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Cytochrome P450s in *Anopheles gambiae* (Diptera: Culicidae) and Insecticide Resistance in Africa: A Mini Review

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Abstract

Cytochrome P450s are known to be critical for the detoxification and/or activation of xenobiotics such as insecticides in all living organisms including *Anopheles gambiae*. Many studies have demonstrated the role of P450s in insecticide resistance in *A. gambiae*. However, little is known about the impact of distribution in the African sub-continent. In this paper therefore, we analyse the P450 clans, the CYP6 family, localisation and function of *A. gambiae* CYPs, their insecticide substrates, regional distribution in the African continent and their role in insecticide resistance. This investigation from published data revealed that in the Central region; CYP6Z3, CYP6Z1, CYP12F2, CYP6P4, CYP6GA1, CYP6Z3 (Yaoundé, Cameroun) have bendiocarb, DDT and pyrethroids as substrates; in the Eastern region: CYP314A1 and CYP12F1 (Tanzania and Zanzibar) have DDT as a substrate, CYP32A3, CYP6Z1 and CYP6Z2 (Western Kenya) have DDT, carbaryl and permethrin; whilst in the Western region: CYP6AG1, CYP6Z2, CYP6Z3, CY6P3, CYP6P4, CYP6M2 (Ghana), CYP6M2, CYP6P3 (Benin), CYP325A3, CYP6P3 and CYP6M2 (Nigeria) all have DDT, carbaryl, permethrin, trans-and cis-permethrin, deltamethrin, bendiocarb as substrates. Additionally, CYP6M2, CYP6P3, CYP6Z3 (Côte d'Ivoire), CYP6P3, CYP6Z2 and CYP9J5 (Burkina Faso) have bendiocarb, DDT plus pyrethroids and only pyrethroids as substrates respectively. Interestingly, CYP6P3 is observed to metabolize all the available insecticides (DDT, pyrethroid, trans- and cis-permethrin, deltamethrin and bendiocarb), indicating possible insecticide cross resistance across all the three regions of Africa. A more detailed understanding of the substrate specificities of various P450s and the geographical distribution of insecticide resistance in Africa is quintessential for an effective resistance management.

Keywords: *Anopheles gambiae*; Cytochrome P450s; Insecticide; Xenobiotics

Introduction

Vector borne diseases (VBDs) are typically of zoonotic importance with a global threat to human life and animal welfare, accounting for more than one million deaths and rendering hundreds of millions of human lives at the risk of infection annually [1]. Anopheles mosquitoes including *A. gambiae* found in Sub-Saharan Africa are the most efficient and predominant vectors, responsible for about 90% of malaria-related deaths [2]. Malaria continues to be a major global public health problem with about half of the global population (3.2 billion people) at risk in more than 106 endemic countries [3]. With an estimated 0.65-1.2 million deaths annually, it accounts for about 40 to 45 million DALYs (Disability-Adjusted Life Years) [4] and the cost to Africa alone in lost Gross Domestic Product (GDP) is estimated at £7.13 billion annually which accounts for 40% of the continent's public health spending [5].

Vector control is a major component of the global strategy for malaria control which aims to prevent parasite transmission mainly through interventions targeting adult *Anopheline* vectors [6]. Different classes of insecticides have been successively used to date, but most current control programmes are largely dependent on synthetic pyrethroids, the only class of insecticide that has been approved by World Health Organisation (WHO) to be used for both Insecticides treated nets (ITNs) and Indoor Residual Spray (IRS) [7]. This is due to their safe, cheap, effective, long lasting nature and minimal mammalian toxicity [5].

The resistance to pyrethroids is mainly due to three mechanisms; reduction in sensitivity of the target site, reduced penetration due to an altered cuticle or increase in enzyme metabolism [8]. The detoxification enzyme-based resistance occurs when increased activity of cytochrome P450 monooxygenases and Glutathione S-Transferases (GSTs) results in sequestration or detoxification of the insecticide thereby impairing the toxicity of the insecticide before it reaches its target site [9].

Cytochromes P450 are one of the largest and most functionally diversified classes of heme-containing enzymes found in nature and are involved mainly in developmental processes and xenobiotic metabolism in insects including *A. gambiae* [10]. It has been well documented that P450-mediated pyrethroid resistant insects strains have higher levels or more efficient enzyme forms of one or more P450s compared to susceptible strains [11-13]. Cytochrome P450s are therefore generally associated with the enzymatic metabolism of insecticides [14]. A number of studies have been established on the characteristic features of P450s in higher mammals including man and a model insect *Drosophila melanogaster*. These features expedite the

identification of these cytochrome P450 enzymes and which among others include; the access paths for substrates and products and the distinctive properties of the active sites with web of water molecules [15]. However, detailed studies on the features of Cytochrome P450s in *A. gambiae* and more significantly their regional distribution which are quintessential for strategic planning of vector control programmes across three different regions of the African continent are scanty. In this paper therefore, we analyse some of the features of cytochrome P450s in *A. gambiae* and their regional distribution in the African sub-continent.

P450 Clans

CYP genes are further classified into clans, families and sub-families based on phylogenetics as well as sequence identity [16]. Clans are defined "as groups of P450 families that consistently cluster together on a phylogenetic tree" [17]. These are higher-order groups and are basically similar to clades, although clades technically refer to species with a common ancestor and not to sequences [18]. The insect CYP6 and CYP9 families belong in a clan with vertebrate CYP3 and CYP5. This has been named the CYP3 clan for the lowest family number in the group. Insects have four clans comprising CYP2, CYP3, CYP4, and mitochondrial CYP11, CYP24, CYP27 families [18].

The CYP6 Family

Although CYP4 and CYP9 families have been earlier reported to display insecticide detoxification activities, the CYP6 family has been implicated in insecticide resistance more often than any other CYP family to date [19]. It is found exclusively in insects and is the most extensively studied P450 group in insects [20]. Within the *A. gambiae* mosquitoes, CYP6 and CYP9 families including; CYP6P3, CYP6M2, CYP6Z2, CYP6P4, CYP6P5 and CYP9J5 have appeared most widely over-transcribed in resistant field populations [21]. More significantly however, only the former two can metabolise the insecticides whilst the later only encodes for enzymes that are able to bind to pyrethroids. Other P450s including CYP3 involved in insecticide metabolism in *A. gambiae* include; CYP6ZI and CYP32SA3. Previous studies also revealed that CYP9M10 for *Culex quinquefasciatus* [20], and CYP6G1 [22] for *D. melanogaster* are repeatedly reported to be involved in insecticide resistance.

Cytochrome P450s in *Anopheles gambiae* and their Regional Distribution in the African Continent

In *Anopheles gambiae* genome alone, there are 111 annotated Cytochromes P450 [5], seven of which are pseudo genes [18]. Of these P450s, CYP314A1, CYP12F1 and CYP6Z1 are involved in the metabolism of DDT in *A. gambiae* in Tanzania and Zanzibar (Eastern Africa) and Western Kenya (Eastern Africa) respectively [11,12,23-25]. In the same vein, permethrin is metabolised by CYP325A3 in *A. gambiae* in Nigeria (Western Africa) and in Western Kenya (Eastern Africa) and hitherto by CYP6Z3 in Ghana (Western Africa) [12,24,25]. Previous investigations further revealed that in Yaoundé, Cameroun (Central Africa) a diverse array of genes including CYP6M2, CYP6P3, CYP6Z3 in *A. gambiae* are involved in DDT or pyrethroid resistance [23]. CYP6M2 and CYP6P3 also appeared as predominant candidate genes conferring bendiocarb resistance in a study conducted in *A. gambiae* Côte d'Ivoire (West Africa) [12,21, 24,25]. Similarly, high levels of CYP6M2 gene expression have been found in a DDT-resistant population of *A. gambiae* from Ghana, (West Africa) using a novel whole genome microarray [9,11,12,23,25]. Further studies also revealed high expression levels of CYP6M2, CYP6Z2 and CYP6Z3 establishing their involvement in pyrethroid resistance in *A. gambiae* [12,23,24]. Investigations conducted in Nigeria (Western Africa) revealed that CYP325A3 (previously not involved in resistance) as constitutively over-expressed in a pyrethroid resistant strain of *A. gambiae* [25]. In the Western African region, studies revealed that CYP6M2, CYP6Z2 and CYP6P3 were repeatedly expressed conferring pyrethroid resistance in *A. gambiae* whilst CYP6P3 and CYP6M2 conferred resistance to bendiocarb [12,21,24,25]. These P450s are also typically involved in insecticide resistance in the Central African region with CYP6M2 reported to confer DDT cross resistance [11,12,21,23,25]. Interestingly, CYP6P3 has been repeatedly expressed in pyrethroid resistance strains across the West African region [11,12,23,25]. The extensive distribution of pyrethroid resistance in the East and West African regions demonstrates that pyrethroid resistance is ubiquitous in *A. gambiae* populations across the three regions of Africa (Table 1).

From Table 1, it can be deduced that permethrin, is the most metabolized insecticide in the West African region (CYP325A3, CYP6Z3, CYP6P3 and CYP6M2) and some parts of East African region (CYP325A3). More significantly however, DDT is observed to be metabolized across all the reviewed regions; Central Africa region (CYP6M2, CYP6P3 and CYP6Z3); East African region (CYP314A1, CYP12F1 and CYP6Z1). More significantly, CYP6P3 is also observed to metabolize all the available insecticides (DDT, pyrethroid, trans and cis-permethrin, deltamethrin and bendiocarb) across all the regions in Africa. These wide scope of resistance screen suggest multiple resistance mechanisms and also demonstrate the possibility of insecticide cross resistance across all the three studied regions.

Role of P450s in Insecticide Resistance

In mosquitoes including *A. gambiae*, resistance is typically associated with combination of target site modification which involves mutations leading to well-defined target site alteration resulting in resistance to chemical insecticides and metabolic resistance [26]. For instance, carbamate resistance reported in Yaoundé, Cameroun (Central African region) was revealed through metabolic resistance [21] and knockdown resistance of *A. gambiae* population to Tiassalé, Ivory Coast (West African region) to Deltamethrin [11]. This metabolic resistance necessitates further precise modifications in the expression of a complex aggregation of enzymes and detoxification pathways [9]. Metabolic resistance involving elevated levels of P450 has been documented across all the regions of the African Continent.

Conclusions and Perspectives

Insecticide resistance, driven by the xenobiotic detoxification role of cytochrome P450s is clearly widespread in *A. gambiae* vectors across the three regions of the African continent. In this review, we revealed the regional distribution of CYP genes and their respective insecticide substrates. The distribution of the CYP6 family members linked to resistance is diverse across the Central, East and West regions of Africa. An understanding of this distribution difference is therefore expected to support the effort to identify new insecticide targets and strategies with which to control mosquitoes and mosquito-borne diseases in Africa by modifying the activities of insecticide usage and preventing cross resistance. This knowledge will help us build a better understanding of the regulatory pathways of insecticide detoxification and evolutionary insecticide selection in mosquitoes in the different

regions. Understanding which insecticides are metabolized by what P450s is important to outline prospective strengths and liabilities of insecticide to guide the development of vector control compounds. These are quintessential for an incisive insecticide resistance management.

Regions	Countries	P450s	Substrates	Sources
Central	Cameroon	CYP6Z3, CYP6Z1, CYP12F2CYP6P4, CYP6GA1	Bendiocarb	22, 26
	Yaoundé, Cameroun	CYP6M2, CYP6P3, CYP6Z3	DDT, Pyrethroid	23
East	Tanzania, Zanzibar	CYP314A1, CYP12F1	DDT	11, 25
East	Western Kenya	CYP325A3	Permethrin	25
East	Western Kenya	CYP6Z1	DDT, carbaryl	11, 25
West	Nigeria	CYP325A3	Permethrin	25
West	Ghana	CYP6Z3	Permethrin	25
	Ghana	CYP6Z2	Carbaryl	24
West	Benin, Ghana, Nigeria	CYP6P3	Trans-and cis-permethrin, deltamethrin	12, 24, 25
West	Ghana	CYP6AG1, CYP6Z3 CYP6P4	DDT, Pyrethroid	24
West	Benin, Ghana, Nigeria	CYP6M2	Permethrin, bendiocarb, DDT, Deltamethrin	25, 24, 12
West	Côte d'Ivoire	CYP6M2, CYP6P3, CYP6Z3	Bendiocarb, DDT, Pyrethroid	12

Table 1: Some common P450s involved in insecticide metabolism and their substrates in *Anopheles gambiae* in the African continent are highlighted.

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References

1. Gubler DJ (2009) Vector-borne diseases. Scientific and Technical Review of the Office. Int Epiz 28: 583-588.
2. Sewe M, Rocklöv J, Williamson J, Hame M, Nyaguara A, et al. (2015) The Association of Weather Variability and Under, Five Malaria Mortality in KEMRI/CDC HDSS in Western Kenya 2003 to 2008: A Time Series Analysis. Int J Environ Res Public Health 2: 1983-1997.
3. WHO. World malaria report (2015) Geneva: World Health Organization; 2015.
4. Zofou D, Nyasa RB, Nsagha DS, Ntiekang F, Meriki HD, et al. (2014) Control of malaria and other vector-borne protozoan diseases in the tropics: enduring challenges despite considerable progress and achievements. Infect Dis Poverty 3: 1.
5. Mohammed BR, Wilding CS, Collier PJ, Deeni YY (2014) Cloning of *Anopheles gambiae* CYP6M2 gene promoter and construction of its luciferase reporter system. Int J Sci Tech Res 3: 7.
6. Gueye CS, Newby G, Gosling RD, Whittaker MA, Chandramohan D, et al. (2016) Strategies and approaches to vector control in nine malaria-eliminating countries: a cross-case study analysis. Mal J 1:1.
7. Bhatt S, Weiss DJ, Cameron E, Bisanzio D, Mappin B, et al. (2015) The effect of malaria control on Plasmodium falciparum in Africa between 2000 and 2015. Nature 7572: 207-211.
8. Li CX, Guo XX, Zhang YM, Xing D, Yan T, et al. (2016) Identification of genes involved in pyrethroid-, propoxur-, and dichlorvos-insecticides resistance in the mosquitoes, Culex pipiens complex (Diptera: Culicidae). Acta Trop 157: 84-95.
9. Mitchell SN, Stevenson BJ, Müller P, Wilding CS, Egyir-Yawson A, et al. (2012) Identification and validation of a gene causing cross-resistance between insecticide classes in *Anopheles gambiae* from Ghana. Proc Natl Acad Sci USA 16: 6147-6152.
10. Guo H, Zhenmin B, Huixia D, Lingling Z, Shi W, et al. (2012) Identification of Cytochrome P450 (CYP) Genes in Zhikong Scallop (Chlamys farreri). J Ocean U China (Oceanic and Coastal Sea Research) 12: 97-102.
11. Edi CV, Djogbenou L, Jenkins AM, Regna K, Muskavitch MA, et al. (2014) CYP6 P450 enzymes and ACE-1 duplication produce extreme and multiple insecticide resistance in the malaria mosquito *Anopheles gambiae*. PLoS Genet 10: 3 pp: 1004236.
12. Mitchell SN, Rigden DJ, Dowd AJ, Lu F, Wilding CS, et al. (2014) Metabolic and target-site mechanisms combine to confer strong DDT resistance in *Anopheles gambiae*. PLoS One 9: 3 p. 92662.
13. Ibrahim SS, Riveron JM, Stott R, Irving H, Wondji CS (2016) The cytochrome P450 CYP6P4 is responsible for the high pyrethroid resistance in knockdown resistance-free Anopheles arabiensis. Insect Biochem Mol Biol 68: 23-32.
14. Wilding CS, Weetman D, Rippon EJ, Steen K, Mawejje HD, et al. (2014) Parallel evolution or purifying selection, not introgression, explains similarity in the pyrethroid detoxification linked GSTE4 of *Anopheles gambiae* and *An. arabiensis*. Mol Genet Genomics 1: 201-215.
15. Cui YL, Xue Q, Zheng QC, Zhang JL, Kong CP, et al. (2015) Structural features and dynamic investigations of the membrane-bound cytochrome P450 17A1. BBA-Biomembranes 10: 2013-2021.
16. Nelson D, Werck-Reichhart D (2011) A P450-centric views of plant evolution. Plant J 1: 194-211.
17. Nelson DR, Phillips IR, Shephard EA (2006) Cytochrome P450 nomenclature, 2004, Cytochrome P450 protocols, Totowa (NJ) Springer 1-10.
18. El Garj FMA, Wajidi MFF (2013) The Cytochrome P450s: Article Review. Asian Pac J Mol Biol Biotechnol 2: 37-41.

19. Zhou D, Liu X, Sun Y, Ma L, Shen B, et al. (2015) Genomic analysis of detoxification supergene families in the mosquito Anopheles sinensis. PloS One 11: e0143387.
20. Mohammed BR, Wilding CS, Amy L, Collier PJ, Deeni YY (2014) Transcriptional regulation of CYP6M2 gene in the mosquito *Anopheles gambiae* cell line. Eur J Biotechnol Biosci 1: 49-55.
21. Antonio-Nkondjio C, Poupardin R, Tene BF, Kopya E, Costantini C, et al. (2016) Investigation of mechanisms of bendiocarb resistance in *Anopheles gambiae* populations from the city of Yaoundé, Cameroon. Malar J 1: 424.
22. Mohammed BR, Kawe MS, Nwachukwu OM, Jegede OC, Agbede RIS, et al. (2017) Understanding the Mechanisms Involved in the Regulation of Cytochrome P450 Gene Expression in Drosophila melanogaster (Diptera: Drosophilidae). Entomol Ornithol Herpetol 189: 2161-0983.
23. Toé KH, N'Falé S, Dabiré RK, Ranson H, Jones CM (2015) The recent escalation in strength of pyrethroid resistance in Anopheles coluzzi in West Africa is linked to increased expression of multiple gene families. BMC Genomics 1: 146.
24. Muller P, Chouaibou M, Pignatelli P, Etang J, Walker ED, et al. (2007) Pyrethroid tolerance is associated with elevated expression of antioxidants and agricultural practice in Anopheles arabiensis sampled from an area of cotton fields in Northern Cameroon. Mol Ecol 17: 1145-1155.
25. David JP, Strode C, Vontas J, Nikou D, Vaughan A, et al. (2005) The *Anopheles gambiae* detoxification chip: a highly specific microarray to study metabolic-based insecticide resistance in malaria vectors. Proc Natl Acad Sci USA 11: 4080-4084.
26. Mohammed BR, Abdulsalam YM, Deeni YY (2015) Insecticide resistance to Anopheles spp. mosquitoes (Diptera: Culicidae) in Nigeria: A. Int J Mosq Res 3: 56-63.