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

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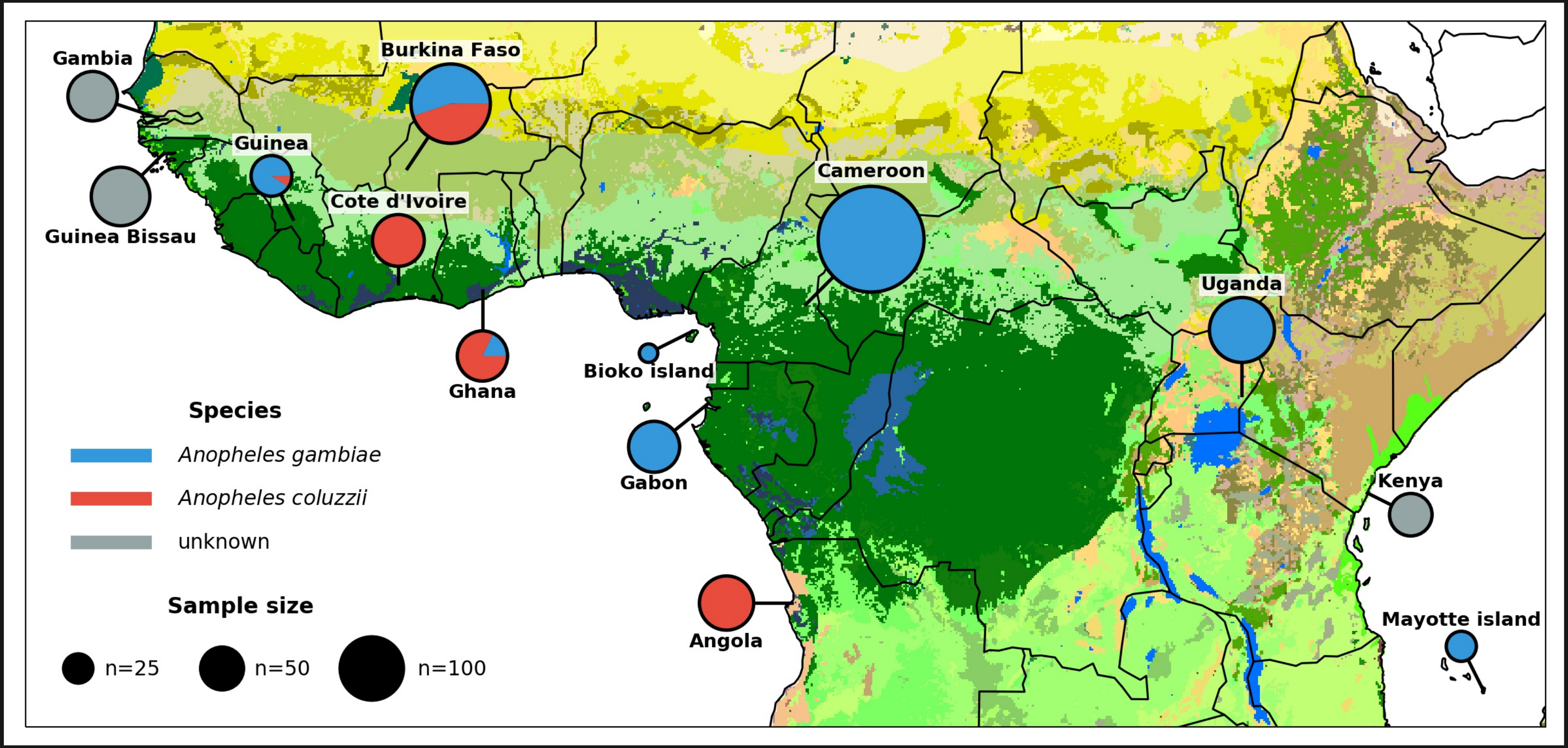
The *Anopheles gambiae* 1000 Genomes Consortium

These slides: alimanfoo.github.io/slides/pamca2019/

Anopheles gambiae 1000 Genomes Project - phase 2

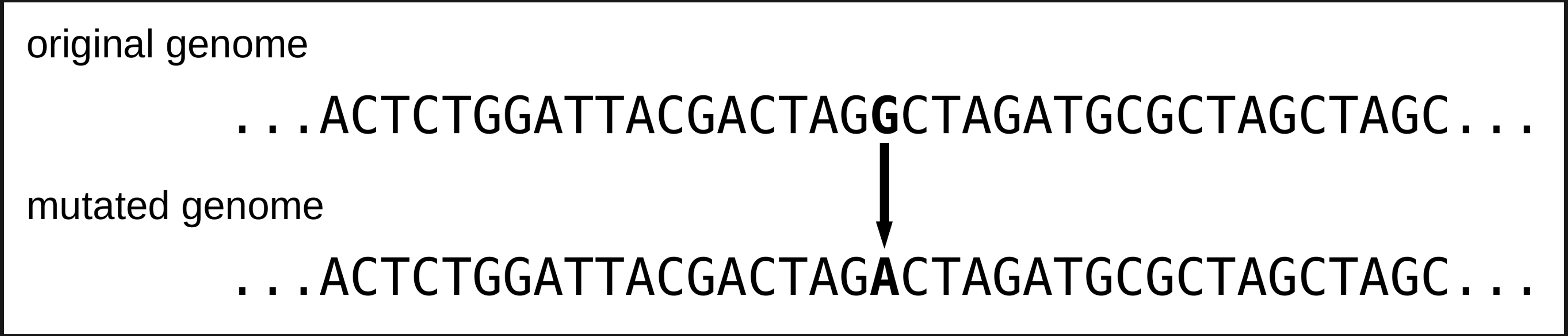


N.B., one location per country; one time point per country; different countries sampled in different years; nothing more recent than 2012.

Methods

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

Single nucleotide polymorphisms (SNPs)



original genome

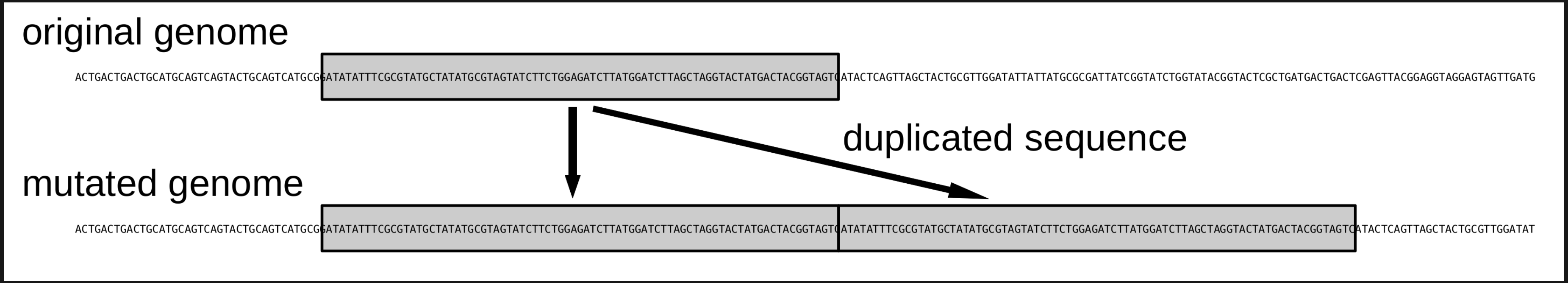
...ACTCTGGATTACGACTAG**G**CTAGATGCGCTAGCTAGC...

mutated genome

...ACTCTGGATTACGACTAG**A**CTAGATGCGCTAGCTAGC...

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

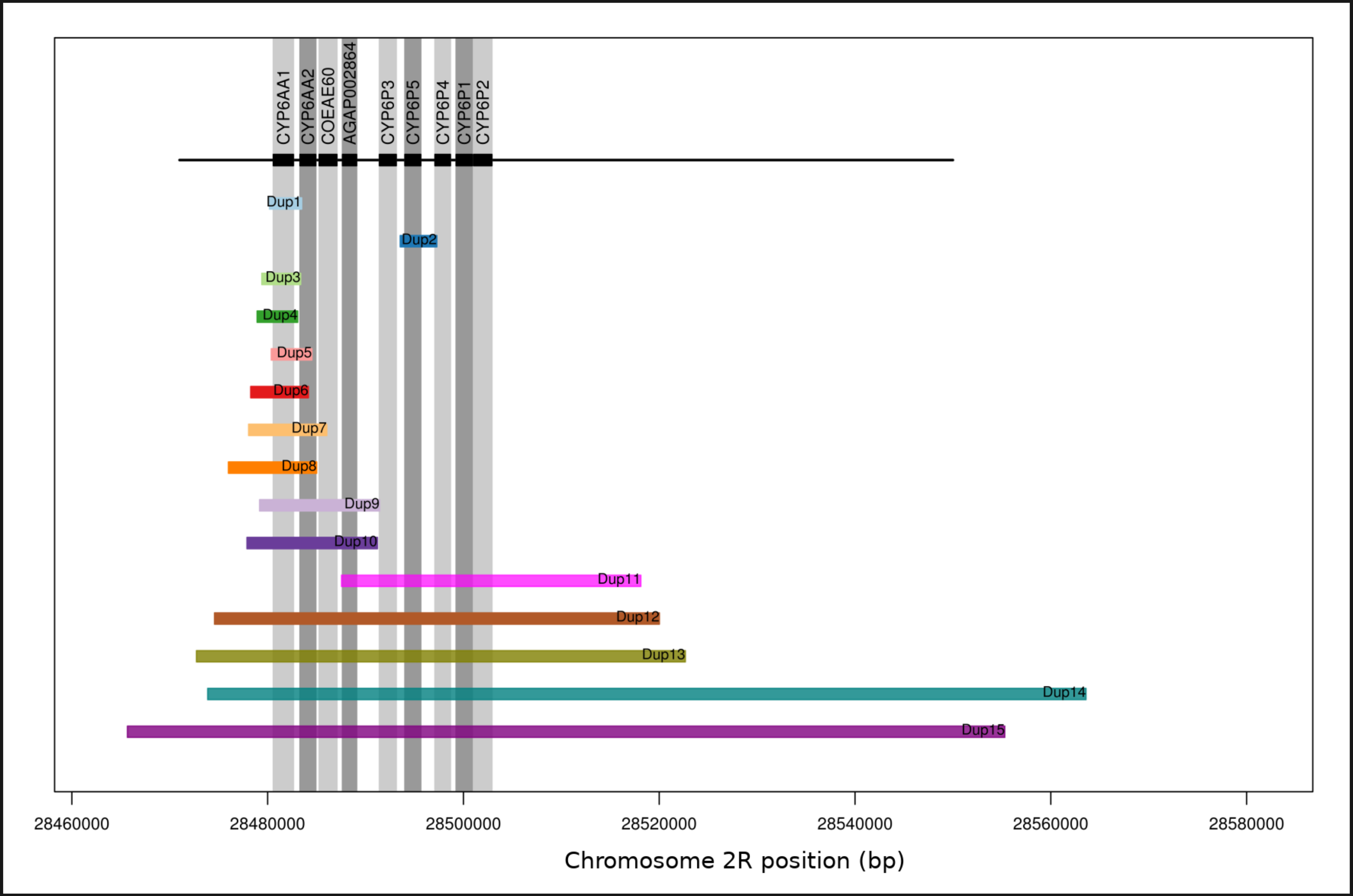
- Pyrethroids are metabolised by cytochrome P450 (CYP) enzymes (a.k.a. mixed-function oxidases; MFOs)
- Increased expression of certain CYP genes causes resistance
- Genetic variants causing metabolic resistance **not previously known** in *An. gambiae* complex
    - Although long suspected that CNVs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

CNV hot spots

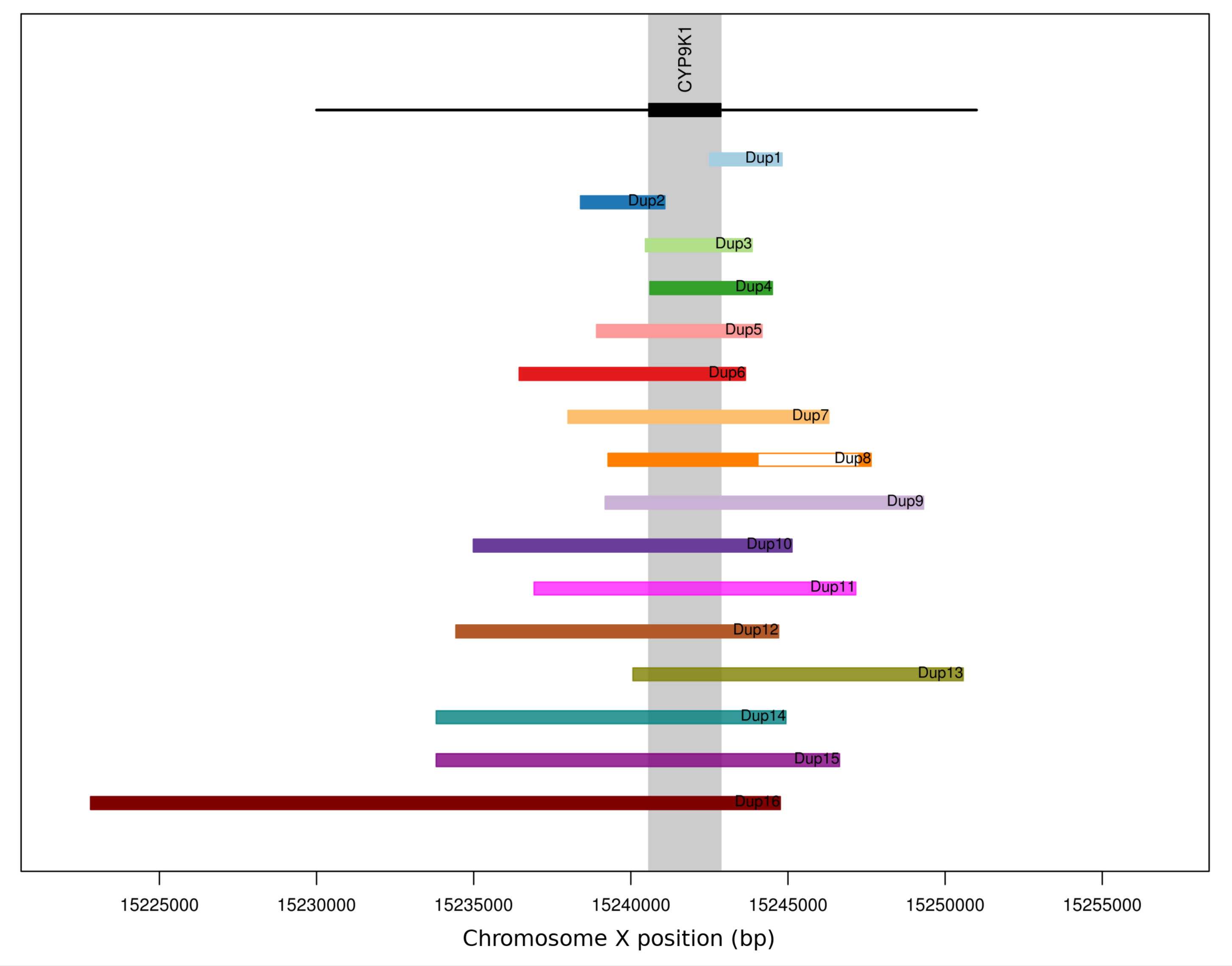
Genome locations where we find a lot of CNVs at high population frequency:

- CYP6P/AA (cytochrome P450 gene cluster)
- CYP9K1 (cytochrome P450 gene)
- GSTE (glutathione S-transferase gene)

CNVs at CYP6P/AA



CNVs at CYP9K1



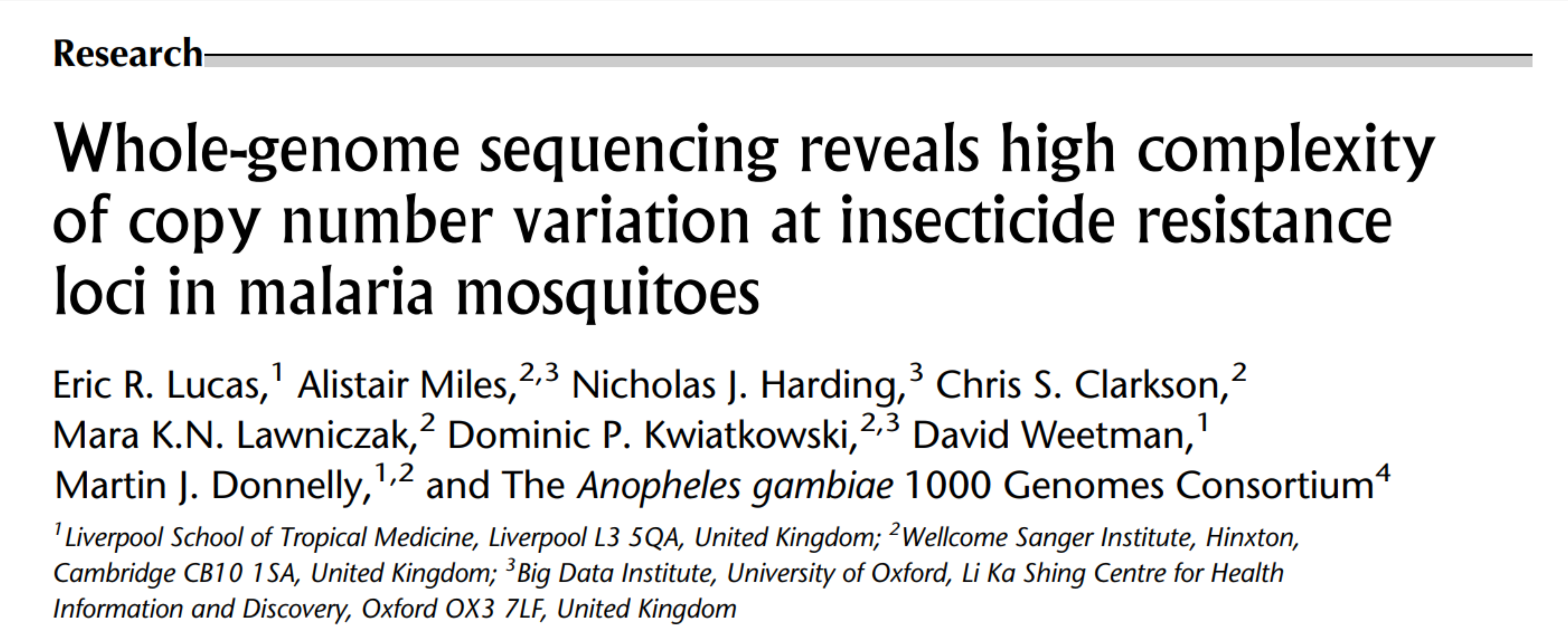
Prevalence and spread of CYP6P/AA CNVs

- 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



Research

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

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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 *kdr* L995F + N1570Y

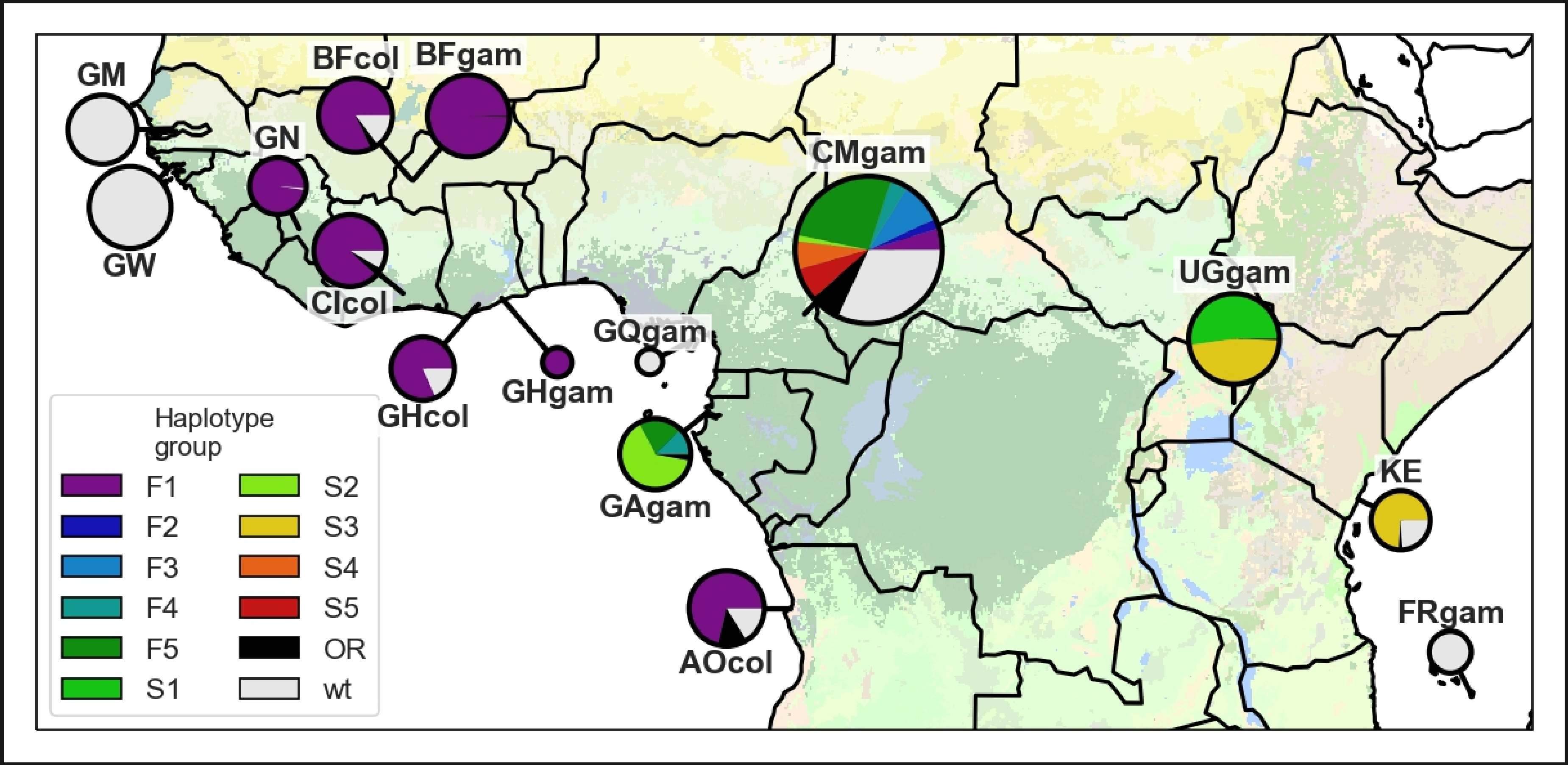
- *An. gambiae*: Cameroon (6%), Ghana (17%), Burkina Faso (21%), Guinea (9%)
- *An. coluzzii*: Burkina Faso (27%)

New resistance SNPs?

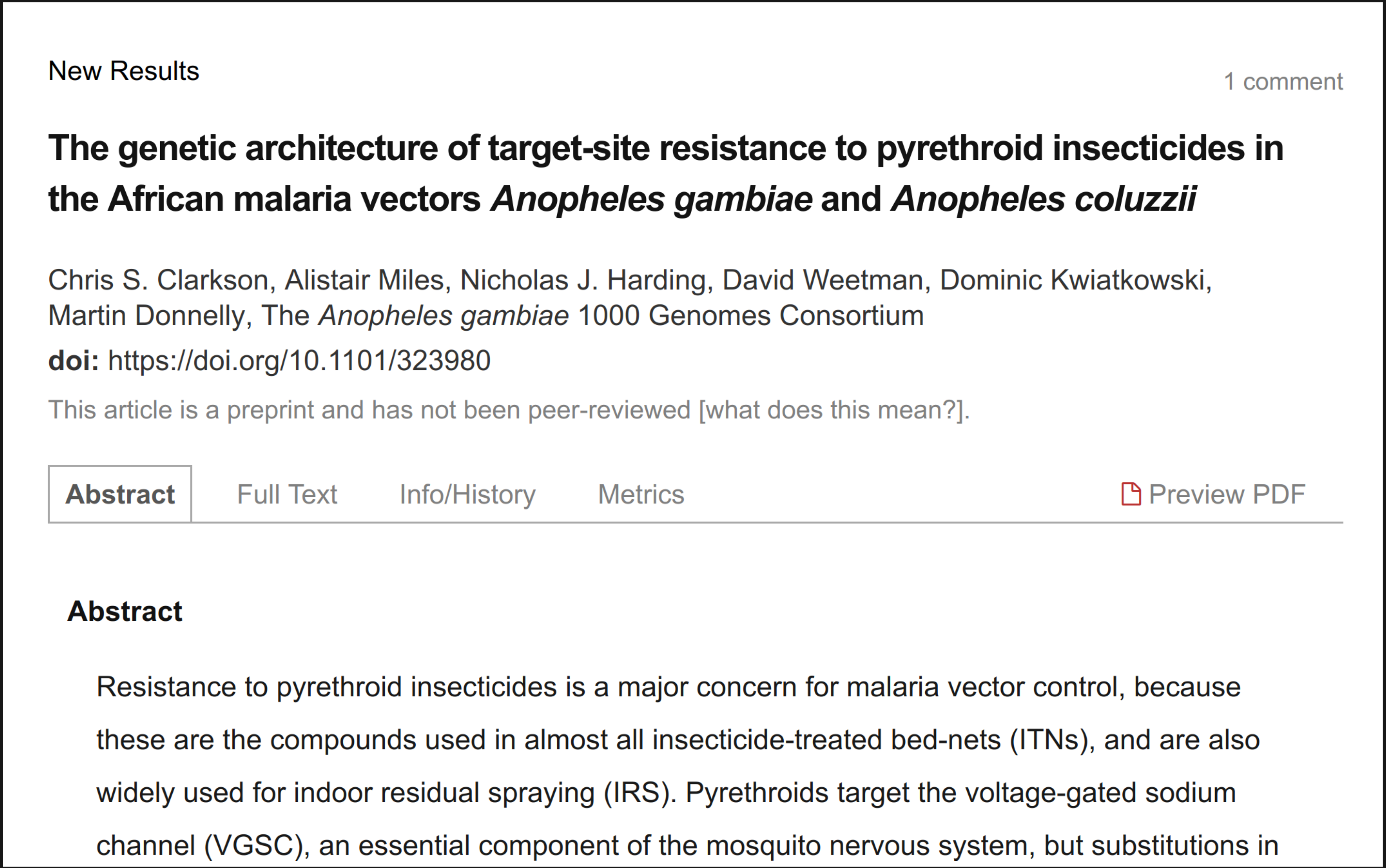
- L995F + R254K
- L995F + D466H + I1940T
- L995F + T791M + A1746S
- L995F + E1597G
- L995F + K1603T
- L995F + V1853I
- L995F + I1868T
- L995F + P1874S
- L995F + P1874L
- L995F + F1920S
- L995F + A1934V
- V402L + I1527T

There is **much** more to pyrethroid target-site resistance than just *kdr*!

Spread of target-site resistance



Use genetic backgrounds (haplotypes) to infer outbreaks of resistance. E.g., "F1" is a major outbreak driven by *kdr* L995F, has spread throughout West and Central Africa, and spread between *An. gambiae* and *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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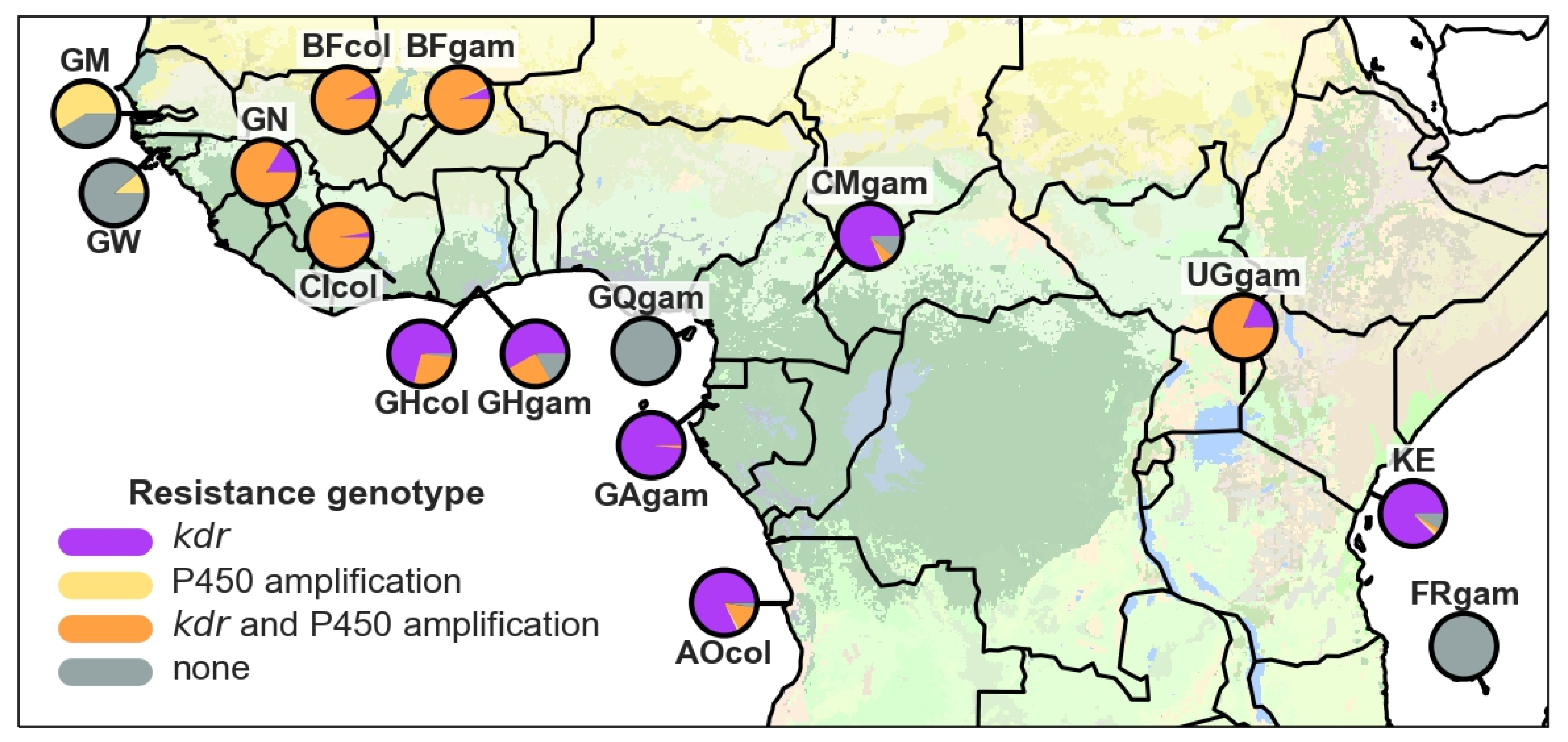
This article is a preprint and has not been peer-reviewed [what does this mean?].

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

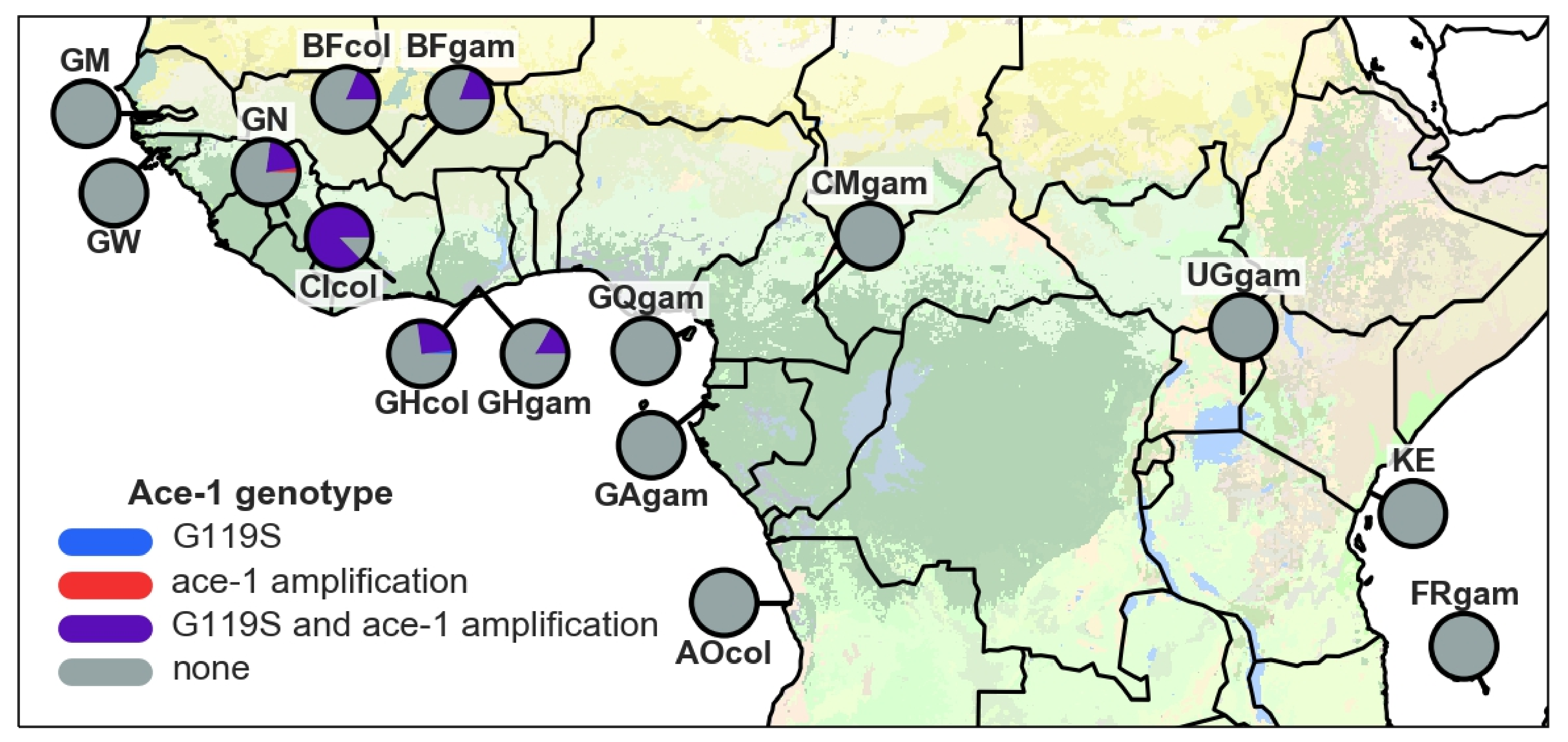


Possible to combine data on target-site and metabolic resistance, to show which molecular mechanisms are present in which populations.

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

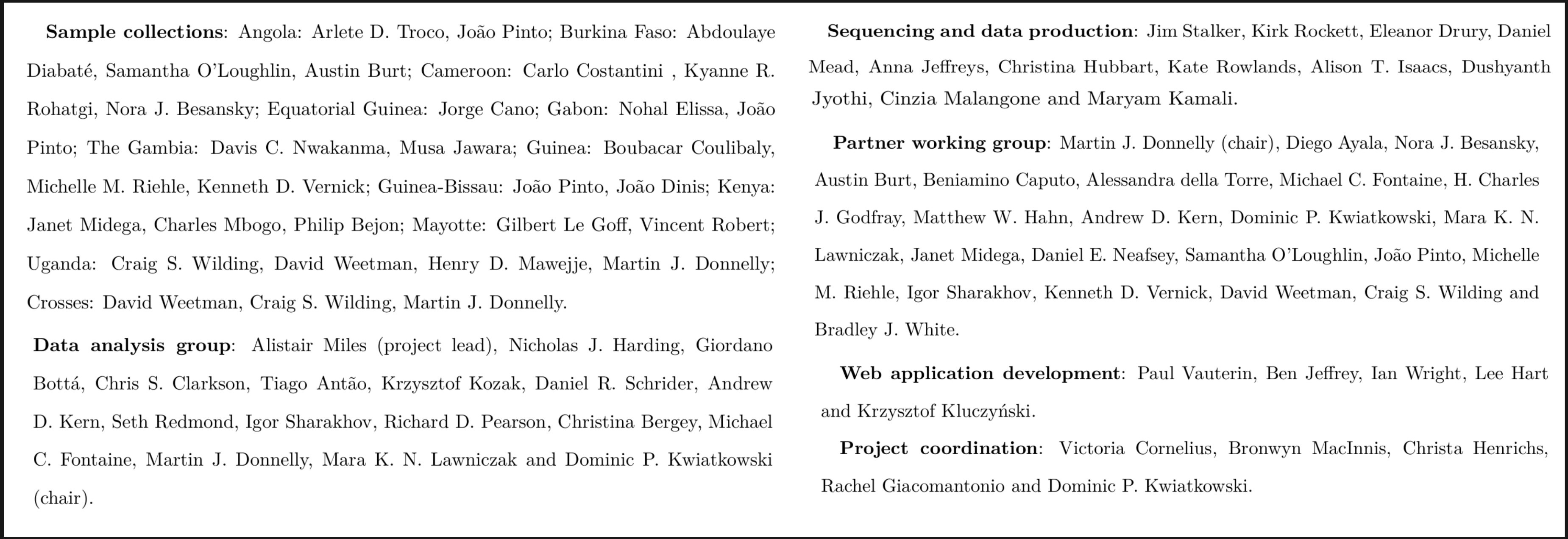
- GSTE CNVs - major role in metabolic resistance of pyrethroids?
- Carboxylesterases - role in organophosphate resistance?
- Emerging signals at other genes in acetylcholine regulation pathway?

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

Acknowledgments

This work was carried out by the *Anopheles gambiae* 1000 Genomes Consortium:



Sample collections: Angola: Arlete D. Troco, João Pinto; Burkina Faso: Abdoulaye Diabaté, Samantha O'Loughlin, Austin Burt; Cameroon: Carlo Costantini , Kyanne R. Rohatgi, Nora J. Besansky; Equatorial Guinea: Jorge Cano; Gabon: Nohal Elissa, João Pinto; The Gambia: Davis C. Nwakanma, Musa Jawara; Guinea: Boubacar Coulibaly, Michelle M. Riehle, Kenneth D. Vernick; Guinea-Bissau: João Pinto, João Dinis; Kenya: Janet Midega, Charles Mbogo, Philip Bejon; Mayotte: Gilbert Le Goff, Vincent Robert; Uganda: Craig S. Wilding, David Weetman, Henry D. Mawejje, Martin J. Donnelly; Crosses: David Weetman, Craig S. Wilding, Martin J. Donnelly.

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Extra slides

Collection						***Anopheles* species counts**		
Country	Location	Site	Year	Latitude	Longitude	*gambiae*	*coluzzii*	Unknown
Angola	Luanda		2009	-8.8210	13.2910	0	78	0
Burkina Faso	Bana		2012	11.2330	-4.4720	20	40	0
	Pala		2012	11.1500	-4.2350	46	10	0
	Souroukoudinga		2012	11.2350	-4.5350	26	25	0
Cameroon	Daiguene		2009	4.7770	13.8440	96	0	0
	Gado Badzere		2009	5.7470	14.4420	73	0	0
	Mayos		2009	4.3410	13.5580	105	0	0
	Zembe Borongo		2009	5.7470	14.4420	23	0	0
Cote d'Ivoire	Tiassale		2012	5.8984	-4.8229	0	71	0
Equatorial Guinea	Bioko		2002	3.7000	8.7000	9	0	0
France	Mayotte	Bouyouni	2011	-12.7378	45.1417	1	0	0
		Combani	2011	-12.7787	45.1429	5	0	0
		Karihani Lake	2011	-12.7965	45.1217	3	0	0
		Mont Benara	2011	-12.8570	45.1552	2	0	0
		Mtsamboro Forest Reserve	2011	-12.7027	45.0811	1	0	0
		Mtsanga Charifou	2011	-12.9907	45.1557	8	0	0
		Sada	2011	-12.8521	45.1039	4	0	0
Gabon	Libreville		2000	0.3840	9.4550	69	0	0
Gambia, The	Njabakunda	Kerr Birom Kardo	2011	13.5500	-15.9000	0	0	19
		Kerr Sama Kuma	2011	13.5500	-15.9000	0	0	8
		Maria Samba Nyado	2011	13.5500	-15.9000	0	0	18
		Sare Illo Buya	2011	13.5500	-15.9000	0	0	20
Ghana	Koforidua		2012	6.0945	-0.2609	0	1	0
	Madina		2012	5.6685	-0.2193	12	12	0
	Takoradi		2012	4.9122	-1.7740	0	20	0
	Twifo Praso		2012	5.6086	-1.5493	0	22	0
Guinea	Koraboh		2012	9.2500	-9.9170	22	0	0
	Koundara		2012	8.5000	-9.4170	18	4	0
Guinea-Bissau	Antula		2010	11.8910	-15.5820	0	0	58
	Safim		2010	11.9569	-15.6492	0	0	33
Kenya	Kilifi	Junju	2012	-3.8620	39.7450	0	0	16
		Mbogolo	2012	-3.6350	39.8580	0	0	32
Uganda	Tororo	Nagongera	2012	0.7700	34.0260	112	0	0