

Tracking the spread of insecticide resistance in *Anopheles gambiae* populations

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These slides: <http://alimanfoo.github.io/slides/20190606-who-geneva.html>



Use cases for genomic surveillance of malaria vector populations

Pyrethroid resistance is widespread in primary African vector species.

How can surveillance improve insecticide resistance management (IRM)?

Use case (1): Procurement and deployment of next-generation LLINs

- "Next-generation" LLINs available, e.g.:
 - Olyset Plus: permethrin + PBO (P450 synergist)
 - Olyset Duo: permethrin + pyriproxyfen (second insecticide)
- More expensive than standard LLINs
- How many to buy?
- Where to deploy them?

Use case (2): IRS deployment strategy

- "Next-generation" IRS formulations available, e.g.:
 - Actellic 300CS: pyrimiphos methyl (organophosphate)
 - SumiShield 50WG: clothianidin (neonicotinoid)
 - Fludora Fusion: deltamethrin + clothianidin
- Preemptive rotation?
- Geographical mosaic?
- Is it working?

Use case (3): Cross-border coordination

- Can countries take decisions in isolation about how to manage insecticide resistance?
- When and where do decisions need to be coordinated across borders?

The *Anopheles gambiae* 1000 Genomes Project (Ag1000G)

- A consortial project using whole-genome sequencing to investigate genetic variation and evolution in natural mosquito populations
- Create an open access data resource to accelerate research and surveillance
- www.malariagen.net/ag1000g

Ag1000G Consortium

Wellcome Sanger Institute / University of Oxford / Liverpool School of Tropical Medicine / Sapienza University of Rome / University of California, Riverside / Liverpool John Moores University / Broad Institute / Institut de Recherche pour le Développement / Virginia Tech / KEMRI Wellcome Trust Research Programme / New Mexico State University / Universidade Nova de Lisboa / University of Minnesota / Université d'Abomey-Calavi, Benin / Indiana University / University of Notre Dame / Washington State University / Imperial College / University of Oregon / University of North Carolina at Chapel Hill / University of Montana / Institut Pasteur / Instituto Nacional de Saúde Pública, Guiné-Bissau / Centre International de Recherches Médicales de Franceville, Gabon / Programa Nacional de Controle da Malária, Angola / Institut de Recherche en Sciences de la Santé, Burkina Faso / University of Bamako, Mali / Infectious Diseases Research Collaboration, Uganda / Organisation de Coordination pour la lutte contre les Endémies en Afrique Centrale, Cameroon

Ag1000G sequencing methods

- Sequence individual mosquitoes collected from the field
- Use whole-genome Illumina (Hi-Seq) sequencing
- Deep coverage (~30X)
- Sequencing performed at and funded by Wellcome Sanger Institute

Ag1000G population sampling

- Aim for broad geographical coverage
 - 18 countries, ~1 site per country
- *An. gambiae*, *An. coluzzii*, *An. arabiensis*
- Sequence >30 individuals per site per species
 - Why 30? Statistical power to make inferences about populations (e.g., gene flow).

Ag1000G data production

- Raw sequence reads →
- Alignment to reference genome →
- Variant calling →
- Variant filtering and annotation →
- Haplotype phasing →
- **Curated "analysis-ready" variant calls and haplotypes**
- Validation, e.g., <1% FDR

Ag1000G data releases

- Phase 1: 765 mosquitoes; 8 countries; *An. gambiae*, *An. coluzzii*
 - Data released 2016
- Phase 2: 1,142 mosquitoes; 13 countries; *An. gambiae*, *An. coluzzii*
 - Data released 2017
- Phase 3: ~4,000 mosquitoes; 18 countries; *An. gambiae*, *An. coluzzii*, *An. arabiensis*
 - Data in production

Ag1000G further information

- www.malariagen.net/ag1000g
- <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6026>

LETTER

doi:10.1038/nature24995

Genetic diversity of the African malaria vector *Anopheles gambiae*

The *Anopheles gambiae* 1000 Genomes Consortium*

The sustainability of malaria control in Africa is threatened by the rise of insecticide resistance in *Anopheles* mosquitoes, which transmit the disease¹. To gain a deeper understanding of how mosquito populations are evolving, here we sequenced the genomes of 765 specimens of *Anopheles gambiae* and *Anopheles coluzzii* sampled from 15 locations across Africa, and identified over 50 million single nucleotide polymorphisms within the accessible genome. These data revealed complex population structure and patterns of gene flow, with evidence of ancient expansions, recent bottlenecks, and local variation in effective population size. Strong signals of recent selection were observed in insecticide-resistance genes, with several sweeps spreading over large geographical distances and between species. The design of new tools for mosquito control using gene-drive systems will need to take account of high levels of genetic diversity in natural mosquito populations.

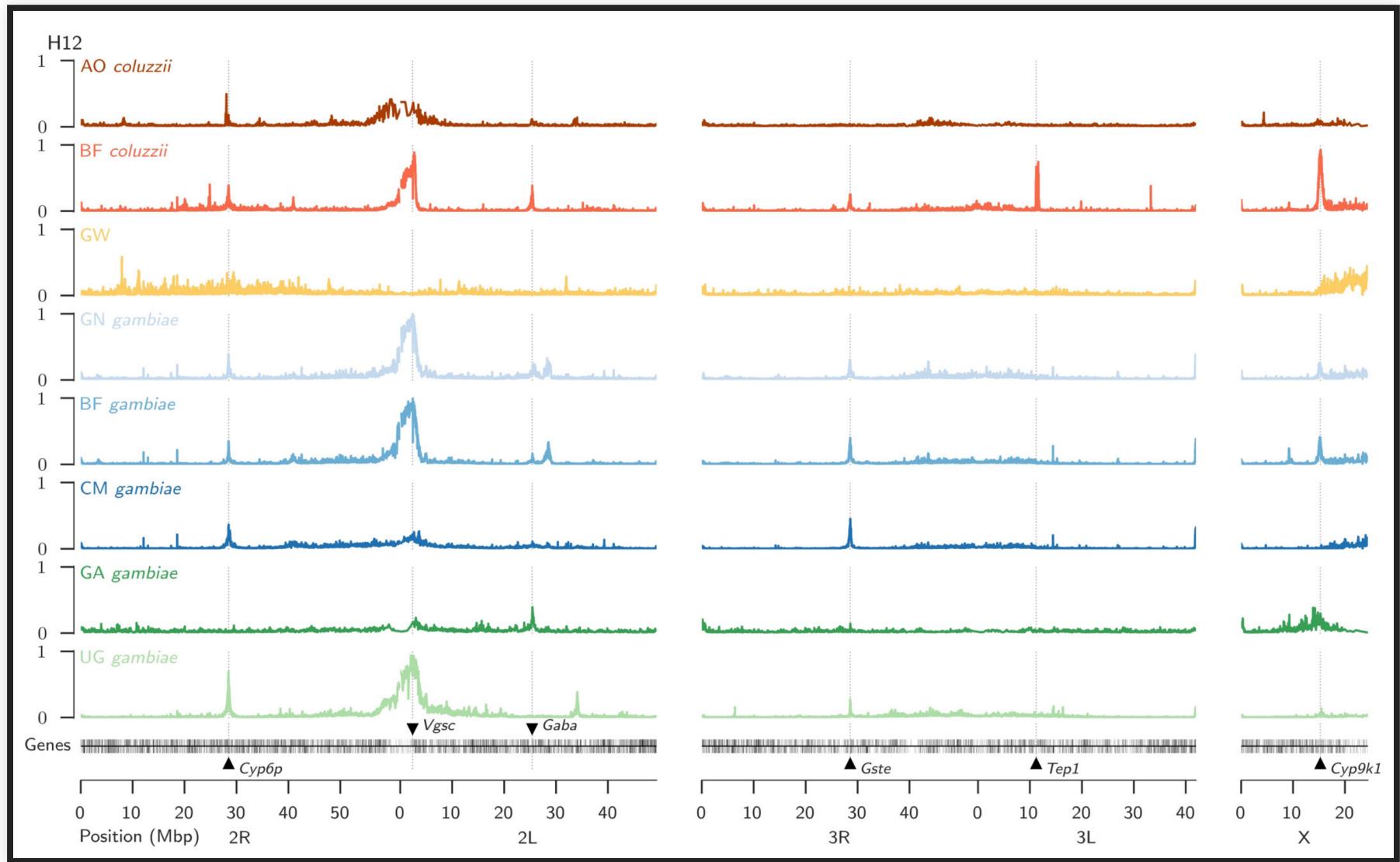
diversity was 1.5% on average (Extended Data Fig. 3b) and more than 3% at synonymous coding sites (Extended Data Fig. 3c), confirming that these are among the most genetically diverse eukaryotic species⁹.

High levels of natural diversity have practical implications for the development of gene-drive technologies for mosquito control¹⁰. CRISPR–Cas9 gene drives can be designed to edit a specific gene and confer a phenotype such as female sterility, which could suppress mosquito populations and thereby reduce disease transmission. However, naturally occurring polymorphisms within the approximately 21-base-pair (bp) Cas9 target site could prevent target recognition, and thus undermine gene-drive efficacy in the field. We found viable Cas9 targets in 11,625 protein-coding genes, but only 5,474 genes remained after excluding target sites with nucleotide variation in any of the 765 genomes sequenced here (Extended Data Fig. 3d; Supplementary Information 5). Resistance to gene drive could be

Gene flow

- Between locations
- Between species
- Between generations (i.e., increasing in frequency,
i.e., under selection)

Genes under selection



Pyrethroid target-site resistance

Spread of "knock-down resistance" (*kdr*) mutations in the voltage-gated sodium channel gene (*Vgsc*).

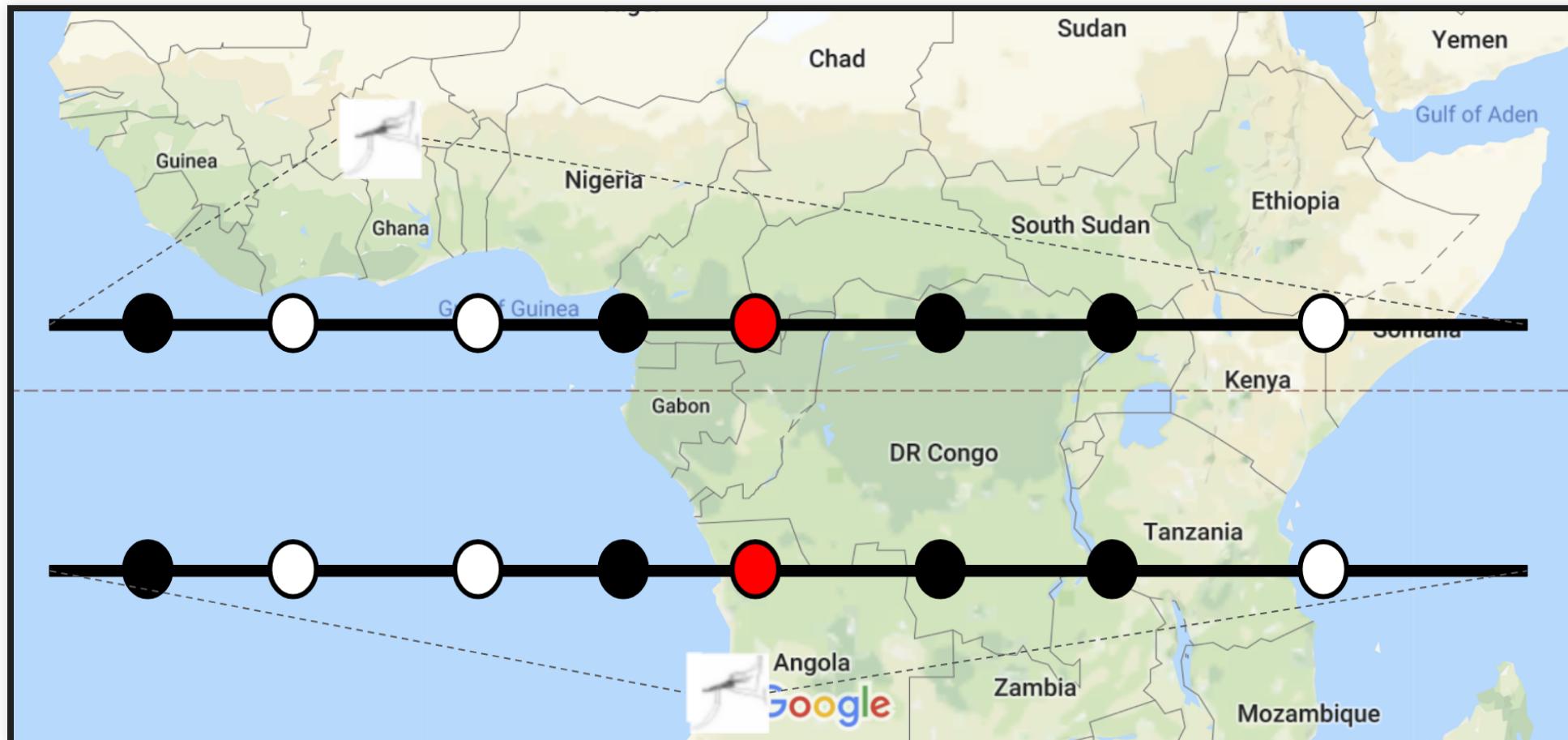
kdr mutations

- Two known *kdr* mutations in *Vgsc* codon 1014
- L1014F found throughout West and Central Africa
- L1014S found throughout East and Central Africa
- Are these mutations spreading?
- Where is gene flow occurring?

Inferring *kdr* gene flow

- Analyse the genetic backgrounds on which *kdr* mutations occur ("*kdr* haplotypes")
- Use all mutations within the *Vgsc* gene
 - 1,710 biallelic SNPs (mostly intronic)
- Same *kdr* haplotype in two different locations:
 - ⇒ gene flow

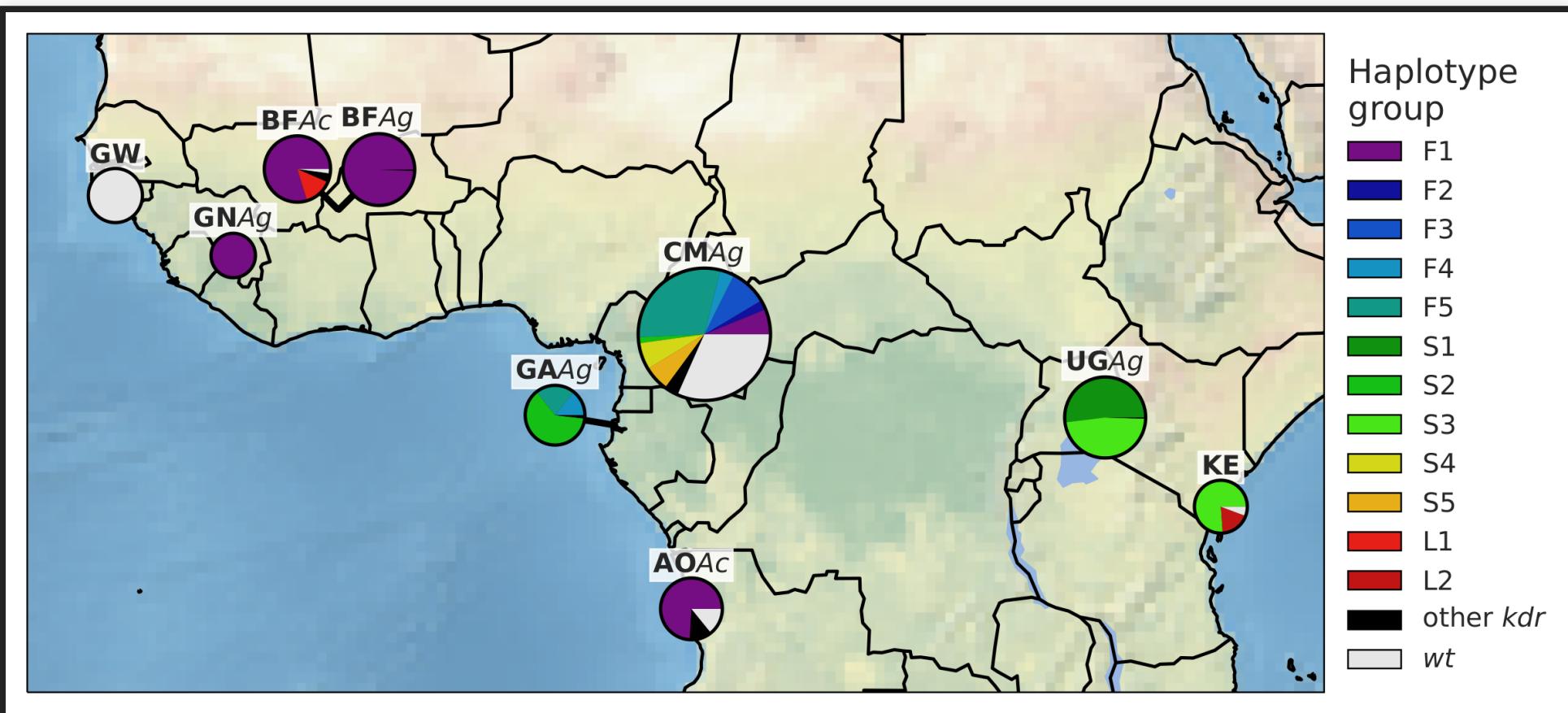
Inferring *kdr* gene flow

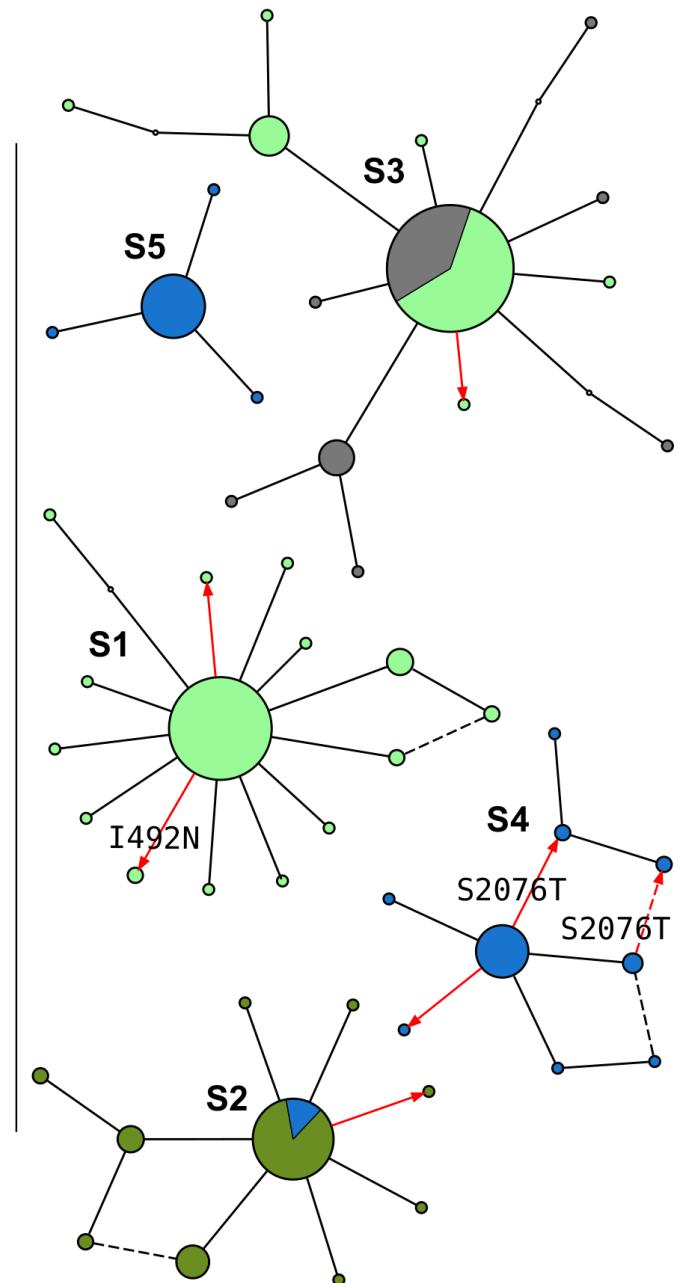
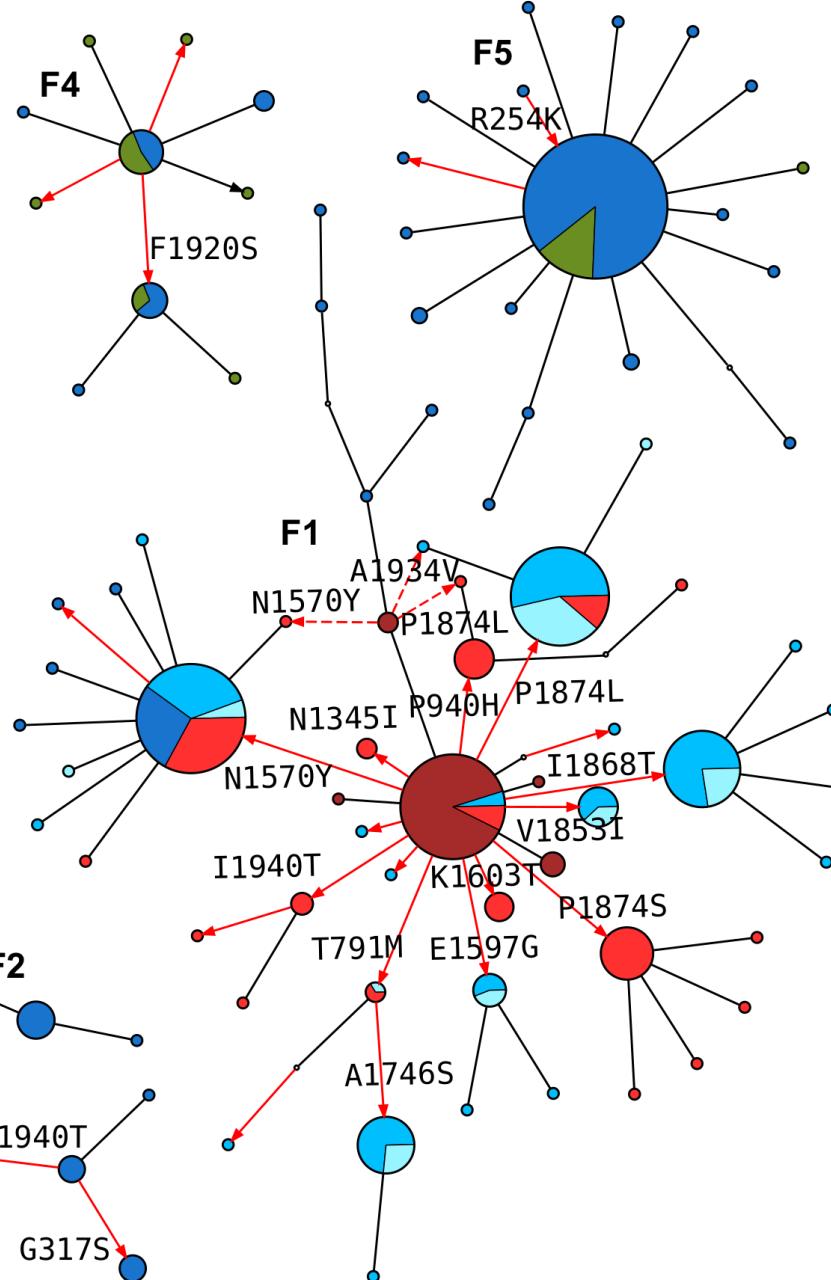
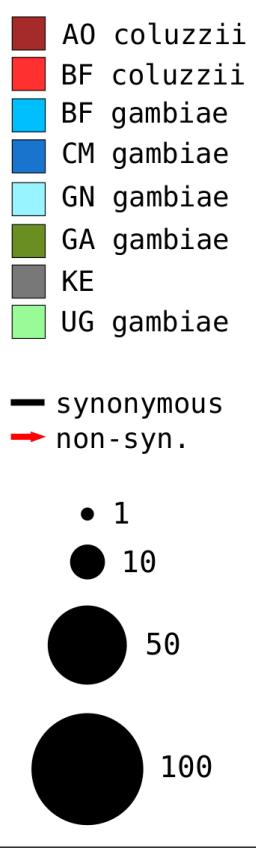


kdr haplotypes

- Analysed data from Ag1000G phase 1 (765 mosquitoes, 8 countries)
- L1014F - 5 major haplotypes (F1-F5)
- L1014S - 5 major haplotypes (S1-S5)

kdr haplotypes





kdr gene flow - further information

<https://doi.org/10.1101/323980>

New Results 1 comment

The genetic architecture of target-site resistance to pyrethroid insecticides in the African malaria vectors *Anopheles gambiae* and *Anopheles coluzzii*

Chris S. Clarkson, Alistair Miles, Nicholas J. Harding, David Weetman, Dominic Kwiatkowski, Martin Donnelly, The *Anopheles gambiae* 1000 Genomes Consortium

doi: <https://doi.org/10.1101/323980>

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Abstract

Resistance to pyrethroid insecticides is a major concern for malaria vector control, because these are the compounds used in almost all insecticide-treated bed-nets (ITNs), and are also widely used for indoor residual spraying (IRS). Pyrethroids target the voltage-gated sodium channel (VGSC), an essential component of the mosquito nervous system, but substitutions in

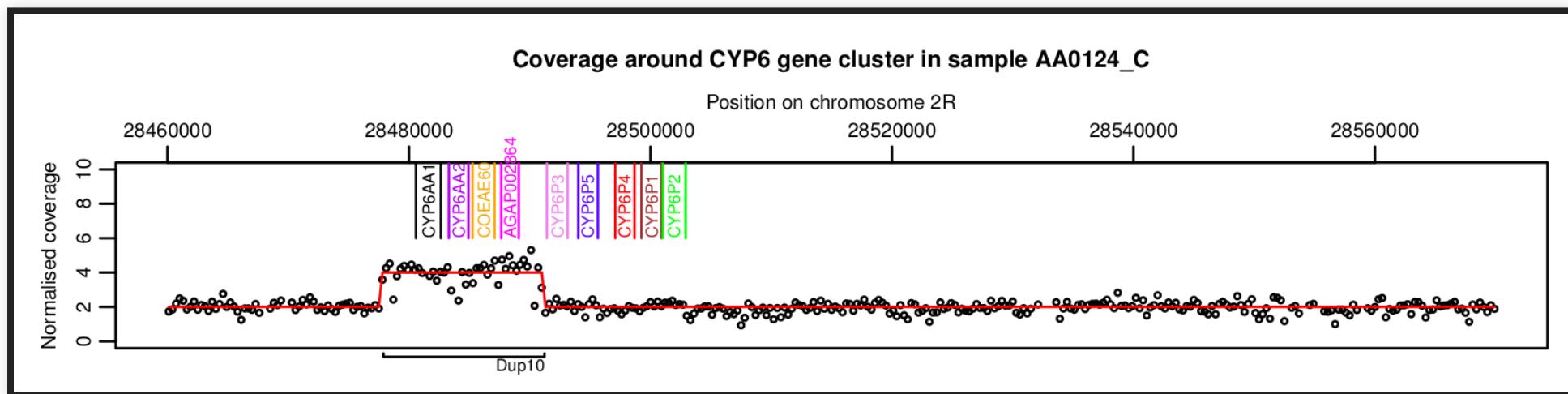
Pyrethroid metabolic resistance

Spread of copy number variations in cytochrome P450 genes.

Cytochrome P450 genes

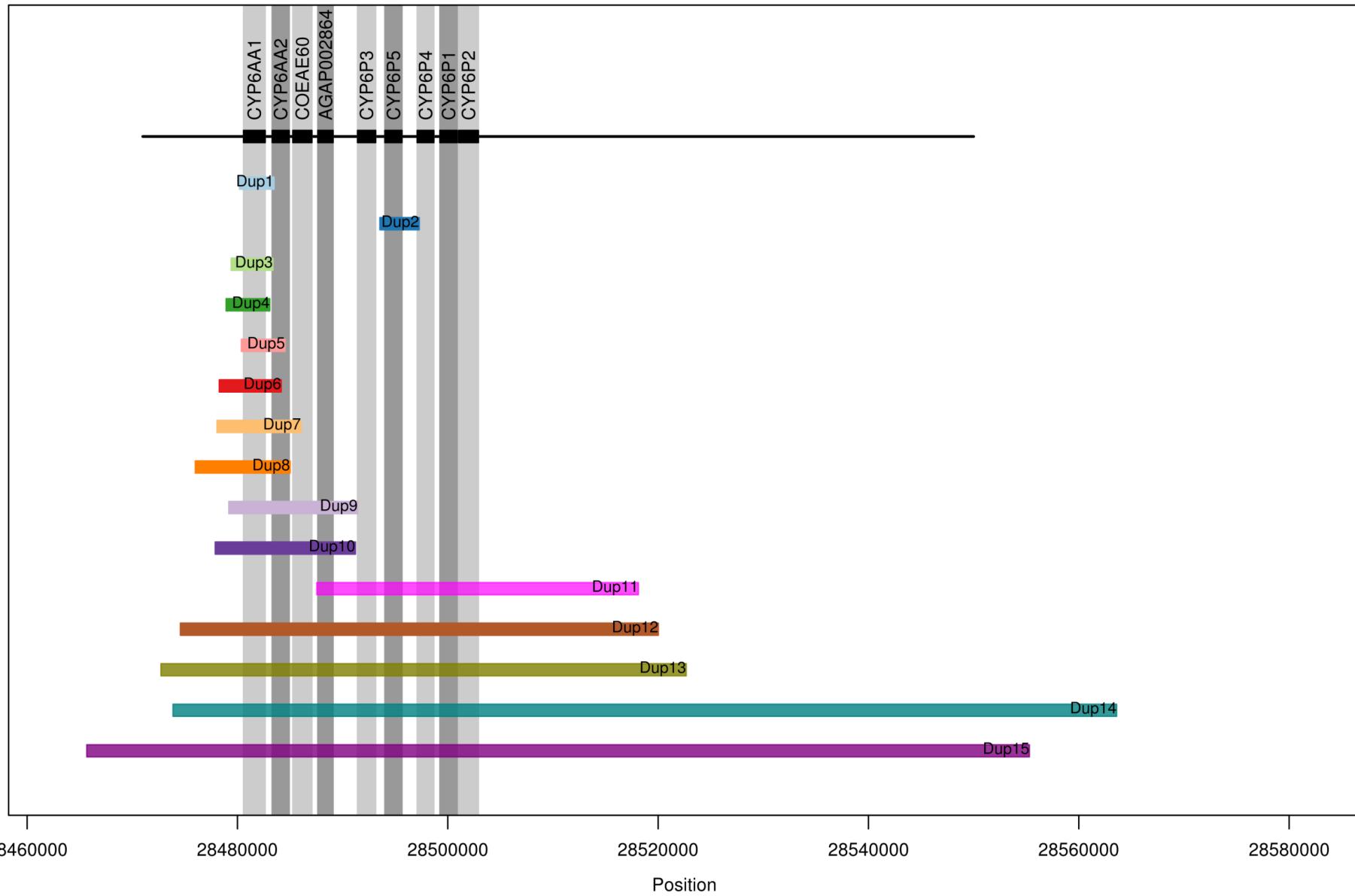
- Known to metabolise pyrethroids
- Neutralised by PBO synergist in next-gen LLINs
- Multiple P450 genes in genome, e.g.:
 - *Cyp6p/aa*
 - *Cyp9k1*
- Increased expression ⇒ pyrethroid resistance
- Increased gene copy number ⇒ increased expression

Detecting copy number variation



P450 copy number variation

- Analysed data from Ag1000G phase 2 (1,142 mosquitoes, 13 countries)
- Gene amplifications are common at two P450 loci:
 - *Cyp6p/aa*
 - *Cyp9k1*



Cyp6p/aa CNV gene flow

- Dup1 - BFcol (8%), UGgam (58%)
- Dup7 - BFcol (44%), Clcol (32%), GHcol (5%), GNcol (75%)
- Dup8 - BFgam (3%), NGam (3%)
- Dup10 - BFcol (49%), GHcol (5%)
- Dup11 - Clcol (41%), GHcol (5%)
- Dup14 - BFcol (3%), Clcol (46%)
- Dup15 - BFcol (1%), Clcol (39%)

CNVs further information

<https://doi.org/10.1101/399568>

New Results Comment on this paper

Whole genome sequencing reveals high complexity of copy number variation at insecticide resistance loci in malaria mosquitoes

 Eric R. Lucas, Alistair Miles, Nicholas J. Harding, Chris S. Clarkson, Mara K. N. Lawniczak, Dominic P. Kwiatkowski, David Weetman, Martin J. Donnelly, The Anopheles gambiae 1000 Genomes Consortium

doi: <https://doi.org/10.1101/399568>

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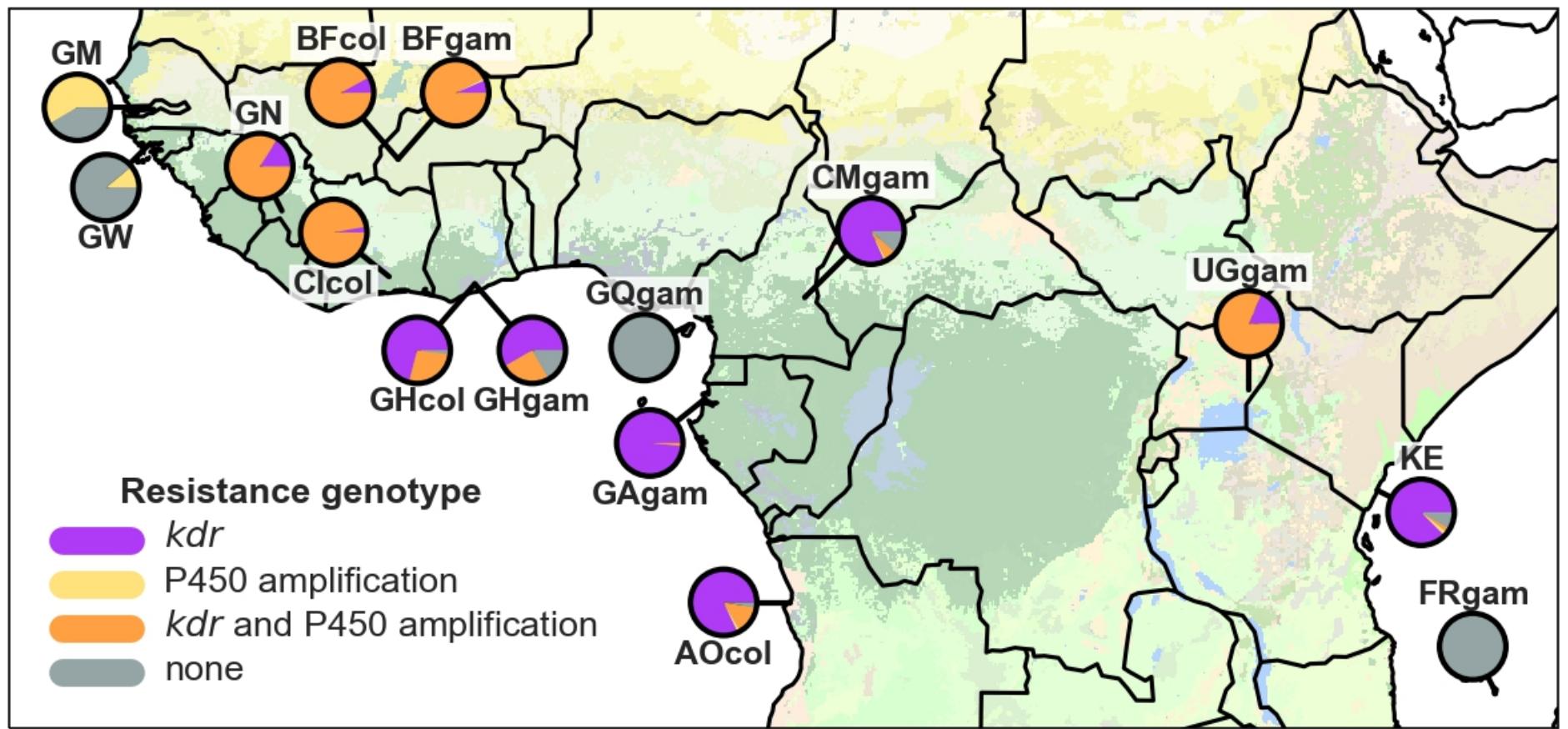
Abstract

Background Polymorphisms in the copy number of a genetic region can influence gene expression, coding sequence and zygosity, making them powerful actors in the evolutionary process. Copy number variants (CNVs) are however understudied, being more difficult to

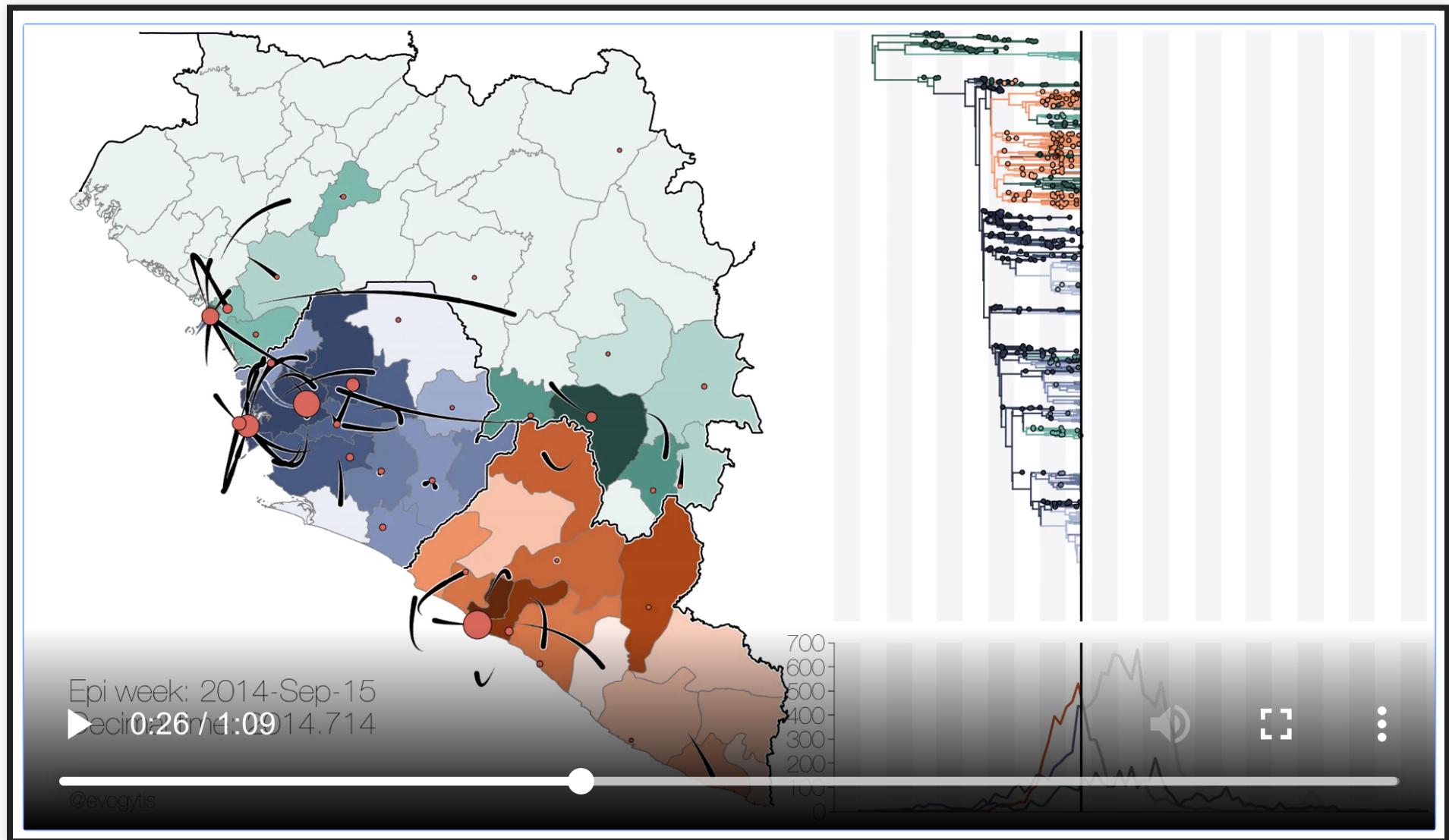
Summary & discussion

- Target-site (*kdr*) and metabolic (P450 CNV) pyrethroid resistance are spreading via gene flow
- Multiple independent outbreaks of resistance
 - Some spreading, some localised
- Long distance gene flow, e.g.:
 - *kdr*-F1 found in GN, BF, CM and AO
 - *Cyp6p*-Dup1 found in BF and UG
- *kdr*, *Cyp6p/aa* and *Cyp9k1* show different patterns of spread

Where to deploy PBO LLINs?



Cf. Ebola outbreaks



Resistance outbreaks

- Geographical origins?
- Direction and routes of gene flow?
- Timing?
- Rate of movement?

Next steps

- Scale up genome sequencing of vector populations
- Increase geographical coverage
- Regular (seasonal) sampling
- Other vector species (e.g., *An. funestus*)

MalariaGEN Vector Observatory

- Aim to sequence 10,000 mosquitoes per year
- Coupled with routine ento surveillance
 - Follow sentinel sites over time
 - Link genomic and epi/ento data
- Partnerships
 - PAMCA/BMGF, GAARDian, Target Malaria, ...
- Open data
- **Bridge the gap** between research and implementation

Acknowledgements

The Anopheles gambiae 1000 Genomes Consortium*

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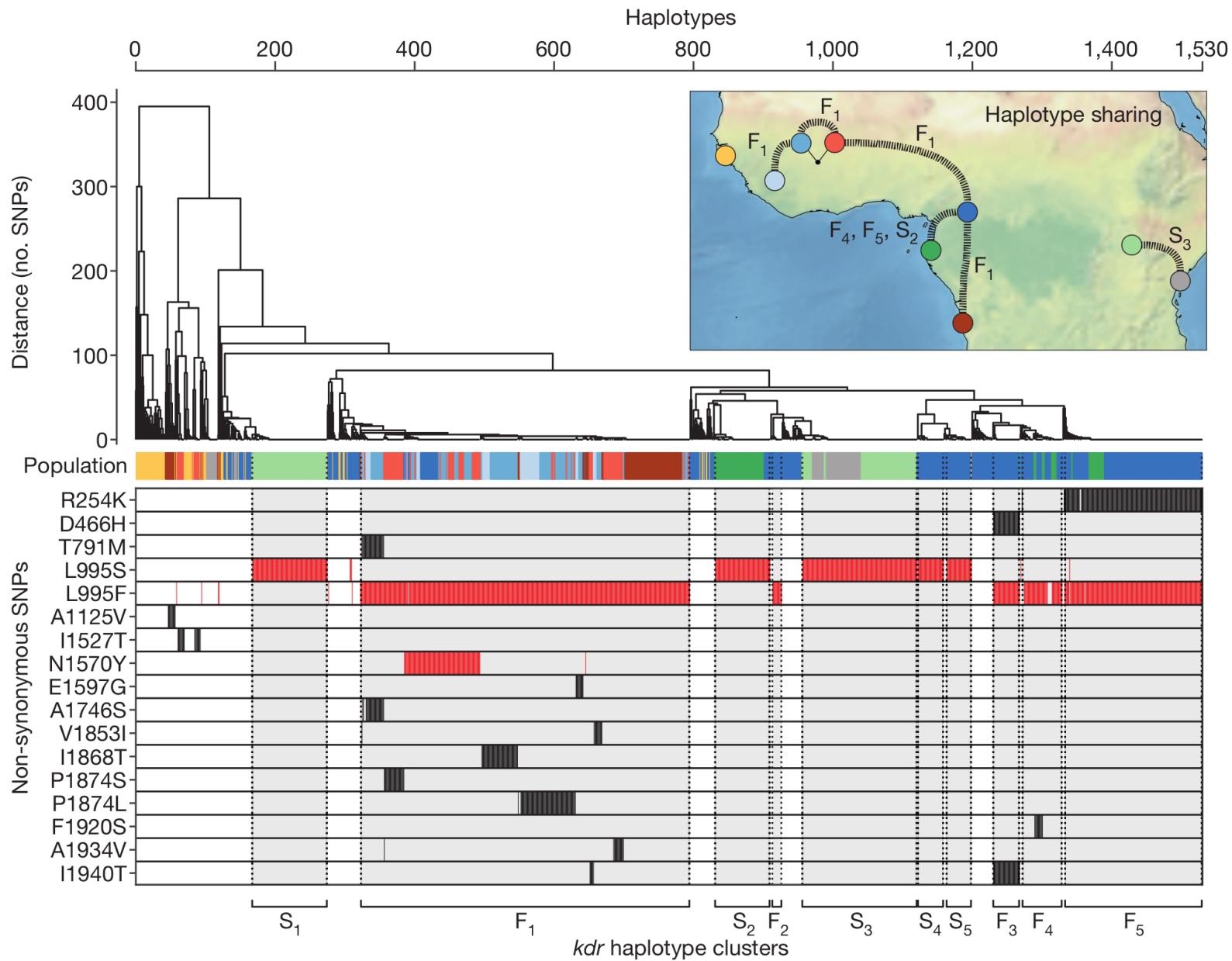
Sample collections. Angola: Arlete D. Troco²³, João Pinto¹⁸; **Burkina Faso:** Abdoulaye Diabaté²⁴, Samantha O'Loughlin¹⁴, Austin Burt¹⁴; **Cameroon:** Carlo Costantini^{13,25}, Kyanne R. Rohatgi¹⁰, Nora J. Besansky¹⁰; **Gabon:** Nohal Elissa¹², João Pinto¹⁸; **Guinea:** Boubacar Coulibaly²⁶, Michelle M. Riehle¹⁹, Kenneth D. Vernick²⁰; **Guinea-Bissau:** João Pinto¹⁸, João Dinis²⁷; **Kenya:** Janet Midega¹⁷, Charles Mbogo¹⁷, Philip Bejon¹⁷; **Uganda:** Craig S. Wilding^{21,3}, David Weetman³, Henry D. Mawejje²⁸, Martin J. Donnelly^{3,1}; **Crosses:** David Weetman³, Craig S. Wilding^{21,3}, Martin J. Donnelly^{3,1}.

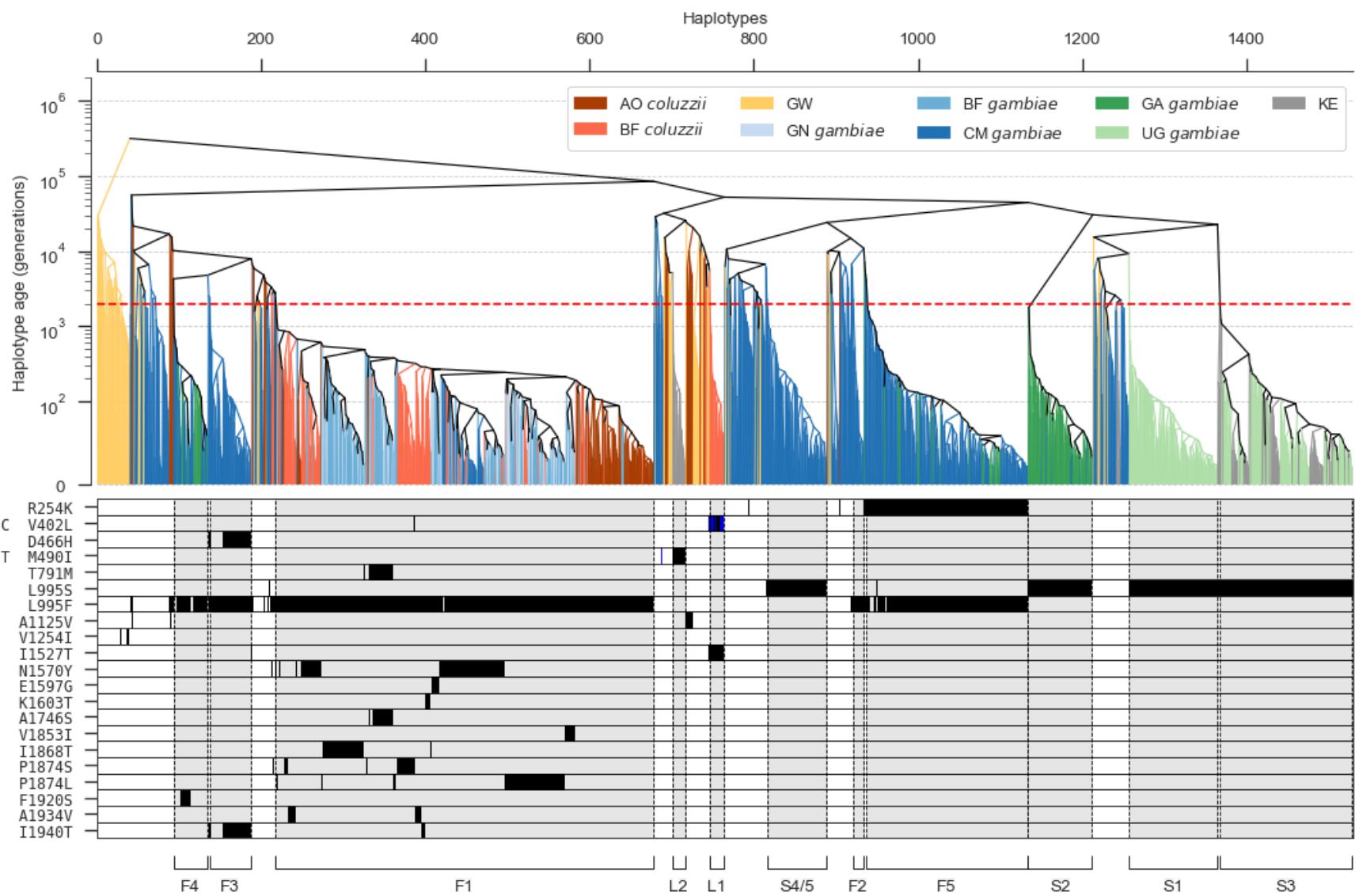
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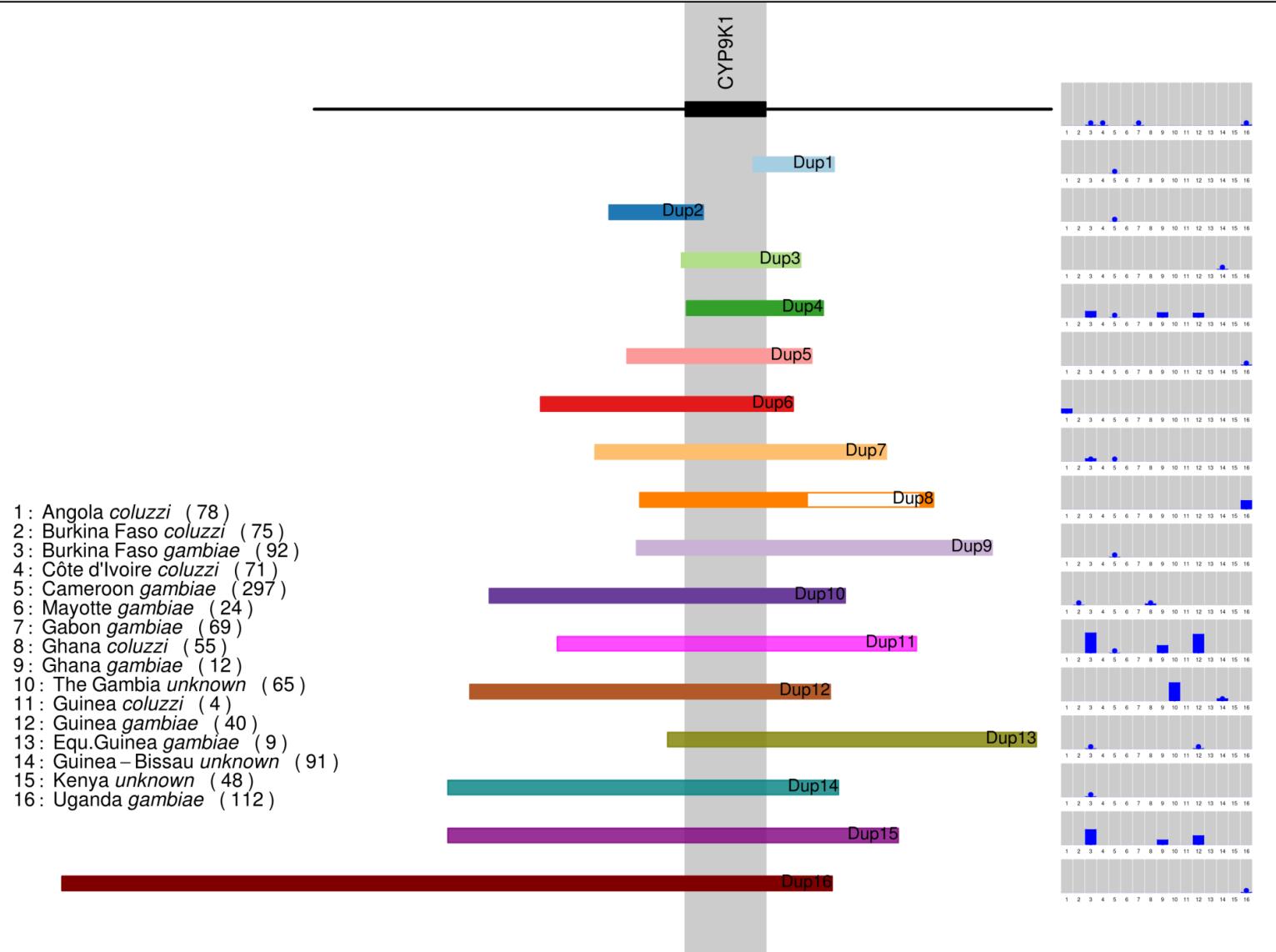
Web application development. Paul Vauterin², Ben Jeffrey², Ian Wright², Lee Hart², Krzysztof Kluczyński².

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Extra slides







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Position

Cyp9k1 CNV gene flow

- Dup4 - BFgam, CMgam, GHgam, GNgam
- Dup7 - BFgam, CMgam
- Dup10 - BFcol, GHcol
- Dup11 - BFgam, CMgam, GHgam, GNgam
- Dup12 - GM, GW
- Dup13 - BFgam, GNgam
- Dup15 - BFgam, GHgam, GNgam

Gene drive use cases

- Design of gene drive constructs - identification of ultra-conserved regions
- Modelling gene drive deployment and spread - estimating rates, ranges and routes of migration
 - Spread of insecticide resistance as a model for spread of gene drive
- Monitoring gene drive in the field - is it spreading as expected, is resistance emerging?