

1 **Supplementary Results and Discussion**

2 **1-Genome-wide transcription microarray analysis**

3 **Up-regulated genes:** Beside the main candidate genes mentioned in the

4 main text, other genes were up-regulated in Benin including a mediator complex gene

5 (FC34) which exhibited the highest fold-change. This gene encodes a multiprotein

6 complex that functions as a transcriptional co-activator in all eukaryotes [1], but no

7 known involvement in insecticide resistance has been previously reported. Another

8 detoxification gene was an alpha-esterase ortholog of AGAP006700 (COEAE1G) in

9 *An. gambiae* (FC5.1). This gene family is more involved in resistance to carbamates

10 or organophosphates. Therefore, the up-regulation of this COEAE1G gene could be

11 associated with the bendiocarb resistance observed in the Pahou mosquitoes [2].

12 However, this hypothesis requires confirmation through further functional analyses.

13 Other probes belonging to genes with known association with insecticide resistance

14 were also up-regulated in Pahou (Table S1). Among these genes were several probes

15 that belong to cuticle protein genes, such as the adult-specific cuticular protein acp-20

16 (FC5.2) ortholog of AGAP006897 in *An. gambiae* (Table S1). Many probes

17 belonging to different short-chain dehydrogenases were also up-regulated, notably the

18 ortholog of AGAP005166 in *An. gambiae* (combined_c738; FC26). Other genes that

19 were up-regulated include the ATP-binding cassette (ABC) transporter gene *ABCB7*

20 (combined_c1762; FC2.8), an odorant receptor gene ortholog of AGAP001012

21 (FC8.2) and an odorant-binding protein (OBP4, FC2.7). Another up-regulated gene

22 was glycoprotein 93 (FC11.6), whose ortholog in *Drosophila* is an ortholog of a

23 mammalian heat shock protein gp96 [3], indicating that this gene could protect

24 mosquitoes against the oxidative stress that could potentially result from exposure to

25 insecticides.

26 The up-regulation of genes belonging to gene families such as ABC
27 transporters, odorant receptors, cuticular proteins, heat shock proteins, serine
28 proteases, short-chain dehydrogenases and UDP-glucosyltransferases is a common
29 observation in genome-wide transcriptional profiling of insecticide resistant strains.
30 Indeed, such up-regulation has previously been observed in different insect species
31 such as *D. melanogaster* [4], *An. gambiae* [5, 6], *Ae. aegypti* [7] and *An. funestus* [8],
32 with genes from these families involved in the various steps of the metabolism
33 pathways of insecticides.

34 **Down-regulated genes:** The most down-regulated probe belonged to the
35 cytochrome c oxidase subunit 3 (CD577188; FC44.3), and this down-regulation is
36 supported by the fact that several probes belonging to this gene were also down-
37 regulated. Other down-regulated probes include several probes for cytochrome b gene
38 (CD577249; FC20.4) and a monkey king protein (combined_c1873; FC 18) (Table
39 S2).

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41 **2-Genetic diversity of GSTe2 across Africa**

42 **Haplotype distribution of GSTe2 across Africa:** A total of 46 *An. funestus*
43 mosquitoes (2n=92) were analyzed across Africa and revealed 37 polymorphic sites,
44 of which eight were singletons (Table 2). One insertion/deletion (T/-) was observed in
45 intron 1. A total of 39 haplotypes were observed for the full gene (Figure S3B), 16 for
46 coding regions (Figure S3C) and 7 protein variants (Figure S3D). When considering
47 the full gene, the resistant haplotype BN23 was predominant, accounting for 34.8% of
48 the total sample due to its nearly fixed frequency in Benin (95.8%) and its presence in
49 Ghana (37.5%) and Cameroon (21.4%). The susceptible haplotype MAL3 was the
50 next highest-frequency haplotype, with 14.1% mainly due to its predominance in

51 Malawi (44.4%) but also due to its broader geographical distribution, with a presence
52 in Mozambique (20%), Cameroon (14.3%) and Uganda (10%). In addition, 31 of the
53 39 haplotypes were singletons, which is indicative of a high haplotypic diversity of
54 GSTe2 across Africa ($0.78 < h_d < 0.97$) but not in Benin, where very low haplotype
55 diversity was observed ($h_d = 0.08$). When considering only the coding region, the
56 frequency of these two haplotypes increased particularly for the susceptible MAL3,
57 which increased from 14.1 to 33.7% while the BN23 increased from 34.8 to 39.1%
58 (Figure S3C). Their frequencies were even higher when only analyzing the non-
59 synonymous substitutions, with 45.6% for the original BN23 and 43.7% for the
60 MAL3 haplotype (Figure S3D).

61 **3-Structural basis of DDT resistance conferred by GSTe2**

62 Attempts to obtain the structure of another allele susceptible L119-GSTe2
63 from Malawi were not successful. The crystal structure of BN-GSTe2 allele was
64 successfully determined without (apo-GSTe2) and with the co-factor glutathione
65 (GSH) (holo-GSTe2), whereas for the UG allele, only the holo structure was
66 determined.

67 **Description of the GSH site:** The GSH molecule lies in a polar cavity with its
68 γ -glutamyl region forming hydrogen bonds with the Glu67 side chain, the main chain
69 amide and the hydroxyl group of Ser68 and the positively charged side chain of the
70 Arg112. The cysteinyl moiety forms two hydrogen bonds with the Ile55 carbonyl and
71 amide main chain. Finally, the glycyl moiety establishes hydrogen bonds with the
72 His53 side chain and main chain and with the Arg112 side chain. Several water
73 molecules also stabilize GSH in the cavity. A 3D hydrogen-bond network stabilizes

74 the cofactor into an extended reactive conformation that enhances the formation of the
75 thiolate anion ($-S^-$) in the active GS^- (Figure S6).

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