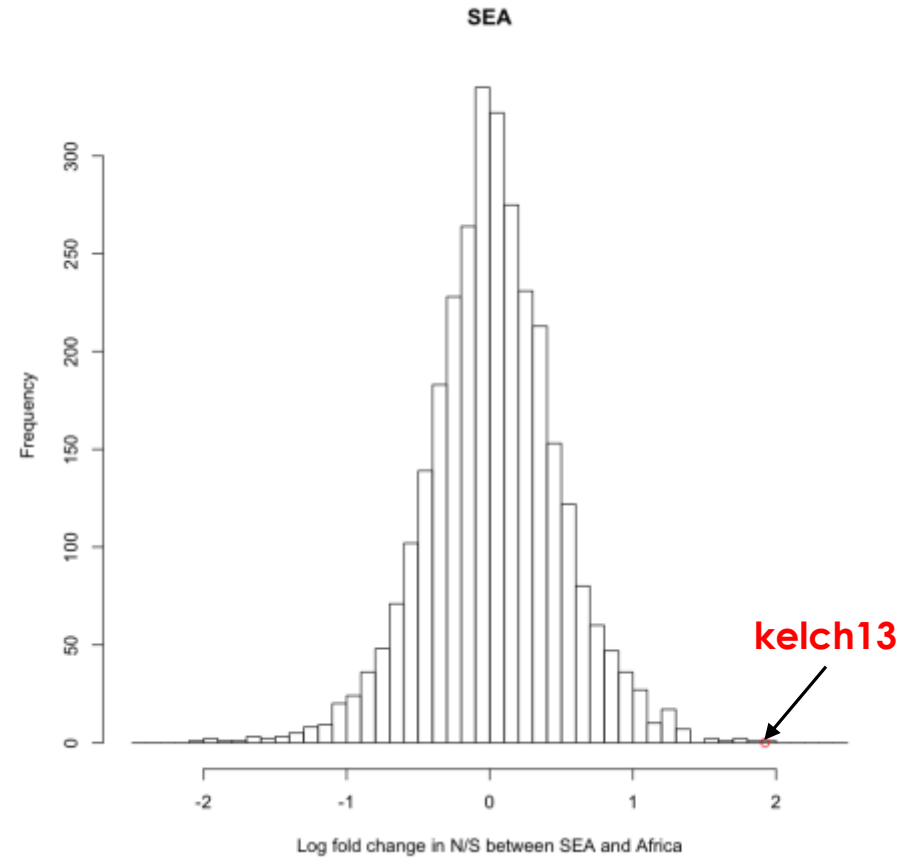
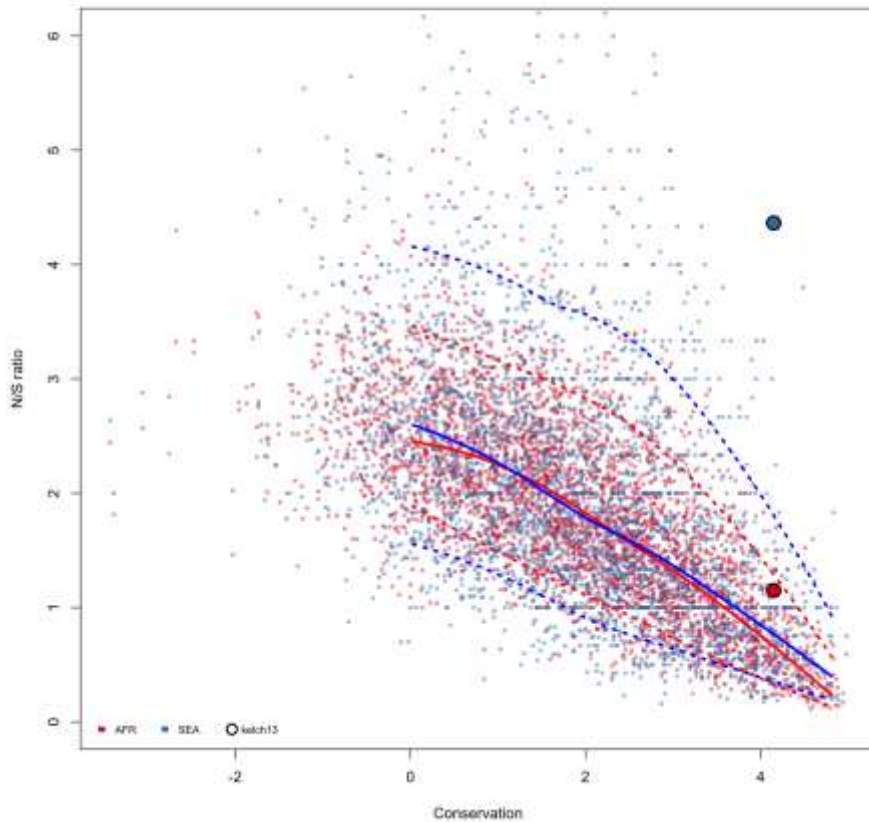
		AFR	SEA	SAS	OCE	SAM
Non-synonymous	Resistance domains	27	33	1	5	0
	Upstream region	42	15	2	4	1
Synonymous	Resistance domains	38	8	1	3	0
	Upstream region	22	3	1	0	0



# Excess of frequent NS mutations in SEA



# N/S vs conservation



■ AFR   ■ SEA   ○ kelch13