Insights into the evolution and spread of insecticide resistance from whole-genome sequencing of 1,142 Anopheles gambiae mosquitoes from 13 countries

Alistair Miles (@alimanfoo) - PAMCA 2019

The Anopheles gambiae 1000 Genomes Consortium

These slides: alimanfoo.github.io/slides/pamca2019/
N.B., one location per country; one time point per country; different countries sampled in different years; nothing more recent than 2012.
Methods

- Collect mosquitoes
- Extract DNA
- Whole-genome sequencing (Illumina Hi-Seq)
- Align sequence reads
- Identify genetic differences
Single nucleotide polymorphisms (SNPs)

If a SNP occurs in a gene coding sequence, it may change the encoded amino acid sequence, which may change the behaviour of the protein.
Copy number variants (CNVs)

If a CNV spans a gene, then the gene will occur twice (two copies) in the mutated genome. More gene copies may mean more protein is produced.
Ag1000G phase 2 data resource

Across the whole genome, we find:

- 57,837,885 SNPs
- 31,335 CNVs
Outline

- Pyrethroid resistance
  - Metabolic resistance
  - Target-site resistance
  - Combined view
- Organophosphate resistance
- Emerging/unknown resistance
- Translation to surveillance
Pyrethroid metabolic resistance

- Pyrethroids are metabolised by cytochrome P450 (CYP) enzymes (a.k.a. mixed-function oxidases; MFOs)
- Increased expression of certain CYP genes causes resistance
- Genetic variants causing metabolic resistance **not previously known** in *An. gambiae* complex
  - Although long suspected that CNVs play a role
  - More gene copies → more protein → faster metabolism → resistance
- N.B., PBO LLINs work by inhibiting CYPs
CNV hot spots

Genome locations where we find a lot of CNVs at high population frequency:

- CYP6P/AA (cytochrome P450 gene cluster)
- CYP9K1 (cytochrome P450 gene)
- GSTE (glutathione S-transferase gene)
CNVs at CYP6P/AA
Prevalence and spread of CYP6P/AA CNVs

- Dup1 - *An. gambiae*: Uganda (58%)
- Dup7 - *An. coluzzii*: Burkina Faso (44%), Cote d'Ivoire (32%), Ghana (5%), Guinea (75%)
- Dup10 - *An. coluzzii*: Burkina Faso (49%), Ghana (5%)
- Dup11 - *An. coluzzii*: Burkina Faso (41%), Ghana (5%)
- Dup14 - *An. coluzzii*: Burkina Faso (3%), Cote d'Ivoire (46%)
- Dup15 - *An. coluzzii*: Burkina Faso (1%), Cote d'Ivoire (39%)

Some CNVs are very common and spreading, especially in West African *An. coluzzii*. 
Surveillance of metabolic resistance

- Next-generation LLINs: how many, what type, where to put them?
- Which populations have CYP-mediated metabolic resistance?
- CNVs are likely to be a good marker of CYP-mediated metabolic resistance
- Although, N.B., there could be other genetic variants causing metabolic resistance
Whole-genome sequencing reveals high complexity of copy number variation at insecticide resistance loci in malaria mosquitoes

Eric R. Lucas,¹ Alistair Miles,²,³ Nicholas J. Harding,³ Chris S. Clarkson,² Mara K.N. Lawniczak,² Dominic P. Kwiatkowski,²,³ David Weetman,¹ Martin J. Donnelly,¹,² and The Anopheles gambiae 1000 Genomes Consortium⁴

¹Liverpool School of Tropical Medicine, Liverpool L3 5QA, United Kingdom; ²Wellcome Sanger Institute, Hinxton, Cambridge CB10 1SA, United Kingdom; ³Big Data Institute, University of Oxford, Li Ka Shing Centre for Health Information and Discovery, Oxford OX3 7LF, United Kingdom

See also "Insecticide resistance gene copy number variation in malaria mosquitoes" on
Pyrethroid target-site resistance

- Pyrethroids bind to the voltage-gated sodium channel (VGSC) protein
- SNPs in the VGSC gene can change the protein and cause resistance
- Known resistance SNPs
  - L995F ("kdr west")
  - L995S ("kdr east")
  - L995F + N1570Y
Prevalence of *kdr* L995F

- *An. gambiae*: Cameroon (53%), Ghana (100%), Burkina Faso (100%), Guinea (100%), Gabon (33%)
- *An. coluzzii*: Angola (84%), Ghana (82%), Burkina Faso (85%), Cote d'Ivoire (91%), Guinea (88%)
Prevalence of *kdr* L995S

- *An. gambiae*: Cameroon (16%), Gabon (67%), Uganda (100%), Kenya (76%)
Prevalence of \textit{kdr} L995F + N1570Y

- \textit{An. gambiae}: Cameroon (6\%), Ghana (17\%), Burkina Faso (21\%), Guinea (9\%)
- \textit{An. coluzzii}: Burkina Faso (27\%)
New resistance SNPs?

- L995F + R254K
- L995F + D466H + I1940T
- L995F + T791M + A1746S
- L995F + E1597G
- L995F + K1603T
- L995F + V1853I
- L995F + I1868T
- L995F + P1874S
- L995F + P1874L
- L995F + F1920S
- L995F + A1934V
- V402L + I1527T

There is much more to pyrethroid target-site resistance than just kdr!
Use genetic backgrounds (haplotypes) to infer outbreaks of resistance. E.g., "F1" is a major outbreak driven by \textit{kdr} L995F, has spread throughout West and Central Africa, and spread between \textit{An. gambiae} and \textit{An. coluzzii}.
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

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This article is a preprint and has not been peer-reviewed [what does this mean?].

**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

(Will be updated with new data from Ag1000G phase 2 soon.)
Pyrethroid resistance mechanisms

Possible to combine data on target-site and metabolic resistance, to show which molecular mechanisms are present in which populations.
Organophosphate resistance

- Organophosphates bind to acetylcholinesterase (ACE1) enzyme
- Genetic changes in ACE1 gene associated with resistance
  - SNPs (G119S) and CNVs
Prevalence of ACE1 SNPs and CNVs

G119S and CNV always found together. Spreading in West Africa, both An. gambiae and An. coluzzii.
Unknown/emerging resistance mechanisms

- GSTE CNVs - major role in metabolic resistance of pyrethroids?
- Carboxylesterases - role in organophosphate resistance?
- Emerging signals at other genes in acetylcholine regulation pathway?
Translation to surveillance

- Use cases in insecticide resistance management
  - Deployment of next-generation LLINs
  - Rotation of next-generation IRS
- Need recent samples
- Need better geographical coverage
- Need more species (e.g., *An. arabiensis*, *An. funestus*)
- Scale up whole-genome sequencing at genome centres
  - N.B., Wellcome Sanger Institute can now sequence 10,000 mosquitoes/year
- Scale up targeted (amplicon) sequencing at local/region labs
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Extra slides
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