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A genomic duplication spanning multiple P450s contributes to insecticide resistance in the dengue mosquito Aedes aegypti

Tiphaine Bacot1 , Chloé Haberkorn§,1, Joseph Guilliet§,1,2, Julien Cattel§,1, Mary Kefi§,3, Louis Nadalin¹ , Jonathan Filee² , Frederic Boyer1 , Thierry Gaude¹ , Frederic Laporte¹ , Jordan Tutagata*, John Vontas^{3,4}, Isabelle Dusfour^v, Jean-Marc Bonneville"[,]*, Jean-Philippe David",**
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equal contribution \S equal contribution
* corresponding auth

 $\stackrel{*}{\rule{0pt}{1.5ex}}$ corresponding auth
 $\stackrel{*}{\rule{0pt}{1.2ex}}$

¹ Laboratoire d'Ecologie Alpine (LECA, UMR 5553), Université Grenoble-Alpes (UGA), Université Savoie Mont-Blanc (USMB), " Laboratoire d'Ecologie Alpine (LECA, UMR 5553), Université Grenoble-Alpes (UGA), Université Savoie Mont-Blanc (USMB),
CNRS, 38041 Grenoble, France.
² Université Paris-Saclay, CNRS, IRD, UMR Évolution, Génomes, Comporte

,
² Université Paris-Saclay, CNRS,
³ Institute of Molecular Biology

Université Paris, Paris, IRD, 2003, IRD, 2003, IRD, 2003, Peripertement et Ecologie, Periodic Paris-Terre, Ten
Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology Hellas, Heraklion, Gre ³ Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology Hellas, Heraklion, Greece.

Institute of Molecular Biology and Biology, Foundation for Molecular Biology (1988), Hellas, America, Biology
Pesticide Science Laboratory, Department of Crop Science, Agricultural University of Athens, Athens, Greece.
Vec ⁻ Pesticide Science Laboratory, Department of Crop Science, Agricultural University of Athens, Athens, Greece.
⁵ Vectopôle Amazonien Emile Abonnenc, Institut Pasteur de la Guyane, Cayenne, France ; Global Health Depart $\frac{5}{1}$ Vector Amazonien Emile Amazonien Emile Abonnenc, Institut Pasteur, Paris, France ; Global Health Department, France ; Institut Pasteur, Paris, France.
* jean-philippe.david@univ-grenoble-alpes.fr

 $\frac{1}{2}$ jean-philippe.david.

Abstract

1 Resistance of mosquitoes to insecticides is one example of rapid adaptation to anthropogenic
2 Selection pressures having a strong impact on human health and activities. Target-site modification 3 and increased insecticide detoxification are the two main mechanisms underlying insecticide 4 resistance in mosquitoes. While target-sites mutations are well characterised and often used to track 5 resistance in the field, the genomic events associated with insecticide detoxification remain partially 5 resistance in the field, the genomic events associated with insecticide detoxification remain partially 6 characterised. Recent studies evidenced the key role of gene duplications in the over-expression of 7 detoxification enzymes and their potential use to track metabolic resistance alleles in the field.
8 However, such genomic events remain difficult to characterise due to their complex genomic 9 architecture and their co-occurrence with other resistance alleles. In this concern, the present work 10 investigated the role of a large genomic duplication affecting a cluster of detoxification enzymes in 10 investigated the role of a large genomic duplication affecting a cluster of detoxification enzymes in 11 conferring resistance to the pyrethroid insecticide deltamethrin in the mosquito Aedes aegypti. 12 Two isofemale lines originating from French Guiana and being deprived from major target-site 13 mutations showed distinct insecticide resistance levels. Combining RNA-seq and whole genome pool-14 seq identified a 220 Kb genomic duplication enhancing the expression of multiple contiguous
15 cytochrome P450s in the resistant line. The genomic architecture of the duplicated loci was 16 elucidated through long read sequencing, evidencing its transposon-mediated evolutionary origin. 17 The involvement of this P450 duplication in deltamethrin survival was supported by a significant 18 phenotypic response to the P450 inhibitor piperonyl butoxide together with genotype-phenotype 19 association and RNA interference. Experimental evolution suggested that this P450 duplication is 19 association and RNA interference. Experimental evolution suggested that this P450 duplication is 20 associated with a significant fitness cost, potentially affecting its adaptive value in presence of other 21 resistance alleles. 22 Overall, this study supports the importance of genomic duplications affecting detoxification enzymes

23 in the rapid adaptation of mosquitoes to insecticides. Deciphering their genomic architecture

24 provides new insights into the evolutionary processes underlying such rapid adaptation. Such

25 findings and provides new tools for the surveillance and management of resistance in the field.

26 Key words: Mosquito; Insecticide resistance; P450; Gene duplication; Kdr mutation; Aedes aegypti

27

Introduction 28

29 Natural populations
29 Natural populations
30 locally adaptive fear
31 driven by human ac
32 machanisms allowir
33 major goal (Hendry
34 example of rapid ev 29 Natural populations experience and the expression of complex phenotypes (Orr, 2005). Among them, those

29 Nature by human activities promote novel and strong selective pressures. Understanding the genetic

29 The chani 31 driven by human activities promote novel and strong selective pressures. Understanding the genetic
32 mechanisms allowing populations to quickly respond to rapid environmental changes has become a
33 major goal (Hendry 32 mechanisms allowing populations to quickly respond to rapid environmental changes has become a
33 major goal (Hendry et al., 2008, 2017; Palumbi, 2001). Resistance of insects to insecticides is a key
34 example of rapid 33 major goal (Hendry et al., 2008, 2017; Palumbi, 2001). Resistance of insects to insecticides is a key
34 example of rapid evolution under strong anthropogenic selective pressures. This adaptation has
35 occurred quickly 34 example of rapid evolution under strong anthropogenic selective pressures. This adaptation has
35 occurred quickly and independently in a large number of taxa with frequent parallel trajectories
36 (Ffrench-Constant et 35 occurred quickly and independently in a large number of taxa with frequent parallel trajectories
36 (Ffrench-Constant et al., 2004; Liu, 2015). As a consequence of local and temporal variations of t
37 selection pressur 36 (Ffrench-Constant et al., 2004; Liu, 2015). As a consequence of local and temporal variations of t
37 selection pressures, the genetic modifications affecting resistant populations are often complex
38 combine several r selection pressures, the genetic modifications affecting resistant populations are often complex and
combine several resistance mechanisms that are potentially additive, each of them bringing variabl-
fitness costs. In add 38 combine several resistance mechanisms that are potentially additive, each of them bringing variable
39 fitness costs. In addition, the very strong selection pressure exerted by insecticides is prone to
30 produce bottle 39 fitness costs. In addition, the very strong selection pressure exerted by insecticides is prone to
39 fitness costs. In addition, the very strong selection pressure exerted by insecticides is prone to
30 produce bottlen 39 and the very strong selection presenting the strong produce bottleneck effects. Such complexity makes the identification of resistance alleles and the assessment of their respective contribution challenging (Ffrench-Con 41 assessment of their respective contribution challenging (Ffrench-Constant et al., 2004; Li et al., 2 Besides the understanding of the genetics of rapid adaptation and the origins of complex traits,

deciphering the molecular bases of insecticide resistance is also essential for improving pest

management strategies (Hawki deciphering the molecular bases of insecticide resistance is also essential for improving pest

management strategies (Hawkins et al., 2018). Among taxa of major economic and medical

importance, mosquitoes represent a maj deciphering the molecular bases of insecticide resistance is also essential for improving pest

management strategies (Hawkins et al., 2018). Among taxa of major economic and medical

importance, mosquitoes represent a maj 14 management strategies (Framment strategies (Hamkins 2014). The limit of magnetic because of the to transmit human viruses and pathogens (Lounibos, 2002). Among them, *Aedes aegypti* is constituent in portance because of 46 to transmit human viruses and pathogens (Lounibos, 2002). Among them, Aedes aegypti is of

47 particular importance because of its wide distribution and its capacity to transmit Yellow fever,

48 Dengue, Zika and Chikun 147 particular importance because of its wide distribution and its capacity to transmit Yellow fever

147 particular importance because of its wide distribution and its capacity to transmit Yellow fever

148 Dengue, Zika a 18 Dengue, Zika and Chikungunya viruses (Brown et al., 2014). These arboviruses are now re-emer,

19 worldwide following the expansion of mosquitoes' distribution area as a consequence of global

19 warming, global transpo 49 worldwide following the expansion of mosquitoes' distribution area as a consequence of global
50 warming, global transportation network and land perturbations (Kraemer et al., 2015). Although
51 efforts are invested in warming, global transportation network and land perturbations (Kraemer et al., 2015). Althought efforts are invested in improving vaccine-based prevention strategies (Carvalho & Long, 2021; G
et al., 2020; Silva et al., 20 51 efforts are invested in improving vaccine-based prevention strategies (Carvalho & Long, 2021; Ga

52 et al., 2020; Silva et al., 2018), vector control remains the cornerstone of arboviral diseases contr

53 However, dec et al., 2020; Silva et al., 2018), vector control remains the cornerstone of arboviral diseases control.

However, decades of insecticide usage have led to the selection and spread of insecticide resistance

in this mosqui 53 However, decades of insecticide usage have led to the selection and spread of insecticide resistance
54 in this mosquito species, affecting all public health insecticides, including the most-used pyrethroids
55 (Moyes e 54 in this mosquito species, affecting all public health insecticides, including the most-used pyrethroids

55 (Moyes et al., 2017). High pyrethroid resistance has been shown to reduce vector control efficacy

56 (Dusfour 55 (Moyes et al., 2017). High pyrethroid resistance has been shown to reduce vector control efficacy
56 (Dusfour et al., 2011; Marcombe, Carron, et al., 2009; Marcombe et al., 2011; Valle et al., 2019).
57 Though greener v (Dusfour et al., 2011; Marcombe, Carron, et al., 2009; Marcombe et al., 2011; Valle et al., 2019).

57 Though greener vector control strategies are being developed, insecticides will likely remain a key

58 component of in 57 Though greener vector control strategies are being developed, insecticides will likely remain a ke
58 component of integrated vector control in high transmission areas for the next decades (Achee e
59 2019). In this con 58 component of integrated vector control in high transmission areas for the next decades (Achee et 2019). In this concern, identifying the genetic factors underlying resistance is crucial for tracking resistance alleles i 2019). In this concern, identifying the genetic factors underlying resistance is crucial for tracking
resistance alleles in the field and making a better use of the few authorised public health insecticides
through resista Fraction in this concerns in this concerns in this concerns in this concerns in the field and making a better use of the few authorised public health insection
61 through resistance management actions (Cattel et al., 2020;

61 through resistance management actions (Cattel et al., 2020; Corbel & N'Guessan, 2013; Dusfour et al., 2019).
62 al., 2019).
63 In mosquitoes, resistance to chemical insecticides is mainly caused by genetic changes decre 62 al., 2019).

In mosquitoes, resistance to chemical insecticides is mainly caused by genetic changes decreasing t

affinity of the insecticide for its target (target-site resistance), decreasing its penetration (cuticula Example 15

63 In mosquit

64 affinity of t

65 resistance

66 resistance

67 (VGSC gen

68 lead to res 64 affinity of the insecticide for its target (target-site resistance), decreasing its penetration (cuticular
65 resistance), or increasing its detoxification through complex biochemical pathways (metabolic
66 resistance) Frankline Controllers and the insecticides target the neuronal pathways (metabolic resistance) (Li et al., 2007). Pyrethroid insecticides target the neuronal voltage-gated sodium channel (VGSC gene), and the selection of resistance) (Li et al., 2007). Pyrethroid insecticides target the neuronal voltage-gated sodium cl

(VGSC gene), and the selection of *knockdown resistance* (*kdr*) mutations affecting this protein (

lead to resistance. M (VGSC gene), and the selection of *knockdown resistance* (*kdr*) mutations affecting this protein can
lead to resistance. Multiple *kdr* mutations, often combined as haplotypes, have been identified in *Ae.*
aegypti with t Fraction of the phenotype (David et al., 2013). Indeed, the complexity and accounts for a significant part of the phenotype (David et al., 2013). Hada et al., 2017; Hirata et al., 2017 Kasai et al., 2022; Saavedra-Rodrigue ead to resistance in South America:

69 aegypti with the following ones playing a major role in pyrethroid resistance in South America:

70 val410Leu, Val1016lle and Phe1534Cys (Brengues et al., 2003; Haddi et al., 2017; H Val410Leu, Val1016lle and Phe1534Cys (Brengues et al., 2003; Haddi et al., 2017; Hirata et al., 2022; Saavedra-Rodriguez et al., 2007; Smith et al., 2016; Yanola et al., 2011). Todat these mutations can be tracked in the f 71 Kasai et al., 2022; Saavedra-Rodriguez et al., 2007; Smith et al., 2016; Yanola et al., 2011). Today,
72 these mutations can be tracked in the field using PCR-based diagnostic assays or mass sequencing
73 approaches, pr These mutations can be tracked in the field using PCR-based diagnostic assays or mass sequencing
approaches, providing essential information for resistance management programmes (Melo Costi
al., 2020).
Conversely, the gene 12 These mutations approaches, providing essential information for resistance management programmes (Melo Costa

21 al., 2020).

21 Conversely, the genetic bases of metabolic resistance are far less understood, though it o

274 al., 2020).
75 Conversely, the genetic bases of metabolic resistance are far less understood, though it often
76 accounts for a significant part of the phenotype (David et al., 2013). Indeed, the complexity and 75 Conversely
76 accounts for
76 accounts fo 75 Conversely, the genetic bases of metabolic resistance are far less understood, though it often
76 accounts for a significant part of the phenotype (David et al., 2013). Indeed, the complexity and $\mathcal{P}(\mathcal{$

278 differ according to the nature and intensity of the selection pressure together with the demograph

279 and ecological context (Feyereisen et al., 2015; Li et al., 2007). Metabolic resistance to pyrethroids

280 usual

279 and ecological context (Feyereisen et al., 2015; Li et al., 2007). Metabolic resistance to pyrethroids

280 usually results from an increased activity of detoxification enzymes such as cytochrome P450

281 monooxygenas 90 usually results from an increased activity of detoxification enzymes such as cytochrome P450

81 monooxygenases (P450s or CYPs for genes), glutathione S-transferases (GSTs)

82 carboxy/cholinesterases (CCEs) and UDP-gly 81 monooxygenases (P450s or CYPs for genes), glutathione S-transferases (GSTs)
82 carboxy/cholinesterases (CCEs) and UDP-glycosyl-transferases (UDPGTs) (David et al., 2013;
83 Hemingway et al., 2004; Smith et al., 2016). A Example, The and Sylvey, The and Sylvey, The anti-The CCP (The CoP) (The CoP) of Hemingway et al., 2004; Smith et al., 2016). At the genetic level, this can result from the sele
enzyme variants showing a higher insecticide

extinguished and the selection of highly resistance and the selection of highly resistance speed and well selection of trans-regulation. Genomic duplications can also contribute to overexpression of trans-regulation. Geno 84 Emission presents showing a higher insecticide metabolism rate, or their overexpression through cis-
85 Expression et al., 2015; Smith et al., 2015; Smith et al., 2018).
86 Experiment et al., 2020, 2021; Faucon et al., 90 characterise. This is the case in French Guiana, where the use of various insecticides, including the 86 affecting detoxification genes appear frequently associated with insecticide resistance in mosquito
87 (Cattel et al., 2020, 2021; Faucon et al., 2015; Weetman et al., 2018).
88 In addition to the genetic complexity of 87 (Cattel et al., 2020, 2021; Faucon et al., 2015; Weetman et al., 2018).
88 In addition to the genetic complexity of metabolic resistance alleles, their frequent co-occurrence
89 with other resistance mechanisms in natur (Cattel et al., 2020, 2021; Faucon et al., 2015; Weetman et al., 2018).

88 In addition to the genetic complexity of metabolic resistance alleles, their frequent co-occurrence

89 with other resistance mechanisms in natura with other resistance mechanisms in natural mosquito populations makes them difficult to

Solon the generatorise. This is the case in French Guiana, where the use of various insecticides, including the

Solon pyrethroid de 89 characterise. This is the case in French Guiana, where the use of various insecticides, includ
81 pyrethroid deltamethrin, for decades has led to the selection of highly resistant Ae. aegypti
82 populations combining me 91 pyrethroid deltamethrin, for decades has led to the selection of highly resistant Ae. aegypti

92 populations combining metabolic resistance alleles and multiple *kdr* mutations such as Val410Leu,

93 Val1016lle or Phe1 propulations combining metabolic resistance alleles and multiple *kdr* mutations such as Val4

91 Val1016lle or Phe1534Cys (Dusfour et al., 2015; Haddi et al., 2017). Although the over-expre

94 several detoxification enzy Val1016lle or Phe1534Cys (Dusfour et al., 2015; Haddi et al., 2017). Although the over-expression c
several detoxification enzymes was supported by both transcriptomics and proteomics (Dusfour et
al., 2015; Epelboin et al. 94 several detoxification enzymes was supported by both transcriptomics and proteomics (Dusfour et al., 2015; Epelboin et al., 2021; Faucon et al., 2017), those most contributing to deltamethrin resistance and the underlyi 95 al., 2015; Epelboin et al., 2021; Faucon et al., 2017), those most contributing to deltamethrin
96 resistance and the underlying genetic events remain to be identified. A recent study using a
97 composite population fro example in the underlying genetic events remain to be identified. A recent study using a

95 composite population from French Guiana resistant to multiple insecticides identified gene co

95 number variations (CNV) as a pr 97 composite population from French Guiana resistant to multiple insecticides identified gene composite population from French Guiana resistant to multiple insecticides identified gene composite remains (CNV) as a probable Frame Premations (CNV) as a probable cause of detoxification enzymes overexpression in this reg

(Cattel et al., 2020). Through a pool-seq approach targeting >300 candidate genes, this study

identified multiple contiguous 99 (Cattel et al., 2020). Through a pool-seq approach targeting >300 candidate genes, this study

199 identified multiple contiguous P450s from the CYP6 family on chromosome 1 showing an apparent

199 identified multiple c 99 101 elevated CNV in association with deltamethrin survival. However, despite the use of controlled

99 crosses, the multigenic resistance phenotype of this population (carrying the three above-mentioned

89 Kdr mutation elevated CNV in association with deltamethrin survival. However, despite the use of controlled

102 crosses, the multigenic resistance phenotype of this population (carrying the three above-mentions

103 Kdr mutations at h 102 crosses, the multigenic resistance phenotype of this population (carrying the three above-ment
103 kdr mutations at high frequency together with multiple metabolic resistance alleles) did not allo
104 conclude about th 103 Kdr mutations at high frequency together with multiple metabolic resistance alleles) did not allow to
104 conclude about their role in deltamethrin resistance. In addition, the exon-based nature of the
105 sequencing d 104 Conclude about their role in deltamethrin resistance. In addition, the exon-based nature of the
105 sequencing data generated together with the complexity of Ae. aegypti genome did not allow
106 resolving the genomic a

105 sequencing data generated together with the complexity of Ae. *aegypti* genome did not allow
106 resolving the genomic architecture of the duplicated locus.
107 In this context, the present study aimed at confirming th 106 resolving the genomic architecture of the duplicated locus.

107 In this context, the present study aimed at confirming the contribution of this P450 duplication

108 resistance of Ae. *aegypti* to deltamethrin and at 107 In this context, the present study aimed at confirming the c
108 resistance of Ae. aegypti to deltamethrin and at deciphering
109 limit confounding effects from other resistance alleles, two
110 Guiana and showing con 107 In this context, the present study aimed at confirming the contribution of this P450 duplication in the resistance of Ae. aegypti to deltamethrin and at deciphering its genomic architecture. In order to limit confoundi 109 limit confounding effects from other resistance alleles, two isofemale lines originating from Frenc Guiana and showing contrasted pyrethroid resistance levels but no apparent resistance to organophosphate and carbamat Guiana and showing contrasted pyrethroid resistance levels but no apparent resistance to

111 organophosphate and carbamate insecticides were used (Epelboin et al., 2021). In addition, both

112 lines lacked the two major 111 organophosphate and carbamate insecticides were used (Epelboin et al., 2021). In additior

112 lines lacked the two major *kdr* mutations Val1016lle and Val410Leu occurring in South American

113 (Epelboin et al., 202 112 lines lacked the two major *kdr* mutations Val1016lle and Val410Leu occurring in South America

113 (Epelboin et al., 2021). Controlled crosses were then used to remove the Phe1534Cys *kdr* mutatio

114 from the resis (Epelboin et al., 2021). Controlled crosses were then used to remove the Phe1534Cys *kdr* mutat

from the resistant line, which retained a P450-mediated resistance phenotype. Then, RNA-seq a

whole genome pool-seq confirme 114 (From the resistant line, which retained a P450-mediated resistance phenotype. Then, RNA-seq and whole genome pool-seq confirmed the presence of a ~200 Kb genomic duplication enhancing the expression of a cluster of mu whole genome pool-seq confirmed the presence of a ~200 Kb genomic duplication enhancing the
116 expression of a cluster of multiple P450 genes in the resistant line. The genomic architecture of the
117 duplicated loci was expression of a cluster of multiple P450 genes in the resistant line. The genomic architecture of th

duplicated loci was elucidated through long read sequencing, providing clues about its evolutional

origin. Association 116 expression of a cluster of multiple P450 genes in the resistant line. The genomic architecture of the

117 duplicated loci was elucidated through long read sequencing, providing clues about its evolutionary

118 origin 118 origin. Association studies, experimental evolution and reverse genetic were then used to further
119 investigate the contribution of this duplicated allele to deltamethrin resistance.
120 **Results**
122 *Deltamethrin r* 119 investigate the contribution of this duplicated allele to deltamethrin resistance.
120
121 **Results** Deltamethrin resistance is associated with P450 activity and is autosomal

120
120 **Results**
122 *Deltamethrin resistance is associated with P450 activity and is autosomal*
123 Two isofemale lines from Ile Royale island (French Guiana) showing contrasted r 121
122
123
124

121 **Results**
122 *Deltameth*
123 Two isofem
124 phenotypes 122 Dentimethrin resistance is associated with P450 activity and is autosomar
123 Two isofemale lines from Ile Royale island (French Guiana) showing contrasted represented phenotypes, namely IR03 and IR13 lines, were used 124 Two isofemale lines from Ile Royale island (French Guiana) showing contrasted resistance
124 Thenotypes, namely IRO3 and IR13 lines, were used as starting material (Table 1). Compar 124 phenotypes, namely IR03 and IR13 lines, were used as starting material (Table 1). Comparative

deltamethrin bioassays confirmed that the IR13 line can be categorized as susceptible to

126 deltamethrin as previously shown (Epelboin et al. 2021), though its susceptibility was slightly lower

127 than the laboratory s

128 genotyped individuals allowed producing the IROF line deprived of the three *kdr* mutations majorly

129 associated with pyrethroid resistance in South America (*i.e.* Val410Leu, Val1016lle, Phe1534Cys).

130 However, 128 genotyped individuals allowed producing the IROF line deprived of the three *kdr* mutations majour associated with pyrethroid resistance in South America (*i.e.* Val410Leu, Val1016lle, Phe1534Cys

130 However, our att 132 (see the IRO3 and the IRO5 and the I

135
136

 1 Mortality rates (mean \pm SD) previously obtained on 3 days-old females using 0.05% deltamethrin for 40 min (N=10).

2 *Kdr* mutation frequencies estimated by qPCR individual genotyping (N=30) and later confirmed by pool-seq whole ge³ *Kdr* mutation frequencies estimated from pool-seq whole genome data.

140 ³ *Kdr* mutation frequen

²³Kdr mutation frequencies estimated by qPCR individual genotyping (N=30) and later confirmed by pool-seq whole genome data.

³Kdr mutation frequencies estimated from pool-seq whole genome data.

141 The involvement of 141
142
143
144
145
146 sequentially exposing adult mosquitoes to the P450 inhibitor piperonyl butoxide (PBO) and to
143 insecticide. Such PBO pre-exposure did not significantly affect deltamethrin survival in the
144 susceptible IR13 line, but i 143 insecticide. Such PBO pre-exposure did not significantly affect deltamethrin survival in the

144 susceptible IR13 line, but increased mortality by 20% in the IR0F line (Wilcoxon test P value = 0.00)

145 supporting t

1. Synepside Critical Cr exposure to 0.03% deltamethrin. Mortality was recorded 24h after
exposure. Mean mortality rates are indicated ± SD, and compared us
Wilcoxon test (N=5, ns: not significant; **: p-value < 0.01).

 exposure. Mean mortality rates are indicated ± SD, and compared Wilcoxon test (N=5, ns: not significant; **: p-value < 0.01).

*

The content of the space of the space of the sex determining

text of our performed on F1 an 1920 exposure the sext of the 193 William 193 William Hagamian, 194 Preasonal external

193 William Hagamian, 194 Preasonal

193 Catated on chromosome 1, which also carries the sex deter

193 September (194 Preasonal Figure 2013).

194 Preasonal For si Bora IR13 IR0F
As the duplicated P450 locus is located on chromosome 1, which also carries the sex determining locus, deltamethrin bioassays were performed on F1 and F2 males and females obtained from both 'Bora-Bora x IROF' reciprocal crosses in order to investigate the mode of transmission of resistance.
Such comparative bioassay 157
158
159
160
161
162
163 159 locus, deltamethrin bioassays were performed on F1 and F2 males and females obtained from bot

160 'Bora-Bora x IROF' reciprocal crosses in order to investigate the mode of transmission of resistance

161 Such comparat 160 *'Bora-Bora x IROF'* reciprocal crosses in order to investigate the mode of transmission of resistance.

161 Such comparative bioassays did not support any significant bias affecting the transmission of

162 resistance 161 Such comparative bioassays did not support any significant bias affecting the transmission of
162 resistance (Supplementary file 1). For both F1 males and females, mortality levels upon deltamethr
163 exposure were sim 162 resistance (Supplementary file 1). For both F1 males and females, mortality levels upon delta
163 exposure were similar in the two reciprocal crosses and intermediate to those of the parental
164 indicating the absence 162 resistance (Supplementary file 1). For both F1 males and females, mortality levels upon detailmenting
163 exposure were similar in the two reciprocal crosses and intermediate to those of the parental lines,
164 indicat 164 indicating the absence of a maternal effect and a semi-dominant phenotype in heterozygotes. Similar indicating the absence of a maternal effect and a semi-dominant phenotype in heterozygotes. Similar indicating the abs 164 indicating the absence of a maternal effect and a semi-dominant phenotype in heterozygotes. Similar $\mathcal{L}(\mathcal{S})$

166 of a sex transmission bias.

167

168 Overexpression of the duplicated CYP6 gene cluster

169 RNA-seq was used to identify differentially transcribed genes between the two resistant lines (IRO3

170 and IROF), and the 167
168 Overexpression of the du
169 RNA-seq was used to ident
170 and IROF), and the two sus
171 genes passing our coverage 168
169
170
171
171
172 168 Overexpression by the duphelied CYP of gene cluster
169 RNA-seq was used to identify differentially transcribed g
170 and IROF), and the two susceptible lines (IR13 and Bora-
171 genes passing our coverage filter (82% and IROF), and the two susceptible lines (IR13 and Bora-Bora). Among the 11268 protein-coding

171 genes passing our coverage filter (82% of all protein coding genes), 84 genes showed a significant are

172 consistent dif quareason and IT all and IROF (82% of all protein coding genes), 84 genes showed a significant
172 consistent differential transcription level in the four *'resistant Vs susceptible'* pairwise compariso
173 (see methods, 172 consistent differential transcription level in the four *'resistant Vs susceptible'* pairwise comparisons

173 (see methods, expression fold change ≥ 1.5 and adjusted P value ≤ 0.0005). The 39 genes under-

17 173 (see methods, expression fold change ≥ 1.5 and adjusted P value ≤ 0.0005). The 39 genes under-

174 transcribed in resistant lines did not include any gene belonging to any family known to be associate

175 wi 173 (see methods) which could change \sim 1.5 and adjusted by \sim 1.5 and \sim 1.5 and 30.005). The 39 genes under-sunder-sunder-sunder-sunder-sunder-sunder-sunder-sunder-sunder-sunder-sunder-sunder-sunder-sunder-sunderwith insecticide resistance. Conversely, the 45 genes over-transcribed in resistant lines included eight
176 genes belonging to gene families associated with metabolic resistance (**Figure 2** and **Supplementary**
177 file 2 176 genes belonging to gene families associated with metabolic resistance (Figure 2 and Supplementary

177 file 2). These included five P450s (CYP6BB2 AAEL014893, CYP6-like AAEL026852, CYP6-like

178 AAEL017061, CYP6P12 AA genes belonging to gene rainmes associated with metabolic resistance (Figure 2 and Supplementary
177 file 2). These included five P450s (CYP6BB2 AAEL014893, CYP6-like AAEL026852, CYP6-like
178 AAEL017061, CYP6P12 AAEL01489

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186
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190
191 Figure 2. Genes over transcribed from RNA.seq are shown (FC≥ 1.5 and corrected P value ≤ 0.0005 in all resistant versus
185 significantly over transcribed from RNA.seq are shown (FC≥ 1.5 and corrected P value ≤ 0.0005 in a 186 significant and the pairwise comparisons). Genes also showing an elevated copy number from WG DNA-seq (FC ≥ 1.5) in all
187 pairwise comparisons are indicated with stars. Genomic coordinates (chromosome and start pos 187 pairwise comparisons are indicated with stars. Genomic coordinates (chromosome and start position), accession number and description are indicated for all genes. Gene descriptions in grey were obtained from a BlastP ag 187 and description are indicated for all genes. Gene descriptions in grey were obtained from a BlastP against NCBI Refseq.

187 Genes in red belong to a single duplicated region on chromosome 1. Pool-seq read coverage pro Genes in red belong to a single duplicated region on chromosome 1. Pool-seq read coverage profiles of the susceptible
and the resistant IROF lines around the duplicated locus are shown. Pie charts show the proportion of P4 190 and the resistant IROF lines around the duplicated locus are shown. Pie charts show the proportion of P450s in each
191 dataset.
192 Whole genome Pool-seq short read data were then used to identify genes affected by co

191 dataset.
192 Whole genome Pool-seq short read data were then used to identify genes affected by copy nun
194 variations in association with resistance base on their normalised exonic coverage. This analysis
194 192
193 Whole
194 variatic 193
194 Whole genome Pool-seq short read data were then used to identify genes affected by copy number
variations in association with resistance base on their normalised exonic coverage. This analysis 194 variations in association with resistance base on their normalised exonic coverage. This analysis

196 $(FC \ge 1.5 \text{ in all } 'resistant \text{ Vs }s$ susceptible' pairwise comparisons). These included seven P450s. One of

197 them (AAEL009018) was located at ~58.8 Mb on chromosome 1 and was not detected as over-

198 transcribed from RNA 197 them (AAEL009018) was located at ~58.8 Mb on chromosome 1 and was not detected as over-

198 transcribed from RNA-seq. The six others were located at ~271 Mb on chromosome 1 and included

199 the five CYP6s found over 203 was not confirmed for the three other 'resistant Vs susceptible comparisons' (1.07, 1.4 and 1.2 fold
204 for IR03/IR13, IR0F/IR13 and IR03/Bora respectively). A closer look at the genomic coverage at the 199 the five CYP6s found over-transcribed in resistant lines (see above) and another CYP6 (CYP6CC1) fro

199 the same gene cluster that was filtered out from RNA-seq analysis due to its low transcription level

199 (Figur 200 the same gene cluster that was filtered out from RNA-seq analysis due to its low transcription level

201 (Figure 2 and Supplementary file 2). The over-transcribed CYP9F-like located on chromosome 3

202 showed a slig 201 (Figure 2 and Supplementary file 2). The over-transcribed CYP9F-like located on chromosome 3
202 showed a slight increased CN in the IROF line Vs the Bora-Bora line (1.6 fold) but such increased CN
203 was not confirm 201 (Figure 2 and Supplementary file 2). The over-transcribed CTP 31-like located on chromosome 3
202 showed a slight increased CN in the IROF line Vs the Bora-Bora line (1.6 fold) but such increased
203 was not confirmed was not confirmed for the three other 'resistant Vs susceptible comparisons' (1.07, 1.4 and 1.2 fold

204 for IR03/IR13, IR0F/IR13 and IR03/Bora respectively). A closer look at the genomic coverage at the

205 CYP6 locus 204 for IRO3/IR13, IROF/IR13 and IRO3/Bora respectively). A closer look at the genomic coverage at the
205 CYP6 locus on chromosome 1 revealed a ~2 folds increased coverage affecting a region of ~200 Kb
206 spanning the e 205 CYP6 locus on chromosome 1 revealed a ~2 folds increased coverage affecting a region of ~200 Kb
206 spanning the entire CYP6 cluster. Such duplication was observed in all resistant *Vs* susceptible
207 comparisons. Alt spanning the entire CYP6 cluster. Such duplication was observed in all resistant Vs susceptible

207 comparisons. Altogether, these genomic data showed that the deltamethrin resistance phenotype

208 observed in the resist

comparisons. Altogether, these genomic data showed that the deltamethrin resistance phenot

208 observed in the resistant lines is associated with the presence of a large duplication on chrome

209 1 affecting the transcr 208 observed in the resistant lines is associated with the presence of a large duplication on chromosom
209 1 affecting the transcription level of six clustered CYP6 genes.
210 Whole genome short reads data confirmed the a 208 observed in the resistant lines is associated with the presence of a large duplication on chromosome

209 1 affecting the transcription level of six clustered CYP6 genes.

210 Whole genome short reads data confirmed th 210 Whole genome short reads data confirmed the absence of the
211 Val1016lle and Phe1534Cys in the IROF resistant line, while an
212 identified at an exact 0.5 frequency (141/282 short reads supp
213 both short read and l

211 Val1016lle and Phe1534Cys in the IROF resistant line, while another *kdr* mutation (Ile1011Met) was

212 identified at an exact 0.5 frequency (141/282 short reads supporting the 1011Met allele). Indeed,

213 both short identified at an exact 0.5 frequency (141/282 short reads supporting the 1011Met allele). Indeed,

213 both short read and long read data confirmed that this Ile1011Met *kdr* mutation is affected by an

214 heterogenous ge 214 heterogenous genomic duplication (see below and in Martins et al., 2013). Therefore, a 0.5
215 frequency indicates that the Ile-Met/Ile-Met genotype is fixed in the IROF line.
216 *Genomic architecture of the duplicate* Letterogenous genomic duplication (see below and in Martins et al., 2013). Therefore, a 0.5

214 beterogenous genomic duplication (see below and in Martins et al., 2013). Therefore, a 0.5

215 frequency indicates that the 215 frequency indicates that the IIe-Met/IIe-Met genotype is fixed in the IROF line.
216 frequency indicates that the IIe-Met/IIe-Met genotype is fixed in the IROF line.
217 *Genomic architecture of the duplicated loci*
21 216
215 frequency indicates that the Ile-Metyle-Metapology per allows the Ile-Metapology
218 Though not the major aim of the present study, our genomic data allowed charachitecture of the duplication affecting the Ile1011M 219
220
221
222 217 Genomic architecture of the duplicated lock
218 Though not the major aim of the present study,
220 read and long read topologies confirmed that the
221 duplication covering the 21 last coding exons of
222 The breakpoin 219 architecture of the duplication affecting the IIe1011Met Kdr mutation in the IROF line. Both she read and long read topologies confirmed that the IIe1011Met *kdr* mutation is part of 125 Kb g duplication covering the read and long read topologies confirmed that the IIe1011Met *kdr* mutation is part of 125 Kb geno

221 duplication covering the 21 last coding exons of the VGSC gene AAEL023266 (**Supplementary file**

222 The breakpoints of duplication covering the 21 last coding exons of the VGSC gene AAEL023266 (**Supplementary file 3**).

222 The breakpoints of this partial gene duplication are located at 315,905,811 bp and 316,030,250 bp on

223 the referen 221 duplication covering the 21 last coding exons of the VGSC gene AAEL023206 (Supplementary the 3).

222 The breakpoints of this partial gene duplication are located at 315,905,811 bp and 316,030,250 bp of

223 the refere 223 the reference genome AaegL5. The partial 3' copy carries the wild type allele (1011lle) while the full-
224 length copy carries the resistant allele (1011Met). The two copies also differed by their intronic
225 sequenc equence with the partial copy bearing a B type intron and the full-length copy bearing a A type

226 intron as previously described (Martins et al., 2013). As expected from an incomplete gene

227 duplication, RNA-seq data 226 intron as previously described (Martins et al., 2013). As expected from an incomplete gene
227 duplication, RNA-seq data supported the sole expression of the 1011Met allele meaning that the
228 IROF line (genotype IIe-

229 The genomic structure of the P450 duplicated region identified on chromosome 1 was further 227 duplication, RNA-seq data supported the sole expression of the 1011Met allele meaning that
228 IROF line (genotype IIe-Met/IIe-Met) express a Met phenotype.
229 The genomic structure of the P450 duplicated region ident 231 line. This revealed the presence of a 220.4 Kb duplication in the IROF line, with the two tandem
232 erepeats separated by a 6 Kb insertion (**Figure 3**). The right breakpoint of the duplication (RB) was
233 found ~50 K 229 The genomic structure of the P450 duplicated region identified
230 Investigated in light of long reads data obtained from the IR13 s
231 Inne. This revealed the presence of a 220.4 Kb duplication in the
232 repeats sep 230 investigated in light of long reads data obtained from the IR13 susceptible line and the IROF res

231 line. This revealed the presence of a 220.4 Kb duplication in the IROF line, with the two tandem

232 repeats sepa 231 ine. This revealed the presence of a 220.4 Kb duplication in the IROF line, with the two tandem

232 repeats separated by a 6 Kb insertion (Figure 3). The right breakpoint of the duplication (RB) was

233 found ~50 Kb repeats separated by a 6 Kb insertion (Figure 3). The right breakpoint of the duplication (RB) wa

found ~50 Kb right to CYP6CC1 at position 271,472,082 bp, and the left breakpoint (LB) ~77 Kb l

CYP6BB2 at 271,251,705 bp. 232 repeats separated by a 6 Kb insertion (Figure 3). The right breakpoint of the duplication (RB) was
233 found ~50 Kb right to CYP6CC1 at position 271,472,082 bp, and the left breakpoint (LB) ~77 Kb lef
234 *CYP6BB2* at 234 *CYP6BB2* at 271,251,705 bp. These breakpoints were supported by split reads from both long and
235 *CYP6BB2* at 271,251,705 bp. These breakpoints were supported by split reads from both long and
235 short read data (S 235 short read data (**Supplementary file 4**). The duplication is flanked by two distinct transposons, each aving its own terminal inverted repeats (TIR): a 2.4 Kb PiggyBac-like transposon on the right (PYL) and 9 Kb hAT-re 233 short read data (Supplementary file 4). The duplication is flamked by two distinct transposons, each having its own terminal inverted repeats (TIR): a 2.4 Kb PiggyBac-like transposon on the right (PYL), and 9 Kb hAT-re 237 and 9 Kb hAT-related transposon on the left (hAT-CYP6). These transposons are described in more
238 detail in Supplementary file 5.
239 238 detail in Supplementary file 5.
239
0 Kb hAT-CYP6 Stated transposed in more described in more described in more described in more described in mor 239 detail in Supplementary file 5.
239

241
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241 Figure 3. Genomic was welongton in the properties are expresented by the during that the site of the duplication in the resistant lines are epresented by ted arrows. Regions containing the emerges rande by the duplica 242

242 fou or partial transpossible sements are shown in greater detail. Orange and yelow triangles denote the inverted terminal

2424

2425 for the tentral sequence joining the tandem copies is chiments, with 60 bp fro 244

2445 repeats from hAT (17 pb) and PiggyBac like (18 pb) e ements respectively. Diagram is not to stale.

246 The central sequence joining the tandem copies is chimenc, with 60 bp from the left end of PYL

247 transpo ²

²¹ The central sequence joining the tandem copies is chimeric, with 60 bp from the ransposon followed by 6001 bp from the right end of the hAT transposon. This 248 from the IR13 susceptible line (no read joining RB The central sequence joining the tandem copies is chimeric, with 60 bp from the left end of PYL

247 transposon followed by 6001 bp from the right end of the hAT transposon. This duplication is absent

248 from the IR13 su 248 from the IR13 susceptible line (no read joining RB and LB sequences and no PYL transposon
249 sequence, neither full nor partial at RB). However, the hAT-CYP6 transposon was identified at the LB
250 in one IR13 haploty 249 sequence, neither full nor partial at RB). However, the hAT-CYP6 transposon was identified a
250 in one IR13 haplotype, much like in the IROF line, while a second haplotype was devoid of ha
251 insertion, much like the 250 in one IR13 haplotype, much like in the IROF line, while a second haplotype was devoid of hAT
251 insertion, much like the reference genome. An excess of tri-allelic SNPs is expected in the duplicated
252 region if the 251 insertion, much like the reference genome. An excess of tri-allelic SNPs is expected in the dupl

252 region if the two copies have diverged. Pool-seq short read data were then used to compare the

253 frequency of tr 252 region if the two copies have diverged. Pool-seq short read data were then used to compare the
253 frequency of tri-allelic loci within the duplication and 100 Kb upward and downward. Among the
254 1401 substitutions s 253 frequency of tri-allelic loci within the duplication and 100 Kb upward and downward. Among the
254 1401 substitutions sites identified within the duplication, five were tri-allelic whereas no such tri-
255 allelic var 254 1401 substitutions sites identified within the duplication, five were tri-allelic whereas no such tri-
255 allelic variant was identified among the 1334 substitutions identified in the flanking regions. This
256 repre 256 represents a slight but significant enrichment in the duplicated region (Fisher test p value = 0.031
257 These tri-allelic sites were all located within a 11.2 Kb intergenic region between the two CYP6-lik
258 genes AA 257 These tri-allelic sites were all located within a 11.2 Kb intergenic region between the two CYP6-like
258 genes AAEL017061 and AAEL014891v1.
260 The P450 duplication and the Kdr 1011 mutation are both associated with d

240

260 The P450 duplication and the Kdr 1011 mutation are both associated with deltamethrin

genes AAEL017061 and AAEL014891v1.
259
260 The P450 duplication and the Kdr 1011 mutation are both associated with deltamethrin
261 Survival
262 The association of the P450 duplication and the kdr Ile1011Met mutation with 259
260 The P450 duplication and the Kdr 101
261 survival
262 The association of the P450 duplication
263 were investigated in F2 individuals obtain 261
262
263
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265 260 The P450 duphcation and the Kdr 1011 mutation are both associated with deltamethrin
261 Survival
262 The association of the P450 duplication and the *kdr* Ile1011Met mutation with deltamethrin sur
263 were investigated 261 survivar
262 The assoc
263 were inverse females
265 CYP6BB2
266 ddPCR (F
267 copy nun were investigated in F2 individuals obtained from both 'Bora-Bora x IROF' reciprocal crosses. F2
264 females were then exposed to a high dose of deltamethrin (85.5% mortality) before quantifying
265 *CYP6BB2* gene copy num 264 females were then exposed to a high dose of deltamethrin (85.5% mortality) before quantifying

265 *CYP6BB2* gene copy number and the frequency of the 1011Met *kdr* allele in dead and survivors

266 ddPCR (**Figure 4**) 265 *CYP6BB2* gene copy number and the frequency of the 1011Met *kdr* allele in dead and survivors u

266 ddPCR (**Figure 4**). As expected in the presence of a fixed duplication, a 2-fold increase of *CYP6BB*

267 copy num 266 ddPCR (Figure 4). As expected in the presence of a fixed duplication, a 2-fold increase of CYP6BB2 copy number was observed in the IROF line (4.31 ± 0.21 copies) as compared to Bora-Bora line (2.07 ± 0.10 copies) whil 267 copy number was observed in the IROF line (4.31 ± 0.21 copies) as compared to Bora-Bora line (2.0
268 0.10 copies) while F1 individuals showed an intermediate copy number (3.58 ± 0.14 copies). In F2
269 individuals, C 268 0.10 copies) while F1 individuals showed an intermediate copy number $(3.58 \pm 0.14 \text{ copies})$. In F2 individuals, CYP6BB2 copy number was higher in survivors than in dead individuals $(4.00 \pm 0.16 \text{ copies}$ versus 3.31 ± 0.15 269 individuals, CYP6BB2 copy number was higher in survivors than in dead individuals $(4.00 \pm 0.16$
270 copies versus 3.31 ± 0.15 copies) supporting a genetic linkage between the P450 duplication and
271 deltamethrin sur 270 copies versus 3.31 ± 0.15 copies) supporting a genetic linkage between the P450 duplication and deltamethrin survival. Insecticide survival was also linked to the *kdr* mutation IIe1011Met on chromosome 3. In line wit 271 deltamethrin survival. Insecticide survival was also linked to the *kdr* mutation Ile1011Met on
272 chromosome 3. In line with an heterogenous *kdr* duplication, the frequency of the 1011Met allele
273 was close to 0. 272 chromosome 3. In line with an heterogenous *kdr* duplication, the frequency of the 1011Met and close to 0.5 in the IROF resistant line (genotype Ile-Met/Ile-Met) and close to 0.33 in F1 was close to 0.5 in the IROF res 273 was close to 0.5 in the IROF resistant line (genotype IIe-Met/IIe-Met) and close to 0.33 in F1 273 was close to 0.5 in the IROF resistant line (genotype III-Met_{/I}le-Met_/III-Met_/III-Metal and close to 0.33 in F1

In deltamethrin-exposed F2

individuals, the Met allele

frequency was higher in survivors

than in dead individuals (0.41 ±

0.02 versus 0.30 ± 0.02).

Figure 4. Genotype-phenotype associatio Individuals, the Met allele
frequency was higher in survey
than in dead individuals (0.4
0.02 versus 0.30 ± 0.02).
Figure 4. Genotype-phenotype ass
study. Top left panel shows the cross

Frequency was higher in su
than in dead individuals (0
0.02 versus 0.30 ± 0.02).
Figure 4. Genotype-phenotype
study. Top left panel shows the
underlying this experiment. Top
shows mortality data obtained b 227 Frequency was engineed and individuals $(0.41 \pm 0.02 \text{ versus } 0.30 \pm 0.02)$.

Figure 4. Genotype-phenotype associatic

study. Top left panel shows the crossings

underlying this experiment. Top right panel

shows mortalit 0.02 versus 0.30 ± 0.02).

Figure 4. Genotype-phenotype associatedly. Top left panel shows the crossing underlying this experiment. Top right physics above smoothline shows mortality data obtained by exposed and their F Figure 4. Genotype-phenotype
study. Top left panel shows the
underlying this experiment. To
shows mortality data obtained
adult females from the IROF are
lines and their F1 and F2 proge
deltamethrin for 1 hour. Mean rigure 4. Genotype-phenotype association
study. Top left panel shows the crossings
underlying this experiment. Top right panel
shows mortality data obtained by exposing
adult females from the IROF and Bora-Bora
lines and t **Example 12**
 Example 12 Shows mortality data obtained by exposing
adult females from the IROF and Bora-Bora
lines and their F1 and F2 progeny to 0.03%
deltamethrin for 1 hour. Mean mortalities ±
SD 24h after insecticide exposure are
shown and di r

adult females from the IROF and Bora-Bora

lines and their F1 and F2 progeny to 0.03%

deltamethrin for 1 hour. Mean mortalities $\frac{3}{2}$

sD 24h after insecticide exposure are

shown and district letters indicate

si lines and their F1 and F2 progeny to 0.03%
deltamethrin for 1 hour. Mean mortalities is
SD 24h after insecticide exposure are
shown and distinct letters indicate
significant differences (Kruskal-Wallis test
followed by po

deltamethrin for 1 hour. Mean mortalities :
 $\frac{3}{2}$ SD 24h after insecticide exposure are

shown and distinct letters indicate

significant differences (Kruskal-Wallis test

followed by post hoc Wilcoxon test with

Bon $\frac{3}{2}$ sp 24h after insecticide exposure are
shown and distinct letters indicate
significant differences (Kruskal-Wallistest
followed by post hoc Wilcoxon test with
Bonferroni-Holm correction, Ne 25, p \leq
0.05). Bot shown and distinct letters indicate
significant differences (Kruskal-Wallis
followed by post hoc Wilcoxon test w
Bonferroni-Holm correction, $N \ge 5$, p :
0.05). Bottom left and right panels sho
the copy number of CYP6BB2 significant differences (Kruskal-Wal
followed by post hoc Wilcoxon test
Bonferroni-Holm correction, $N \ge 5$,
0.05). Bottom left and right panels
the copy number of *CYP6BB2* and t
frequency of the *kdr* 1011Met allel
resp followed by post hoc Wilcoxon test with
Bonferroni-Holm correction, $N \ge 5$, $p \le 0.05$). Bottom left and right panels show
the copy number of *CYP6BB2* and the
frequency of the *kdr* 1011Met allele
respectively as inferr Bonferroni-Holm correction, $N \ge 5$, $p \le 0.05$). Bottom left and right panels show
the copy number of *CYP6BB2* and the
frequency of the *kdr* 1011Met allele
respectively as inferred by ddPCR from
pools of individuals (m 0.05). Bottom left and right panels shot the copy number of CYP6BB2 and the frequency of the *kdr* 1011Met allele respectively as inferred by ddPCR from pools of individuals (mean ± 95% Cl, Bc gF2 individuals 24h after in the copy number of *CYP6BB2* and the
frequency of the *kdr* 1011Met allele
respectively as inferred by ddPCR from
pools of individuals (mean ± 95% Cl, Bora
g F2 individuals 24h after insecticide exposure are
are are
ation Fractively as inferred by ddPCR from
Fractively as inferred by ddPCR from
pools of individuals (mean ± 95% Cl, B
g F2 individuals 24h after insecticide exposure
ation in deltamethrin survival was ther
adult females of the respectively as inferred by ddPCR frequency as inferred by ddPCR frequency of F258 frequency of the production of F23 individuals 24h after insecticide exposure
ation in deltamethrin survival was the adult females of the F25 pools of individuals (mean ± 95% Cl, Bo
ng F2 individuals 24h after insecticide exposure at
ation in deltamethrin survival was then
adult females of the IROF line were used
cient expression to be detected by RNA-
CYP6 ng F2 individuals 24h after insecticide exposure are

29 postpaid and the insecticide exposure are

29 contribute to resistance

29 contribute to resistance

20 cient expression to be detected by RNA-sec

20 cient expressi 298 noted F2D and F2S respectively.

299 noted F2D and F2S respectively.

300 Multiple CYP genes carried by the P450 duplication may contribute to resistance

302 The relative importance of the P450s carried by the duplic 300

201 Multiple CYP genes carric

202 The relative importance of

203 examined by RNA interfere

204 specifically knock down the

205 (CYP6BB2, CYP6-like AAELC

206 approach allowed reaching

207 (from 42.2% for AAEL0170 300
301
302
303
304
305
306 301 Multiple CYP genes carried by the P450 duplication may contribute to resistance
302 The relative importance of the P450s carried by the duplication in deltamethrin survival was then
303 examined by RNA interference. Di EXERCISED and GYPG SPREAT CONCERS and GYPG SPREAT CONCERS CONDUCTS CONDUCTS CONDUCTS and CONCERT CONCERT CONDUCTS of the ROF line were used specifically knock down the five CYP6 genes showing sufficient expression to be de specifically knock down the five *CYP6* genes showing sufficient expression to be detected by RNA-seq

(*CYP6BB2, CYP6-like* AAEL026582, *CYP6-like* AAEL017061, *CYP6P12v1* and *CYP6P12v2*). Such

approach allowed reaching (CYP6BB2, CYP6-like AAEL026582, CYP6-like AAEL017061, CYP6P12v1 and CYP6P12v2). Such
approach allowed reaching an acceptable silencing specificity and a moderate silencing efficiency
(from 42.2% for AAEL017061 to 69.7% for approach allowed reaching an acceptable silencing specificity and a moderate silencing efficity (from 42.2% for AAEL017061 to 69.7% for AAEL026582). Comparative deltamethrin bioassay performed on dsCYP6-injected mosquitoes 307 (from 42.2% for AAEL017061 to 69.7% for AAEL026582). Comparative deltamethrin bioassays
308 performed on dsCYP6-injected mosquitoes and dsGFP-injected controls suggested that the two fir
309 CYP gene of the duplicated 308 performed on dsCYP6-injected mosquitoes and dsGFP-injected controls suggested that the tw
309 CYP gene of the duplicated cluster (CYP6BB2 and CYP6-like AAEL026582) contribute to deltam
310 survival (see methods and res CYP gene of the duplicated cluster (CYP6BB2 and CYP6-like AAEL026582) contribute to deltamethrin
310 survival (see methods and results in **Supplementary file 6**). However, data generated across multiple
311 injection exper survival (see methods and results in **Supplementary file 6**). However, data generated across multiple
injection experiments were not fully conclusive because a high mortality was frequently observed in
dsGFP-injected contr 311 survival (see methods and results in **Supplementary me** 0). However, data generated across multiple injection experiments were not fully conclusive because a high mortality was frequently observed in dsGFP-injected con 312 dsGFP-injected controls and because the increased mortalities observed in dsCYP-injected
313 dsGFP-injected controls and because the increased mortalities observed in dsCYP-injected
314 to the moderate silencing effici mosquitoes were relatively low. Such low mortality variations upon dsCYP6 injection were
314 to the moderate silencing efficiency and the presence of the *kdr* 1011Met allele in the IROF
315 The P450 duplication is hardly

314 to the moderate silencing efficiency and the presence of the *kdr* 1011Met allele in the IROF line.
315 The P450 duplication is hardly retained by selection in presence of the *Kdr* 1011 allele
317 The co-occurence of 315
315 The P450 duplication is hardly retained by selection in presence of the Kdr 1011 allele
317 The co-occurence of the P450 duplication and the Ile1011Met kdr mutation in the IROF resistant
318 was taken as an opportu 315
316
317
318
319
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321 317 The reason duplication is hardly retained by selection in presence by the Kdr 1011 different 318 The co-occurence of the P450 duplication and the Ile1011Met *kdr* mutation in the IROF resistation was taken as an opport was taken as an opportunity to compare their responses to deltamethrin selection. Four independen
318 was taken as an opportunity to compare their responses to deltamethrin selection. Four independen
320 (selected lines: S 319 lines obtained from 'Bora-Bora x IROF' F3 offspring and representing two replicated selection regimes
320 (selected lines: SelA and SelB, non-selected lines: NSA and NSB) were compared for their resistance
321 level, C 320 linested lines: SelA and SelB, non-selected lines: NSA and NSB) were compared for their resistance
321 lievel, *CYP6BB2* copy number and *kdr* 1011Met allele frequency (**Figure 5**).
322 321 (sevel, *CYP6BB2* copy number and *kdr* 1011Met allele frequency (**Figure 5**).
322
And Non-Selected for the selected for the selection of the selected for the selected for the selected for the S21 level, CYP6BB2 copy number and kdr 1011Met allele frequency (Figure 5).

duplication and the *kdr* 1011M
to deltamethrin selection. Top I
panel shows the crossings under
this experiment. Top right panel
the resistance of each line as me
by bioassays on adult females (C
deltamethrin for 1h expos to deltamethrin selection. Top left
to deltamethrin selection. Top left
panel shows the crossings underlying
this experiment. Top right panel shows
the resistance of each line as measured
by bioassays on adult females (0.0 Figure 1.1 The selection. The selection of the selection of the selection of the resistance of each line as measured by bioassays on adult females (0.03 deltamethrin for 1h exposure, meal mortality ± SD). Bottom left and r this experiment. Top right panel show
the resistance of each line as measure
by bioassays on adult females (0.03%
deltamethrin for 1h exposure, mean
mortality ± SD). Bottom left and right
panels show CYP6BB2 copy number a
 the resistance of each line as measured
by bioassays on adult females (0.03%
deltamethrin for 1h exposure, mean
mortality ± SD). Bottom left and right
panels show CYP6BB2 copy number and
individuals (mean ± 95% Cl, N ≥ 30) By bioassays on adult females (0.03%
deltamethrin for 1h exposure, mean
mortality ± SD). Bottom left and right
panels show CYP6BB2 copy number and
Kdr 1011Met frequency respectively as
inferred by ddPCR from pools of
indiv deltamethrin for 1h exposure, mean
mortality ± SD). Bottom left and right
panels show CYP6BB2 copy number and
Kdr 1011Met frequency respectively a
inferred by ddPCR from pools of
individuals (mean ± 95% Cl, N ≥ 30). Ti
Sel mortality ± SD). Bottom left and right
panels show CYP6BB2 copy number and right
water of the state of the state of the state of the state of the initial
inferred by ddPCR from pools of
individuals (mean ± 95% Cl, N ≥ 30) panels show CYP6BB2 copy number a

side 1011Met frequency respectively a

inferred by ddPCR from pools of

individuals (mean ± 95% Cl, N ≥ 30). T

selB CYP6BB2 copy number data poin

marked with a '!' was caused by an

un

EXECT THE EXECT THE SET SET SET AND SET SET AND SET SET SET AND A SUPPOSE STATE SHOW AND SUPPOSE SHOW THAND SUPPOSE SHOW THAND SUPPOSE SUPPO inferred by ddPCR from pools of
individuals (mean \pm 95% Cl, N \geq 30). The
SelB CYP6BB2 copy number data point
marked with a '!' was caused by an
unexpected sampling error and was
therefore excluded from the statisti individuals (mean \pm 95% CI, N \ge 3

SelB CYP6BB2 copy number data

marked with a 'I' was caused by

unexpected sampling error and v

therefore excluded from the stat

analysis comparing CYP6BB2 cop

number between Se SelB CYP6BB2 copy number data point

marked with a '!' was caused by an

unexpected sampling error and was

therefore excluded from the statistical

analysis comparing CYP6BB2 copy

number between Sel and NS lines

across marked with a ''' was caused by an
unexpected sampling error and was
therefore excluded from the statistical
analysis comparing *CYP6BB2* copy
number between Sel and NS lines
across generations. Grey dashed lines
indicate unexpected sampling error and was
therefore excluded from the statisti
analysis comparing CYP6BB2 copy
number between Sel and NS lines
across generations. Grey dashed lin
indicate the generations for which s
lines were sel therefore excluded from the statistic
analysis comparing *CYP6BB2* copy
number between Sel and NS lines
across generations. Grey dashed line
indicate the generations for which S
lines were selected with deltamethr
F13
5
1
 analysis comparing *CYP6BB2* copy
analysis comparing *CYP6BB2* copy
arrows generations. Grey dashed lines
indicate the generations for which Sel
lines were selected with deltamethrin.
Fix
 $\frac{6}{11}$
 $\frac{1}{21}$
 $\frac{1}{21}$
number between Sel and NS lines
across generations. Grey dashed li
indicate the generations for which
lines were selected with deltamet
Fi3
Fi3
3
1
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5
5 in F9 of both Sel lines, supporting
freq across generations. Grey dashed lindicate the generations for which
lines were selected with deltamet
lines were selected with deltamet
F13
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1247
12 indicate the generations for which Sel
lines were selected with deltamethrin.
F13
17
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19 across generations
agesting that the resistance phenotype
howed that the *kdr* 1011Met allele was
1.5 in F9 of lines were selected with deltamethrin.

This were selected with deltamethrin.

This 344

A Sel lines, with mortality dropping fron

rotion for three successive generations

ggesting that the resistance phenotype

howed tha 345

113

113

113

114,

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116 Sel lines, with mortality dropping from

116 Sel lines successive generations

116 howed that the *kdr* 1011Met allele was

1.5 in F9 of both Sel lines, supporting the

frequenc F3 F5 F7 F9 F11 F13

An increase of deltamethrin resistance was observed for both Sel lines, with mortality dropping from

~80% in F4 to less than 20% in F9. Relaxing deltamethrin selection for three successive generation h Sel
ctio
gges
how
).5 ir
freq ²³⁴⁹ \sim 80% in F4 to less than 20% in F9. Relaxing deltamethrin selection for three successive generations
350 (F9 to F11) lead to an increased mortality in both Sel lines, suggesting that the resistance phenotype
351 350 (F9 to F11) lead to an increased mortality in both Sel lines, suggesting that the resistance phenotype
351 is costly. Though subjected to sampling effects, ddPCR data showed that the *kdr* 1011Met allele was
352 rapid is costly. Though subjected to sampling effects, ddPCR data showed that the *kdr* 1011Met allele was
352 rapidly selected by deltamethrin. Its frequency was close to 0.5 in F9 of both Sel lines, supporting the
353 near fix rapidly selected by deltamethrin. Its frequency was close to 0.5 in F9 of both Sel lines, supporting the

near fixation of the IIe-Met duplicated allele. Such increased frequency was not seen in NS lines

where Met allele The mean fixation of the IIe-Met duplicated allele. Such increased frequency was not seen in NS lines
354 where Met allele frequency decreased to less than 10% in one line and fluctuated under 0.25 in the
355 other line. N where Met allele frequency decreased to less than 10% in one line and fluctuated under 0.25 in
355 other line. No such strong response to selection was observed for the P450 duplication, with
356 *CYP6BB2* copy number show 355 other line. No such strong response to selection was observed for the P450 duplication, with
356 *CYP6BB2* copy number showing a gradual decrease through generations in both Sel and NS lines.
357 However, *CYP6BB2* mea 356 CYP6BB2 copy number showing a gradual decrease through generations in both Sel and NS line.
357 However, CYP6BB2 mean copy number were significantly higher in Sel lines than in NS lines a
358 generations, supporting a 359 (Linear Mixed Effect Model, p value = 0.0088).
360
361 **Discussion**
362 In line with their high probability of occurrence, genomic duplications have been shown to frequentl

357 However, CYP6BB2 mean copy number were significantly higher in Sel lines than in NS lines acros
358 generations, supporting a higher retention rate of the duplicated allele upon deltamethrin selecti
359 (Linear Mixed E generations, supporting a higher retention rate of the duplicated allele upon deltamethrin selectio

359 (Linear Mixed Effect Model, p value = 0.0088).

360

361 **Discussion**

362 In line with their high probability of occ 360
361 **Discussion**
362 In line with their high probability of occurrence
363 contribute to short-term adaptation in various 360
361
362
363
364
365 362 In line with their
363 contribute to sh
364 strong selection
365 mechanisms (Ba
366 genes and tethe
367 fitness costs (Lal 363 contribute to short-term adaptation in various organisms (Kondrashov, 2012). In arthropods facing
364 strong selection pressures from insecticides, duplications are known to be adaptive via two distinct
365 mechanisms 364 strong selection pressures from insecticides, duplications are known to be adaptive via two distinct
365 mechanisms (Bass & Field, 2011). First, heterogeneous duplications affecting key insecticide target
366 genes and 365 mechanisms (Bass & Field, 2011). First, heterogeneous duplications affecting key insecticide target
366 genes and tethering both susceptible and resistant alleles have been associated with a reduction of
367 fitness co 366 genes and tethering both susceptible and resistant alleles have been associated with a reduction of
367 fitness costs (Labbe et al., 2007). Second, duplications of detoxification genes can contribute
368 quantitatively 367 fitness costs (Labbe et al., 2007). Second, duplications of detoxification genes can contribute
368 quantitatively to their over-expression, leading to resistance through increased insecticide
369 metabolism (Faucon et 368 quantitatively to their over-expression, leading to resistance through increased insecticide
369 metabolism (Faucon et al., 2015; Weetman et al., 2018). As opposed to target-site resistance
370 mutations that can be ea 369 metabolism (Faucon et al., 2015; Weetman et al., 2018). As opposed to target-site resistant mutations that can be easily tracked by PCR in natural populations, the paucity of DNA mand underlying metabolic resistance hi 370 mutations that can be easily tracked by PCR in natural populations, the paucity of DNA marke
371 underlying metabolic resistance hinders their tracking in the field. In this concern, identifying
372 duplications associ 371 underlying metabolic resistance hinders their tracking in the field. In this concern, identifying geally duplications associated with metabolic resistance can provide new tools to monitor the dynamic
373 esistance alle 372 duplications associated with metabolic resistance can provide new tools to monitor the dynamics of
373 resistance alleles (Cattel et al., 2021).
This concerns general general provide new tools to monitor the dynamics o associations associated with metabolic resistance alleles (Cattel et al., 2021).
The dynamics of monoide new tools to monitor the dynamics of monoide new tools to monitor the dynamics of monitor the dynamics of monoide new 373 resistance alleles (Cattel et al., 2021).

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380 The P450 duplication affects a cluster of genes previously associated with resistance
376 The present study confirmed the occurrence of a large genomic duplication affecting a cluster of six
377 P450s from the CYP6 family duplication was previously suspected though the structure of the duplicated loci could not be
379 sesolved from targeted-sequencing data (Faucon et al., 2015). The association of this P450
380 duplication with deltamethrin duplication was previously suspected though the structure of the duplicated loci could not be
resolved from targeted-sequencing data (Faucon et al., 2015). The association of this P450
duplication with deltamethrin resista 378 duplication was previously suspected though the structure of the duplicated loci could not be 380 duplication with deltamethrin resistance was supported by controlled crosses, although the
381 of other resistance alleles limited the association power (Cattel et al., 2020). A duplication
382 encompassing the CYP6BB2 381 of other resistance alleles limited the association power (Cattel et al., 2020). A duplication
382 encompassing the CYP6BB2 gene was also associated with pyrethroid resistance in Lao PDR
383 (Marcombe et al., 2019). Th encompassing the CYP6BB2 gene was also associated with pyrethroid resistance in Lao PDR

383 (Marcombe et al., 2019). Though gene duplication was not looked for, the over-transcription

384 genes from this cluster (e.g. CY (Marcombe et al., 2019). Though gene duplication was not looked for, the over-transcription
383 (Marcombe et al., 2019). Though gene duplication was not looked for, the over-transcription
384 pyrethroid resistance worldwid Sastigates from this cluster (e.g. CYP6BB2, CYP6P12 and CYP6CC1) was frequently associated with

senes from this cluster (e.g. CYP6BB2, CYP6P12 and CYP6CC1) was frequently associated with

pyrethroid resistance worldwide (pyrethroid resistance worldwide (Bariami et al., 2012; Dusfour et al., 2015; Goindin et al., 2014; Marcombe, Poupardin, et al., 2009; Moyes et al., 2017; Reid et al., 2014; Saavedra-Rodriguez et al., 2012; Seixas et al., 2 286 et al., 2014; Marcombe, Poupardin, et al., 2009; Moyes et al., 2017; Reid et al., 2014; Saavedra-

387 Rodriguez et al., 2012; Seixas et al., 2017). These genes were also found under directional selection in

388 ssoci 393 responded to selection with the neonicotinoid imidacloprid, suggesting that P450s from this cluster 388 association with pyrethroid survival in a Mexican resistant line from whole exome SNP data
389 (Saavedra-Rodriguez et al., 2021). In addition, *CYP6BB2* was also shown capable of metabolising the
390 pyrethroid permeth Sameta and Sandar (Saavedra-Rodriguez et al., 2021). In addition, CYP6BB2 was also shown capable of metaboli

390 pyrethroid permethrin (Kasai et al., 2014). By comparing its copy number and transcriptiona

391 across diff 391 across different lines, Faucon et al. (2017) suggested that both transcriptional regulation and
392 genomic duplication contribute to its over-expression in South America. Interestingly, this gene als
393 responded to 392 genomic duplication contribute to its over-expression in South America. Interestingly, this ger
393 responded to selection with the neonicotinoid imidacloprid, suggesting that P450s from this c
394 can be selected by a

by prethroid permethrin (Kasai et al., 2014). By comparing its copy number and transcriptional level
391 across different lines, Faucon et al. (2017) suggested that both transcriptional regulation and
392 genomic duplicati The sponded to selection with the neonicotinoid imidacloprid, suggesting that P450s from this cluster

394 can be selected by and metabolise other insecticides (Riaz et al., 2013; Zoh et al., 2021).

395 Our attempt to iso can be selected by and metabolise other insecticides (Riaz et al., 2013; Zoh et al., 2021).
395 Our attempt to isolate this P450 duplication from other resistance alleles occurring in French Guiana
396 was partially succes can be selected by and metabolise other insecticides (Riaz et al., 2013; Zoh et al., 2021).

395 Our attempt to isolate this P450 duplication from other resistance alleles occurring in French Guiana

396 was partially succ 399 Ile1011Met *kdr* mutation as a fixed heterogenous duplication (Ile-Met/Ile-Met genotype, Met/Met
400 phenotype). There is evidence that this mutation confers some resistance to pyrethroids in the field
401 (Brengues et 397 Val1016lso and Val410Leu *kdr* mutations, and controlled crosses allowed removing the Phe15
398 mutation. However, the resulting IROF resistant line carrying the P450 duplication still carried
399 Ile1011Met *kdr* mut 398 mutation. However, the resulting IROF resistant line carrying the P450 duplication still carried the
399 Ile1011Met *kdr* mutation as a fixed heterogenous duplication (Ile-Met/Ile-Met genotype, Met/Met
400 phenotype). 399 Ile1011Met *kdr* mutation as a fixed heterogenous duplication (Ile-Met/Ile-Met genotype, Met/Me

400 phenotype). There is evidence that this mutation confers some resistance to pyrethroids in the fie

401 (Brengues et provided a Final Experimentally associated that mutation confers some resistance to pyrethroids in the field

401 (Brengues et al., 2003; Brito et al., 2018; Martins et al., 2009). Nevertheless, the use of the P450

402 in (Brengues et al., 2003; Brito et al., 2018; Martins et al., 2009). Nevertheless, the use of the P450

inhibitor PBO showed that the resistance phenotype was still associated with P450-mediated

metabolism. Considering that inhibitor PBO showed that the resistance phenotype was still associated with P450-mediated

403 metabolism. Considering that four of the five P450s over-transcribed in resistant lines belong to

404 duplicated locus, the c metabolism. Considering that four of the five P450s over-transcribed in resistant lines belong

404 duplicated locus, the contribution of this P450 duplication to the resistance phenotype is likel

405 Studying the mode of 404 duplicated locus, the contribution of this P450 duplication to the resistance phenotype is likely.
405 studying the mode of transmission of resistance did not allow evidencing any significant sexual
406 transmission bi 405 Studying the mode of transmission of resistance did not allow evidencing any significant sexual
406 transmission bias potentially associated with resistance. In line with this, this P450 duplication is
407 located outs transmission bias potentially associated with resistance. In line with this, this P450 duplication i

407 located outside of a 210 Mb region of chromosome 1 showing a low recombination rate and a h

408 genetic differentia Franchischer Hersen, and the Franchischer Hersen as a bounded outside of a 210 Mb region of chromosome 1 showing a low recombination rate and a higher genetic differentiation between Ae. *aegypti* males and females (Fontai

Figure 407 located outside of a 210 Mb region of chromosome 1 showing a low recombination rate and a high
408 genetic differentiation between Ae. *aegypti* males and females (Fontaine et al., 2017).
409 Contrasted architec 409

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408 Contrasted architectures of the duplications affecting the P450 and the Kdr loci

11 The presence of two distinct transposons at the P450 duplication breakpoints implies t

412 played an active role in the ge 412
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415 410 Contrasted dremettures by the dupheditions affecting the P450 and the Kdr lock
411 The presence of two distinct transposons at the P450 duplication breakpoints implies t
412 played an active role in the generation of t Figure 2112 played an active role in the generation of this genomic event. Transposon-mediated duplication
413 often arises from ectopic recombination between two homologous transposon copies inserted in the
414 same orien 413 often arises from ectopic recombination between two homologous transposon copies inserted in
414 same orientation at distinct positions of a same chromosome (Baker et al., 1996). However, such
415 event would leave a s same orientation at distinct positions of a same chromosome (Baker et al., 1996). However, such an
event would leave a single full-length transposon copy between the two duplicated genomic regions
with no transposon at oth event would leave a single full-length transposon copy between the two duplicated genomic regions

416 with no transposon at other breakpoints (Remnant et al., 2013). Here, a more complex scenario mus

417 have taken place with no transposon at other breakpoints (Remnant et al., 2013). Here, a more complex scenario must
have taken place because two distinct transposons are flanking the duplication, and because the
sequence joining the two co have taken place because two distinct transposons are flanking the duplication, and because the
418 sequence joining the two copies is chimeric. A possible scenario involves independent insertion of
419 the two distinct tr At the sequence joining the two distinct transposons are flanking the duplication, and because the

418 sequence joining the two copies is chimeric. A possible scenario involves independent insertion of

419 the two distin the two distinct transposons, then transposition of the hAT into the PiggyBac-like transposon
420 followed by a deletion event, and finally an ectopic homologous recombination event between the
421 two duplicated hAT seque 420 followed by a deletion event, and finally an ectopic homologous recombination event betwee
421 two duplicated hAT sequences (**Supplementary File 7**). One transposon involved is related to
422 superfamily while the othe 421 two duplicated hAT sequences (**Supplementary File 7**). One transposon involved is related to the h/
422 superfamily while the other one is distantly related to PiggyBac (see **Supplementary file 5** for more
423 details) 421 two duplicated hAT sequences (Supplementary The 7). One transposed involved is related to the hAT
422 superfamily while the other one is distantly related to PiggyBac (see **Supplementary file 5** for more
423 details), 422 superfamily while the other one is distantly related to Piggybac (see Supplementary file 5 for more
details), both being cut and paste elements having multiple copies and being active in the Ae. *degyp*
details), both

 \mathcal{A}_μ , both being cut and paste elements having multiple copies and being active in the Ae. aegyptic in the Ae. a.

Figure 1992 and the two copies substitution sites within the duplicated region, most likely im

425 identification of a few tri-allelic substitution sites within the duplicated region, most likely im

427 those, because th mutations occurring post-duplication. The few tri-allelic sites observed represent only a fraction of

427 those, because they imply a pre-duplication mutation at the same position, and because mutations

428 reverting to

those, because they imply a pre-duplication mutation at the same position, and because mutations

reverting to the reference allele are not detected as such. Nevertheless, such low divergence rate

between the two copies s Figure the efference allele are not detected as such. Nevertheless, such low divergence rate

429 between the two copies supports the recent age of this duplication event.

430 In contrast, the genomic duplication affectin between the two copies supports the recent age of this duplication event.

430 In contrast, the genomic duplication affecting the VGSC gene at the kdr Ile1011Met locus shows not

431 Temnant of any transposon at breakpoint 1429 between the two coppers supports the terms and produces in the kdr lle:

1431 between the recent age of this duplication affecting the VGSC gene at the kdr lle:

1432 It is likely to be an earlier event, since the cop For the general dumped and the general duplication and the pass of the general duplication

432 In the general duplication after the copy divergence is much more pronounced, particularly in

433 Intronic regions (Martins e 1432 It is likely to be an earlier event, since the copy divergence is much more pronounced, particularly in

1433 It is likely to be an earlier event, since the copy divergence is much more pronounced, particularly in

14 intronic regions (Martins et al, 2013). Also, it differs from other heterogenous duplications implicated
so far in insecticide resistance because the incomplete copy carrying the susceptible Ile1011
pseudoallele, does not 434 so far in insecticide resistance because the incomplete copy carrying the susceptible Ile1011
435 pseudoallele, does not contribute to gene expression. It therefore cannot modulate the resistance
436 level provided by pseudoallele, does not contribute to gene expression. It therefore cannot modulate the resis

436 level provided by the 1011Met allele carried by the full copy, nor its fitness. This contrasts wi

437 heterogenous duplicat

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- Figure 1343 Pure provided by the 1011Met allele carried by the full copy, nor its fitness. This contrasts with the

437 heterogenous duplications affecting the Ace1 target-site mutation observed in *Culex pipiens sp.* and
 436 level provided by the 1011Met allele carried by the full copy, nor its fitness. This contrasts with the

437 heterogenous duplications affecting the Ace1 target-site mutation observed in *Culex pipiens sp.* and

438 *A* 2009; Labbe et al., 2007). In this regard, further investigating the evolutionary origin and dynamic
441 this *kdr* heterogenous duplication deserves further attention.
442 The P450 duplication has a limited adaptive value
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An *Anopheles gambiae*, where both copies can contribute to the phenotype, thus allowing a reduction

433 *Anopheles gambiae*, where both copies can contribute to the phenotype, thus allowing a reduction

440 **2009**; Labbe of the fitness cost associated with the resistant allele in absence of insecticide (Djogbenou et al.,

2009; Labbe et al., 2007). In this regard, further investigating the evolutionary origin and dynamics c

this *kdr* het this *kdr* heterogenous duplication deserves further attention.

442

443 The P450 duplication has a limited adaptive value in presence of the Kdr 1011 mutation

444 The association study performed on F2 individuals suppor The P450 duplication has a limited adaptive value in pres

442 The P450 duplication has a limited adaptive value in pres

444 The association study performed on F2 individuals supported

445 with deltamethrin survival toge 443
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448 The P450 duplication has a limited adaptive value in presence of the Kdr 1011 mutation

The association study performed on F2 individuals supported the association of the P450 duplicati

with deltamethrin survival togethe mutations is located on chromosome 3, a genetic linkage with the P450 duplicated loci is excluded.

Therefore, the association of the P450 duplication in F2, following limited recombination events,

implies a resistance l 1446 IIe1011Met *kdr* mutation with deltamethrin survival was expected. As the *VGSC* gene carrying

1447 mutations is located on chromosome 3, a genetic linkage with the P450 duplicated loci is excluded

1448 Therefore, mutations is located on chromosome 3, a genetic linkage with the P450 duplicated loci is excluded

Therefore, the association of the P450 duplication in F2, following limited recombination events,

implies a resistance lo Therefore, the association of the P450 duplication in F2, following limited recombination events,

449 implies a resistance locus located on chromosome 1, either the P450 duplication itself or another

450 genetically lin implies a resistance locus located on chromosome 1, either the P450 duplication itself or another

genetically linked locus. However, no other known resistance gene was identified in this genomic

region. The relative imp Fraction 1450 and the locus. However, no other known resistance gene was identified in this genomic
1451 region. The relative importance of the different CYP6 genes carried by the P450 duplication in
1452 deltamethrin surv Frame in the relative importance of the different CYP6 genes carried by the P450 duplication in
452 deltamethrin survival was investigated through RNA interference. Despite a limited statistical pow
453 likely due to the

deltamethrin survival was investigated through RNA interference. Despite a limited statistical likely due to the presence of the *kdr* Ile1011Met resistance allele and moderate knock down efficiencies, these data suggest Fixely due to the presence of the *kdr* Ile1011Met resistance allele and moderate knock down
454 efficiencies, these data suggest that at least two *CYP6* genes *(CYP6BB2* and AAEL026582) carried by
455 the duplication may 454 efficiencies, these data suggest that at least two *CYP6* genes (*CYP6BB2* and AAEL026582) carr
455 efficiencies, these data suggest that at least two *CYP6* genes (*CYP6BB2* and AAEL026582) carr
455 experimental evol the duplication may contribute to deltamethrin detoxification.

455 the duplication may contribute to deltamethrin detoxification.

456 Experimental evolution confirmed the strong positive response of the *kdr* 1011Met all Experimental evolution confirmed the strong positive response

455 with a high dose of deltamethrin. Such experiment also evident

458 absence of insecticide selection, supporting a significant fitness

459 kdr mutations (with a high dose of deltamethrin. Such experiment also evidenced a gradual decrease of this allele in
458 absence of insecticide selection, supporting a significant fitness cost as previously shown for other
459 kdr mutati 458 absence of insecticide selection, supporting a significant fitness cost as previously shown for other
459 kdr mutations (Rigby et al., 2020; Uemura et al., 2023). The response of the P450 gene duplication to
460 deltam *Absonce of insection*
459 *kdr* mutations (Rigby et al., 2020; Uemura et al., 2023). The response of the P450 gene duplication
460 deltamethrin selection was less clear. A gradual decrease of *CYP6BB2* copy number was obs deltamethrin selection was less clear. A gradual decrease of CYP6BB2 copy number was observed in

both selected and non-selected lines, even though such decay was slower in the selected lines. Such

result supports a lower both selected and non-selected lines, even though such decay was slower in the selected lines. Such result supports a lower adaptive value of the P450 duplication in our experimental conditions. Such unexpected response ma Figures 162 are sult supports a lower adaptive value of the P450 duplication in our experimental conditions. Such
463 unexpected response may indicate the presence of a significant fitness cost associated with this large
4 et al., 2013): first, gene duplications can alter the gene dosage balance which can lead to metabolic
466 cost. Second, genomic duplications allow the accumulation of deleterious mutations that are less
467 cleared off by genomic duplication. Indeed, genomic duplications can be detrimental for various reasons (Schrider

et al., 2013): first, gene duplications can alter the gene dosage balance which can lead to metabolic

cost. Second, genom 465 et al., 2013): first, gene duplications can alter the gene dosage balance which can lead to metabolic

466 cost. Second, genomic duplications allow the accumulation of deleterious mutations that are less

467 cleared o cost. Second, genomic duplications allow the accumulation of deleterious mutations that are less
deared off by background selection because of the functional redundancy of duplicated copies.
Third, large duplications can i cleared off by background selection because of the functional redundancy of duplicated copies.

468 Third, large duplications can impair recombination locally. In the present case, metabolic costs due

469 gene dosage bala Third, large duplications can impair recombination locally. In the present case, metabolic costs d

gene dosage balance and/or a low recombination rate at this locus between the Bora-Bora line

adapted to laboratory condit gene dosage balance and/or a low recombination rate at this locus between the Bora-Bora line (well
470 adapted to laboratory conditions) and the IROF line (less fitted to our laboratory conditions) may
471 have impaired th adapted to laboratory conditions) and the IROF line (less fitted to our laboratory conditions) may
471 have impaired the selection of the P450 duplication and favoured the selection of other resistance
471 have impaired th 471 have impaired the selection of the P450 duplication and favoured the selection of other resistances.
471 have impaired the selection of the P450 duplication and favoured the selection of other resistances. 471 have impaired the selection of the P450 duplication and favoured the selection of other resistance

Figure 2014 consistance alleles *in natura*, leading to distinct adaptive trajectories depending on selection pressure

475 conclusions

479 *Conclusions*

50 Following previous work, the present study supports the contrib

kdr mutation, its additive cost with the P450 duplication may have prevented their concomitant
473 kdr mutation, its additive cost with the P450 duplication may have prevented their concomitant
474 selection with the use selection with the use of a high insecticide dose favouring the selection of the former. Such fitne

475 cost balance at two distinct resistance loci may reflect the complex interactions occurring betwe

476 resistance all Following previous work, the present study supports the contribution of this P450 gene duplication in and demographic effects.

478 Conclusions

480 Following previous work, the present study supports the contribution of this P450 gene duplication in

481 pyrethroid resistance. However, deciphering its adaptive value versu 478
479 Conclusions
480 Following previous work, t
481 pyrethroid resistance. How
482 different CVPC cance coming 479
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484 475 Concrussions

480 Following pre

481 pyrethroid re

482 dynamics in n

483 different CYP

484 insecticides) a

485 P450s affecte pyrethroid resistance. However, deciphering its adaptive value versus other resistance alleles and its
dynamics in natural mosquito populations deserves further work. The relative importance of the
different CYP6 genes car dynamics in natural mosquito populations deserves further work. The relative importance of the
different CYP6 genes carried by this duplication in resistance to pyrethroids (and possibly to other
insecticides) also deserve design of a specific PCR diagnostic assay targeting the breakpoints, the dual-colour quantitative insecticides) also deserves further validation. Despite this incomplete picture, the nature of the P450s affected by this la 484 insecticides) also deserves further validation. Despite this incomplete picture, the nature of the
485 p450s affected by this large genomic duplication and their frequent association with insecticide
486 resistance cal Matter of this large genomic duplication and their frequent association with insecticide

486 resistance call for further studying its evolutionary origin and dynamics in response to xenobioti

487 this context, though the resistance call for further studying its evolutionary origin and dynamics in response to xenobioti

this context, though the flanking of the duplication by repeated transposable elements prevente

488 the design of a speci this context, though the flanking of the duplication by repeated transposable elements prevented
the design of a specific PCR diagnostic assay targeting the breakpoints, the dual-colour quantitative
TaqMan ddPCR assay we d 488 the design of a specific PCR diagnostic assay targeting the breakpoints, the dual-colour quantitative

489 TaqMan ddPCR assay we developed (see **Supplementary file 8**) represents a good alternative to tr

490 this resi TaqMan ddPCR assay we developed (see **Supplementary file 8**) represents a good alternative to tract
490 this resistance allele in natural mosquito populations.
491 **Methods**
493 Mesquitess 1489 Taqman ddPcR assay we developed (see Supplementary file 8) represents a good alternative to track

1490 this resistance allele in natural mosquito populations.

1492 Methods

1493 Mosquitoes

491 this resistance allele in natural mosquito populations.
492 **Methods**
494 The *Ae. aegypti* laboratory strain Bora-Bora, fully susce 492
493
494
495
496 493 Mosquitoes
494 The Ae. aegyp
495 the present str
496 collected in 20
497 described in E 493 Mosquitoes
494 The Ae. aegy
495 the present s
496 collected in 2
498 IR13 and IR03
499 resistant and 495 the present study. The isofemale lines used in the present study were derived from a field population
496 collected in 2015 in the IIe Royale (IR) island (5.287° N; 52.590° W) off the coast of French Guiana as
497 des 495 the present study. The isofemale lines used in the present study were derived from a field population
496 collected in 2015 in the lle Royale (IR) island (5.287° N; 52.590° W) off the coast of French Guiana as
497 des described in Epelboin et al. (2021). Among the isofemale lines isolated from this population, the line:

IR13 and IR03 showed contrasted pyrethroid resistance levels with the IR03 line being highly

resistant and the IR13 1981 IR13 and IR03 showed contrasted pyrethroid resistance levels with the IR03 line being highly

1992 resistant and the IR13 line being susceptible as defined by WHO diagnostic dose bioassay (Table 1)

1993 and Epelboin resistant and the IR13 line being susceptible as defined by WHO diagnostic dose bioassay (Tal

and Epelboin et al. (2021). Both lines were shown to lack the two major voltage-gated sodium

channel *kdr* mutations occurrin 499 and Epelboin et al. (2021). Both lines were shown to lack the two major voltage-gated sodium

501 channel *kdr* mutations occurring in this geographical area (Val410Leu and Val1016lle), yet both

502 carried the Phe15 501 channel *kdr* mutations occurring in this geographical area (Val410Leu and Val1016lle), yet both carried the Phe1534Cys mutation at a moderate frequency (Epelboin et al., 2021). The proteor profiling of these lines id 502 carried the Phe1534Cys mutation at a moderate frequency (Epelboin et al., 2021). The proteom
503 profiling of these lines identified multiple cytochrome P450s enriched in the IR03 resistant line
504 compared to a susc 503 profiling of these lines identified multiple cytochrome P450s enriched in the IR03 resistant line as
504 compared to a susceptible line, supporting the presence of metabolic resistance alleles (Epelboin
505 al., 2021) 504 compared to a susceptible line, supporting the presence of metabolic resistance alleles (Epelboin of al., 2021). All mosquito lines were maintained under standard laboratory conditions (27 ± 2°C, 70
506 10 % relative 505 al., 2021). All mosquito lines were maintained under standard laboratory conditions (27 ± 2°C, 70 ± 10 % relative humidity, light/dark cycle 14:10h) and large population size to limit drift effects (N>1000). Larvae we 506 10% relative humidity, light/dark cycle 14:10h) and large population size to limit drift effects
507 (N>1000). Larvae were reared in tap water and fed with hay pellets. Adults were maintained in mes
508 cages and fed w 507 (N>1000). Larvae were reared in tap water and fed with hay pellets. Adults were maintained is
508 cages and fed with a 10% honey solution. Blood feedings were performed on mice.
509 Removal of the Phe1534Cys mutation f

cages and fed with a 10% honey solution. Blood feedings were performed on mice.
509
510 Removal of the Phe1534Cys mutation from the IR03 line
511 In an attempt to isolate metabolic resistance alleles, inter-crosses assiste 509
509 Removal of the Phe1534Cys mutation from the IRO3 line
511 In an attempt to isolate metabolic resistance alleles, inter-crosses assisted by genot
512 performed on the IRO3 resistant line to create the IROF line depl 509
510
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515 510 Removal of the Phe1534Cys mutation from the IR03 line
511 In an attempt to isolate metabolic resistance alleles, inter-cro
512 performed on the IR03 resistant line to create the IR0F line de
513 mutation. A total of 24 Fig. The formed on the IRO3 resistant line to create the IROF line depleted from the Phe1534Cys *kdr* mutation. A total of 245 IRO3 couples were individualised at the pupal stage in eppendorf tubes placed in plastic cups c mutation. A total of 245 IR03 couples were individualised at the pupal stage in eppendorf tube.

placed in plastic cups covered by a nylon mesh and allowed to emerge and reproduce. The

Phe1534Cys Kdr mutation was first se mutation. The United States were topics when the pupal states of the 1534 Cys Kdr mutation was first searched for from male exuvia for all couples and then from
515 Phe1534 Cys Kdr mutation was first searched for from male Fig. 2015
515 Phe1534Cys Kdr mutation was first searched for from male exuvia for all couples and then 1
516 female exuvia of the 58 couples from which males were negatives for the 1534Cys allele. G
517 was performed on qP

516 female exuvia of the 58 couples from which males were negatives for the 1534Cys allele. Genoty
517 was performed on qPCR using the High Resolution Melt Curve Analysis method as described in
518 Saavedra-Rodriguez et al

-
- 517 was performed on qPCR using the High Resolution Melt Curve Analysis method as described in
518 Saavedra-Rodriguez et al. (2007). The six couples in which both parents were homozygous wild type
514 Saavedra-Rodriguez et 518 Saavedra-Rodriguez et al. (2007). The six couples in which both parents were homozygous wild
518 Saavedra-Rodriguez et al. (2007). The six couples in which both parents were homozygous wild $\frac{1}{\sqrt{2}}$ Saavedra-Rodriguez et al. (2007). The six couples in which both parents wild type wild t

520 1534Cys allele in the offspring was confirmed by genotyping 40 individuals of both sexes. Adults were then exposed to 0.05% deltamethrin and survivors were allowed to freely reproduce to generate the IROF resistant lin 521 then exposed to 0.05% deltamethrin and survivors were allowed to freely reproduce to generate the
522 IROF resistant line. The IROF line was then maintained in standard insectary conditions under
523 moderate deltameth

522 IROF resistant line. The IROF line was then maintained in standard insectary conditions under
523 moderate deltamethrin selection pressure (*i.e.* 50% mortality every two generations).
525 Deltamethrin bioassays
526 A 523 IRC resistant line may be the IRC resistent line. The IRO mortality every two generations).
524 ISO Deltamethrin bioassays
525 Deltamethrin bioassays
526 All bioassays were performed by exposing replicates of 20-25 thr 525 Deltamethrin bioassays

525 All bioassays were performed by exposing replicates of 20-25 three days-old non-bloom

527 deltamethrin impregnated filter papers following the standard WHO procedure, with

528 recorded 24h 524
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526
527
528
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530 S25 Dentamethrin biotossuys
526 All bioassays were perforn
527 deltamethrin impregnated
528 recorded 24h after insective
530 sex, use of enzymatic inhit
531 mortality variations betwe deltamethrin impregnated filter papers following the standard WHO procedure, with mortality being

528 recorded 24h after insecticide exposure (WHO, 2022). Different insecticide doses and exposure times

529 were used acco Following the method is Filter and the standard filter paper in the standard filter paper in the standard filter with the standard filter with the standard filter paper in the standard SS0 sex, use of enzymatic inhibitor o were used according to the nature of the experiment and the mosquito tested (susceptibility status,

sex, use of enzymatic inhibitor or not) in order to keep mortality in the dynamic range and maximise

mortality variation 530 sex, use of enzymatic inhibitor or not) in order to keep mortality in the dynamic range and maximise
531 mortality variations between conditions. The bioassays used for assessing the resistance level of the
532 differe 531 mortality variations between conditions. The bioassays used for assessing the resistance level of the
532 different lines presented in Table 1 were performed on females using 10 replicates per line and a
533 dose of 0. 532 different lines presented in Table 1 were performed on females using 10 replicates per line and a
533 dose of 0.05% deltamethrin for 40 min. The involvement of P450s in the resistance phenotype
534 presented in Figure dose of 0.05% deltamethrin for 40 min. The involvement of P450s in the resistance phenotype

presented in Figure 1 was assessed by bioassays on 5 replicates of females exposed or not to 4%

piperonyl butoxide (PBO) for 1h For the internal was assessed by bioassays on 5 replicates of females exposed or not to 4¹

535 pieronyl butoxide (PBO) for 1h prior to deltamethrin exposure using a dose of 0.03% deltame

for 30 min. Bioassays used to i presented in Bioassays used to investigate the mode of inheritance of resistance presented in
535 priperonyl butoxide (PBO) for 1h prior to deltamethrin exposure using a dose of 0.03% deltameth
536 for 30 min. Bioassays us 536 for 30 min. Bioassays used to investigate the mode of inheritance of resistance presented in
537 Supplementary Figure 1 were performed on males and females obtained from the susceptible Bora-
538 Bora line and the resi Samplementary Figure 1 were performed on males and females obtained from the susceptible

Bora line and the resistant IROF line together with F1 and F2 individuals obtained from each

reciprocal crosses (see below). As mal Supplementary Supplementary Supplementary Supplementary Supplementary Supplementary Supplementary Supplementary

544 Supplementary Figure 1 were performed on 5 to 7 replicates per line per generation using a dose of 0.03% Factor 2.1

19 Bora line and females naturally show different tolerance to

19 Insecticides and F1/F2 individuals were expected to be more susceptible than IROF individua

19 deltamethrin exposure conditions were according Face insecticides and F1/F2 individuals were expected to be more susceptible than IROF individual

deltamethrin exposure conditions were accordingly (0.03% deltamethrin for 30 min for fem

0.015% deltamethrin for 25 min). 641 deltamethrin exposure conditions were accordingly (0.03% deltamethrin for 30 min for female.

542 0.015% deltamethrin for 25 min). Finally, bioassays used for monitoring the resistance level of

543 selected and non-se 542 0.015% deltamethrin for 25 min). Finally, bioassays used for monitoring the resistance level of the
543 selected and non-selected lines through the experimental evolution experiment presented in Figure
544 5 were perfo selected and non-selected lines through the experimental evolution experiment presented in Figure 5 were performed on 5 to 7 replicates per line per generation using a dose of 0.03% deltamethrin for the resistance level of

545 1h.
546 *Controlled crosses and association studies*
547 *Controlled crosses and association studies*
549 susceptible Bora-Bora line and the resistant IROF line were performed (cross A: 'Bora-Bora females x
550 suscept 546
547 *Con*
548 In.o
550 IROI
550 IROI 547
548
549
550
551
552 547 Controlled crosses and association states
548 In order to investigate the mode of inheritance
550 IROF males' and cross B: 'IROF females x Bora-F
551 females and males of each line. The susceptibi
552 were then compare susceptible Bora-Bora line and the resistant IROF line were performed (cross A: 'Bora-Bora females

IROF males' and cross B: 'IROF females x Bora-Bora males'). Crosses were performed with 200 virgi

females and males of ea 1995 S50 IROF males' and cross B: 'IROF females x Bora-Bora males'). Crosses were performed with 200 virgin

1995 females and males of each line. The susceptibility of F1 and F2 males and females from each cross

1995 were For the males and males of each line. The susceptibility of F1 and F2 males and females from each cross

state then compared using deltamethrin bioassays as described above. The association of the P450

duplication with de 551 females and males of each line. The susceptibility of F1 and F2 males and females from each cross
552 were then compared using deltamethrin bioassays as described above. The association of the P450
553 duplication with were then compared using denamethrin bioassays as described above. The association of the P450

duplication with deltamethrin survival was studied in F2 females obtained from both reciprocal

S53 are exposed to 0.03% delta ESSA crosses pooled in equal quantities. F2 females were exposed to 0.03% deltamethrin for 1h leadi

S55 88.5% mortality after a 24h recovery time. Dead and surviving females were sampled and stored

20°C until molecular a 555 88.5% mortality after a 24h recovery time. Dead and surviving females were sampled and stored at -
556 20°C until molecular analyses. The response of the P450 duplication and the Ile1011Met *kdr*
557 mutation were furt 20°C until molecular analyses. The response of the P450 duplication and the Ile1011Met *kdr*
557 mutation were further studied across multiple generations using experimental selection. F3 eggs
558 obtained from both recipr 557 mutation were further studied across multiple generations using experimental selection. F3
558 obtained from both reciprocal crosses were pooled in equal quantity and then randomly split
1559 lines: the first two lines 558 obtained from both reciprocal crosses were pooled in equal quantity and then randomly split in 4
559 lines: the first two lines (NSA and NSB) were maintained without insecticide selection, while the t
560 other lines (559 lines: the first two lines (NSA and NSB) were maintained without insecticide selection, while the tv
560 other lines (SelA and SelB) were selected with deltamethrin. This duplicated line setup was used in
561 order to other lines (SelA and SelB) were selected with deltamethrin. This duplicated line setup was used in

561 order to control for genetic drift that may occur across generations. Deltamethrin mass selection was

562 performed order to control for genetic drift that may occur across generations. Deltamethrin mass selection w

selection were prior mating on both virgin males and virgin females (N>1000 for each line) at generation

F4, F6, F8, F12 performed prior mating on both virgin males and virgin females (N>1000 for each line) at generations

563 F4, F6, F8, F12 and F13. A constant dose of deltamethrin was used through the selection process

564 leading to ~70% F4, F6, F8, F12 and F13. A constant dose of deltamethrin was used through the selection process

leading to ~70% mortality in each sex at generation F3 (females: 0.03% for 1h; males: 0.015% for 30

min). The resistance lev 564 Ieading to ~70% mortality in each sex at generation F3 (females: 0.03% for 1h; males: 0.015% for
565 min). The resistance level of each line was monitored at generations F5, F9, F12, F13 and F14 on
566 females prior t min). The resistance level of each line was monitored at generations F5, F9, F12, F13 and F14 on
566 females prior to insecticide selection using standard bioassays (see above). Unexposed adult females:
567 from each line 566 females prior to insecticide selection using standard bioassays (see above). Unexposed adult fem
567 from each line were sampled at the same generations and stored at -20°C until molecular analysi
568 566 females prior to insecticide selection using standard bioassays (see above). Unexposed adult femal
567 from each line were sampled at the same generations and stored at -20°C until molecular analysis.
568 568 from each line were sampled at the same generations and stored at -20°C until molecular analysis.

565 RNA-sequencing
570 Gene transcription
571 RNA-seq. For each
572 three-day-old non-
573 TRIzol (Thermo Fis
574 treated with RNass
575 columns (Qiagen) 571 RNA-seq. For each line, four RNA-seq libraries were prepared from distinct batches of 25 calibrated

572 three-day-old non-blood-fed females not exposed to insecticide. Total RNA was extracted using

573 TRIzol (Therm three-day-old non-blood-fed females not exposed to insecticide. Total RNA was extracted using

573 TRIzol (Thermo Fisher Scientific) following manufacturer's instructions. RNA samples were then

574 treated with RNase-fre TRIzol (Thermo Fisher Scientific) following manufacturer's instructions. RNA samples were then

treated with RNase-free DNase set (Qiagen) to remove gDNA contamination, purified on RNeasy

columns (Qiagen) and QC checked TRIzol (Thermo Fisher Scientific) following manufacturer's instructions. RNA samples were then

574 treated with RNase-free DNase set (Qiagen) to remove gDNA contamination, purified on RNeasy

575 columns (Qiagen) and QC c s columns (Qiagen) and QC checked using Qubit (Thermo Fisher Scientific) and bioanalyzer (Agilent).

ST6 RNA-seq libraries were prepared from 500 ng total RNA using the NEBNext® Ultra™ II directional RNA

library Prep Kit 576 RNA-seq libraries were prepared from 500 ng total RNA using the NEBNext® Ultra™ II directional RN
577 library Prep Kit for Illumina (New England Biolabs) following manufacturer's instructions. Briefly,
578 mRNAs were For starting of the Communist Co FRICE CONSIDER THE SURVEY ON STATE STATES AND THE STATES AND THE STATES AND A SURVEY THAT STATES AND A SURVEY OF THE STATES ARE purified before QC check using Qubit and Bioanalyzer. Libraries were then sequenced in music s 580 adaptors were incorporated at both ends. Libraries were then amplified by PCR for 10 cycles and
581 purified before QC check using Qubit and Bioanalyzer. Libraries were then sequenced in multiple
582 single 75 bp read 581 purified before QC check using Qubit and Bioanalyzer. Libraries were then sequenced in multiplex as

582 single 75 bp reads using a NextSeq500 sequencer (Illumina). An average of 51.4 M reads was

583 generated per li 582 single 75 bp reads using a NextSeq500 sequencer (Illumina). An average of 51.4 M reads was
583 generated per library. After unplexing and removing adaptors, sequenced reads from each library
584 were loaded into Stran 583 generated per library. After unplexing and removing adaptors, sequenced reads from each lib

584 were loaded into Strand NGS V3.2 (Strand Life Science) and mapped to the latest Ae. aegypti

585 assembly (Aaeg L5) usin S84 were loaded into Strand NGS V3.2 (Strand Life Science) and mapped to the latest Ae. aegypti geno

585 assembly (Aaeg L5) using the following parameters: min identity = 90%, max gaps = 5%, min aligne

586 read length = 355 assembly (Aaeg L5) using the following parameters: min identity = 90%, max gaps = 5%, min aligned
366 assembly (Aaeg L5) using the following parameters: min identity = 90%, max gaps = 5%, min aligned
368 read length = Following the following the following parameters: $\frac{1}{2}$ and read trimming if quality < 20, mismatch

star penalty = 4, gap opening penalty = 6, gap extension penalty = 1. Mapped reads were then filtered

based on thei For a read transition of 588 based on their quality and alignment score as follows: mean read quality > 25, max N allowed per
589 read = 5, alignment score ≥ 90, mapping quality ≥ 120, no multiple match allowed, read length ≥ 35.
590 These filter For search is an interval on the intervalsty and alignization of the set filtering steps allowed retaining ~75% of reads. Quantification of transcription levels was

580 ference on the 13614 protein-coding genes using the 590 These filtering steps allowed retaining ~75% of reads. Quantification of transcription levels was
591 performed on the 13614 protein-coding genes using the DESeq2 method with 1000 iterations
592 (Anders & Huber, 2010, Formed on the 13614 protein-coding genes using the DESeq2 method with 1000 iterations

592 (Anders & Huber, 2010, https://bioconductor.org/packages/release/bioc/html/DESeq2.html). O

593 the 11268 protein-coding genes show 592 (Anders & Huber, 2010, https://bioconductor.org/packages/release/bioc/html/DESeq2.html).
593 the 11268 protein-coding genes showing a normalised expression level ≥ 0.5 (~0.05 RPKM) in a
594 replicates for all line 593 (And 11268 protein-coding genes showing a normalised expression level ≥ 0.5 (~0.05 RPKM) in all replicates for all lines were retained for further analysis. Differential gene transcription levels between each line For the 11268 protein-coding a normalised expression levels
595 between each line across all replicates were then computed using a one-way ANOVA followed by
596 Tukey post hoc test and p values were corrected using the Be 595 between each line across all replicates were then computed using a one-way ANOVA followed
596 Tukey post hoc test and p values were corrected using the Benjamini and Hochberg multiple t
597 correction (Benjamini & Hoc Tukey post hoc test and p values were corrected using the Benjamini and Hochberg multiple testing

597 correction (Benjamini & Hochberg, 1995). Genes showing a transcription variation ≥ 1.5 fold (in

598 either direct For the system of Benjamini & Hochberg, 1995). Genes showing a transcription variation ≥ 1.5 fold (in either direction) and an adjusted p value ≤ 0.0005 in the four pairwise comparisons between resistant and susce 598 either direction) and an adjusted p value \leq 0.0005 in the four pairwise comparisons between
599 resistant and susceptible lines (*i.e.* IR03 Vs Bora-Bora, IR03 Vs IR13, IR0F Vs Bora-Bora and IR0F
600 IR13), were c 599 resistant and susceptible lines (*i.e.* IRO3 Vs Bora-Bora, IRO3 Vs IR13, IROF Vs Bora-Bora and IRO
600 IR13), were considered differentially transcribed in association with deltamethrin resistance.
601 Whole genome Poo

1999 Factor in a sociation with deltamethrin resistance.

1999 Factor in a sociation with deltamethrin resistance.

1999 The genome Pool-sequencing

1999 The genome of the four lines were compared using short read whole ge Whole genome Pool-sequencing
602 Mhole genome Pool-sequencing
603 The genome of the four lines were compared using short read whole genome pool-seq. For each
604 genomic DNA was extracted from two batches of 50 adult femal 602
603
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607 Fractional The genome Pool-sequencing
603 The genome of the four lines were of
604 genomic DNA was extracted from ty
605 (Qiagen) and the two gDNA extracts
606 library. Whole genome sequencing
607 prepared according to the genomic DNA was extracted from two batches of 50 adult females using the PureGene Core Kit A

(Qiagen) and the two gDNA extracts were then pooled in equal proportion into a single sequencing

library. Whole genome sequenci 605 (Qiagen) and the two gDNA extracts were then pooled in equal proportion into a single sequencin
606 library. Whole genome sequencing was performed from 200 ng gDNA. Sequencing libraries were
607 prepared according to 606 library. Whole genome sequencing was performed from 200 ng gDNA. Sequencing libraries were
607 prepared according to the TruSeq DNA Nano Reference guide for Illumina Paired-end Indexed
608 sequencing (version oct. 201 For the cording to the TruSeq DNA Nano Reference guide for Illumina Paired-end Indexed

sequencing (version oct. 2017) with an insert size of 550 bp. Sequencing was performed on a

NextSeq 550 (Illumina) as 150 bp paired-608 sequencing (version oct. 2017) with an insert size of 550 bp. Sequencing was performed on a
609 NextSeq 550 (Illumina) as 150 bp paired-reads. Sequencing depth was adjusted to reach an ave
610 coverage $\geq 80X$ leadi Fraction of the sequencing of \sim 713 M reads per library. Reads obtained from e

609 NextSeq 550 (Illumina) as 150 bp paired-reads. Sequencing depth was adjusted to reach an ave

610 coverage \geq 80X leading to the seq 609 NextSeq 550 (Illumina) as 150 bp paired-reads. Sequencing depth was adjusted to reach an average
610 coverage \geq 80X leading to the sequencing of ~713 M reads per library. Reads obtained from each line
611 were loa were loaded into Strand NGS V3.2 and mapped to Ae. *aegypti* genome (Aaeg L5) using the following

parameters: min identity = 90%, max gaps = 5%, min aligned read length = 25, ignore reads with > 5

matches, 3^p end read 612 parameters: min identity = 90%, max gaps = 5%, min aligned read length = 25, ignore reads with > 5
613 matches, 3 \overline{a} end read trimming if quality < 15, mismatch penalty = 4, gap opening penalty = 6, gap
614 exten matches, 3^m end read trimming if quality < 15, mismatch penalty = 4, gap opening penalty = 6, gap
614 extension penalty = 1. Mapped reads were then filtered based on their quality and alignment score
615 as follows: mea Extension penalty = 1. Mapped reads were then filtered based on their quality and alignment score

as follows: mean read quality > 25, max N allowed per read = 5, alignment score ≥ 90, mapping

quality ≥ 60, no multiple m 615 as follows: mean read quality > 25, max N allowed per read = 5, alignment score ≥ 90, mapping
616 quality ≥ 60, no multiple match allowed, read length ≥ 100. Finally, interchromosomal split reads an
617 PCR duplicates 616 quality ≥ 60, no multiple match allowed, read length ≥ 100. Finally, interchromosomal split read PCR duplicates were removed. These filtering steps allowed retaining ~53% of reads. 617 PCR duplicates were removed. These filtering steps allowed retaining ~53% of reads. 617 PCR duplicates were removed. These filtering steps allowed retaining ~53% of reads.

analysis was performed on exons of all protein-coding genes. For each gene, raw coverages were
620 obtained by dividing the number of reads mapped to all (non-overlapping) exons by total exon
621 length. Normalised gene c 620 obtained by dividing the number of reads mapped to all (non-overlapping) exons by total exon
621 length. Normalised gene coverages were then obtained by dividing raw gene coverages by the tot
622 number of reads passi Example 10. September of reads passing mapping and quality filters from each sequenced library. In order to limit filters from the number of reads passing mapping and quality filters from each sequenced library. In order 621 length. Normalised gene coverages were then obtained by dividing raw gene coverages by the total
622 number of reads passing mapping and quality filters from each sequenced library. In order to limit
623 false positiv False positives in low coverage regions, genes showing a normalised coverage value < 1E-8 in at leads to read one line were filtered out (12145 genes retained). As for RNA-seq, genes showing a CNV \geq 1.5 fold either di 624 one line were filtered out (12145 genes retained). As for RNA-seq, genes showing a CNV \geq 1.5 fold (in either direction) in the four pairwise 'resistant *vs* susceptible' comparisons were considered affected by CNV extra the direction) in the four pairwise 'resistant vs susceptible' comparisons were considered affected
626 by CNV in association with resistance.
627 The divergence between the two copies of the P450 duplication was inv by CNV in association with resistance.

626 by CNV in association with resistance.

628 detection of tri-allelic substitution sites in the IROF resistant line. Substitution were called from reads

629 mapped within a regio

627 by Chernal The divergence between the two copies
628 detection of tri-allelic substitution site
629 mapped within a region encompassing
630 (Chr1:271151705 - Chr1:271572082).
631 allele frequency = 10% were called, al detection of tri-allelic substitution sites in the IROF resistant line. Substitution were called from
629 mapped within a region encompassing the P450 duplication extended by 100 kb on both sides
630 (Chr1:271151705 - Chr1

mapped within a region encompassing the P450 duplication extended by 100 kb on both sides
630 (Chr1:271151705 - Chr1:271572082). Only substitutions showing a coverage >40 reads and minimum
631 allele frequency = 10% were c

630 (Chr1:271151705 - Chr1:271572082). Only substitutions showing a coverage >40 reads and minimallele frequency = 10% were called, allowing the detection of 1401 and 1334 substitution sites
and outside the duplicated regi Finally and outside the duplicated region respectively. Substitution sites with and outside the duplicated region respectively. Substitution sites were then considered as tri-allelic if they carried the reference allele t and outside the duplicated region respectively. Substitution sites were then considered as tri-allelic

for the same position,

for the same position,

for the same position,

for the detection of the P450 duplication

for

-
-

633 if they carried the reference allele together with two distinct variant alleles at the same position,
634 each showing a frequency > 20%.
635 Long read sequencing and de novo assembly of the P450 duplication
637 The g each showing a frequency > 20%.
635 if the reference allele to reference allele to reference allele to reference as
636 if the genomic architecture of the duplicated locus was further examined in the duplicated IROF line
6 each showing a frequency > 20%.

635

636 Long read sequencing and de novo assembly of the P450 duplication

637 The genomic architecture of the duplicated locus was further examined in the duplicated IROF line

638 versus 636
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641 The genomic architecture of the duplicated locus was further examined in the sequencial contract of the duplicated locus was further examined in the secrets of the non-duplicated IR13 line. Both lines were submitted to lo For the genomic architecture of the genomic products are submitted to long read sequencing using Oxform Nahappore technology. Genomic DNA was extracted from pools of 75 adult females using the Gentral Puregene Tissue kit (Nanopore technology. Genomic DNA was extracted from pools of 75 adult females using the Gentra
640 Puregene Tissue kit (Qiagen) with 3 mL cell lysis buffer. Genomic DNA was quantified using Qubit
641 DNA Broad Range assay For Puregene Tissue kit (Qiagen) with 3 mL cell lysis buffer. Genomic DNA was quantified using Qubit

EXALD DNA Broad Range assay (Qiagen) and quality-checked by agarose gel electrophoresis before adjusting

concentration 641 DNA Broad Range assay (Qiagen) and quality-checked by agarose gel electrophoresis before adjus
642 concentration to 150 ng/µl. Long genomic DNA fragments were selected using the Short Read
643 Eliminator kit (Circulom 642 concentration to 150 ng/µl. Long genomic DNA fragments were selected using the Short Read
643 Eliminator kit (Circulomics). DNA libraries were prepared using 1 µg gDNA with the ligation
644 sequencing kit SQK-LSK109 k Eliminator kit (Circulomics). DNA libraries were prepared using 1 µg gDNA with the ligation
644 sequencing kit SQK-LSK109 kit (Oxford Nanopore) following manufacturer's instructions. Finall
645 fmoles of the resulting DNA 644 sequencing kit SQK-LSK109 kit (Oxford Nanopore) following manufacturer's instructions. Fi
645 fmoles of the resulting DNA library were loaded on a R9.4.1 Minlon flow cell. Long read seq
646 were collected from 2 indep 644 sequencing kit SQK-LSK109 kit (Oxford Nanopore) following manufacturer's instructions. Finally, 50
645 fmoles of the resulting DNA library were loaded on a R9.4.1 MinIon flow cell. Long read sequences
646 were collect were collected from 2 independent runs of 48 h for each line. Sequencing generated a total of
647 515K/1017K reads with an average length of 13.5/9.8 Kb, representing 3.4/4.8-fold genome
648 coverage, for IROF and IR13 lin 515K/1017K reads with an average length of 13.5/9.8 Kb, representing 3.4/4.8-fold genome
648 coverage, for IROF and IR13 lines respectively. Reads were mapped on the AaegL5 reference ge
650 using Winnowmap (Jain et al., 20 coverage, for IROF and IR13 lines respectively. Reads were mapped on the AaegL5 reference
649 using Winnowmap (Jain et al., 2022) and alignments were visualised using IGV (Robinson et a
650 2011). Finally, de novo genomes coverage, and in the IROF and IR13 lines were visualised using IGV (Robinson et al., 2011). Finally, de novo genomes of the IROF and IR13 lines were assembled using both short and long reads using MaSuRCA (Zimin et al., 20 2011). Finally, de novo genomes of the IROF and IR13 lines were assembled using both short and
651 reads using MaSuRCA (Zimin et al., 2013). This de novo assembly resulted into 17346/13160 co
652 with a median size of 40/ reads using MaSuRCA (Zimin et al., 2013). This de novo assembly resulted into 17346/13160 contigs,

with a median size of 40/55 kb for each line respectively. Transposable elements were identified

using BLAST against the with a median size of 40/55 kb for each line respectively. Transposable elements were identified
653 using BLAST against the RepBase library (10/12/2021 version).
654 Quantification of the P450 duplication
656 The detectio

652 with a median size of 40/55 kb for each line respectively. Transposable elements were identified Example Blast and Maritim Higherian and North Carporal Control.
654 Quantification of the P450 duplication
656 The detection of the P450 duplication was achieved through th
657 the cetyltrimethylammonium bromide (CTAB) met 655
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660 G55 Quantification of the P450 duplication
656 The detection of the P450 duplication was
657 number by digital droplet PCR (ddPCR). Ge
658 the cetyltrimethylammonium bromide (CT
659 free water and quantified using Qubit DN 657 number by digital droplet PCR (ddPCR). Genomic DNA was extracted from pools of mosquitoes use the cetyltrimethylammonium bromide (CTAB) method (Collins et al 1987), resuspended in nuclear free water and quantified usi the cetyltrimethylammonium bromide (CTAB) method (Collins et al 1987), resuspended in nuclease-
free water and quantified using Qubit DNA Broad Range assay (Qiagen). The duplex ddPCR
quantification assay was based on the c 664 2015). Before amplification, gDNA samples were digested with Xhol for 15 min at 37°C directly within
665 the ddPCR reaction mixture. This reaction mixture was partitioned into up to 20,000 nanoliter-sized quantification assay was based on the co-amplification of two target genes each detected v
specific Taqman probes: the gene CYP6BB2 (AAEL014893, Fam) was used to quantify the P4
duplication copy number while the gene CYP4 specific Taqman probes: the gene CYP6BB2 (AAEL014893, Fam) was used to quantify the P450
662 duplication copy number while the gene CYP4D39 (AAEL007808, Hex) always found as a single in various Ae. aegypti lines was used a duplication copy number while the gene CYP4D39 (AAEL007808, Hex) always found as a single
663 in various Ae. *aegypti* lines was used as control for normalisation of gDNA quantities (Faucon e
664 2015). Before amplificati 663 in various Ae. *aegypti* lines was used as control for normalisation of gDNA quantities (Faucon et al.,
664 2015). Before amplification, gDNA samples were digested with Xhol for 15 min at 37°C directly withi
665 the d 664 2015). Before amplification, gDNA samples were digested with Xhol for 15 min at 37°C directly with
the ddPCR reaction mixture. This reaction mixture was partitioned into up to 20,000 nanoliter-sized
for the ddPCR reac the ddPCR reaction mixture. This reaction mixture was partitioned into up to 20,000 nanoliter-sized
the ddPCR reaction mixture. This reaction mixture was partitioned into up to 20,000 nanoliter-sized
of the ddPCR reaction 665 the ddPCR reaction mixture. This reaction mixture was partitioned into up to 20,000 nanoliter-sized

mix. Then, 40μL of the partitioned reaction was amplified for 40 cycles with an annealing
temperature of 60°C (see **Supplementary file 8** for protocol details and primers/probes). After
amplification, the number of posit mix. Then, 40µL of the partitioned reaction was amplified for 40 cycles with an annealing
668 temperature of 60°C (see **Supplementary file 8** for protocol details and primers/probes). After
669 amplification, the number o Emperature of 60°C (see **Supplementary** the 8 for protocol details and primers/probes). After
amplification, the number of positive and negative droplets was quantified for both Fam and H
channels using the QX200 droplet r channels using the QX200 droplet reader (Bio-Rad) and the positive/negative ratio was used to
671 estimate the initial gDNA concentration of each gene assuming a Poisson distribution. After
672 normalisation for gDNA quant estimate the initial gDNA concentration of each gene assuming a Poisson distribution. After
672 normalisation for gDNA quantity, CYP6BB2 copy number were expressed as mean copy number
673 95% CI).
675 Quantification of the Frammer in the initial graduated at the inguitarium graduated as mean copy num
673 95% CI).
674 Quantification of the Ile1011Met kdr mutation frequency
676 The frequency of the Ile1011Met kdr mutation was quantified on poo

673 95% CI).
673 95% CI).
674 Quantification of the Ile1011Met kdr mutation frequency
676 The frequency of the Ile1011Met kdr mutation was quantified on pools of mosquitoes using ddPCR
677 Genomic DNA was extracted as des 674
675 Quantifi
676 The freq
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678 two Team 675
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680 677 Genomic DNA was extracted as described above and digested with Xhol for 15 min at 37°C before
678 partitioning. The duplex TaqMan reaction mixture contained two amplification primers together wit
679 two Taqman probes partitioning. The duplex TaqMan reaction mixture contained two amplification primers together w
two Taqman probes labelled with Fam and Hex fluorophores corresponding to the Met and Iso alle
respectively (see **Supplementar**

two Taqman probes labelled with Fam and Hex fluorophores corresponding to the Met and Iso alleles

respectively (see **Supplementary file 8** for protocol and primer/probes details). The duplex reaction

mixture was emulsion

Eduality Cation of the Ile1011Met kar mutation frequency
676 The frequency of the Ile1011Met kdr mutation was quantified
677 Genomic DNA was extracted as described above and digested
679 two Taqman probes labelled with Fam

For the spectively (see **Supplementary file 8** for protocol and primer/probes details). The duplex reaction

681 mixture was emulsioned as described above and amplified for 40 cycles with an annealing

682 temperature of mixture was emulsioned as described above and amplified for 40 cycles with an annealing

temperature of 59°C. After droplet reading, the positive/negative ratio obtained for each channel

was visualised as a scatterplot an Frame in the set of 59°C. After droplet reading, the positive/negative ratio obtained for each cost was visualised as a scatterplot and was used to estimate the frequency of each allele assur
Poisson distribution with 95% 683 was visualised as a scatterplot and was used to estimate the frequency of each allele assuming a
684 Poisson distribution with 95% CI.
685 **Aknowledgments**
687 We thank the Institut Pasteur de La Guvane for providing t

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687 we thank the Institut Pasteur de La Guyane for providing the isofemale lines IR03 and IR13. We also was lo
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686 **Aknowledgments**
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694 **Authors's contributions**

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695 **Authors's contributions
696 JMB and JPD designed the study. TB, analysed data. JF, FB, JV, JMB and JPD
698 data. JMB, JF and JPD acquired the fu** 695
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696 JMB and JPD designed the study
697 analysed data. JF, FB, JV, JMB and
698 data. JMB, JF and JPD acquired
699 **Data availability** For the share study. The study, they beyen, they below the study. The study of the study analysed data. JF, FB, JV, JMB and JPD supervised data analysis. ID provided biological materials and data. JMB, JF and JPD acquired

data. JMB, JF and JPD acquired the funding. TB, JMB and JPD wrote and revised the paper.
1998 **Data availability**
1908 **Data availability**
1902 13325. DNA-seq short and long reads have been deposited at EBI short read arch

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700 **Data availability**
701 RNA-seq data have been deposited at EBI short read archive (SRA) under accession number
702 13325. DNA-seq short and long reads have been deposited at EBI short read archive (SRA) is 700
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704 700 **Data availability**
701 RNA-seq data have be
702 13325. DNA-seq short
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704 **Ethical aspects**

- Mice were kept in the animal facility of the Biology department of the University of Grenoble-Alpes, 2022 1322-2022 13325. DNA-seq short and long reads have been deposited at EBI short reads having provided at a
1332 13326. To accession number E-MTAB-13310.
1342 13326. Discussed are archive (SRA) under a read archive (SRA TO4
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- 705 **Ethical aspects**
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708 accordance with the 207 approved by the French Ministry of Animal Welfare (agreement no. B 38 421 10 001) and used in
708 accordance with the laws of the European Union (directive 2010/63/EU). The use of animals for this
708 accordance with the laws of the European Union (directive 2010/63/EU). The use of animals for this 708 accordance with the laws of the European Union (directive 2010/63/EU). The use of animals for this

- 709 study was approved by the ComEth Grenoble-C2EA-12 ethics committee mandated by the French
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713 **Funding**
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716 agreement no. 731060
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- 713 Funding
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716 agreement
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- under the *ZIKAlliance* project [grant agreement no. 734548] and the *Infravec2* project [grant
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718 agreement no. CA17108] and the MSCA *INOVEC* project [grant agreement no. 101086257]. Views
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724 Supplementary file 1 (.tiff image)

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726 generate F1 and F2 individuals use

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728 order to account for their inheren 724
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729 724 Supplementary file 1 (.tiff image). Bioassays data from F1 and F2 offspring used to investigate the
725 mode of inheritance of the resistance phenotype. The reciprocal controlled crosses used to
726 generate F1 and F2 The reciprocal controlled crosses used to

generate F1 and F2 individuals used for bioasays are indicated. Deltamethrin exposure condit

were 0.03% deltamethrin for 30 min for females and 0.015% deltamethrin for 25 min fo were 0.03% deltamethrin for 30 min for females and 0.015% deltamethrin for 25 min for males in

order to account for their inherent lower insecticide tolerance. Mortality rates are indicated as me
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T29 and the SD. Distinct letters indicate significantly distinct means (Kruskal-Wallis test followed by post

 \pm SD. Distinct letters indicate significantly distinct means (Kruskal-Wallis test followed by post hoc
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 Supplementary file 2 (.xlsx file). RNA-seq and CNV data sets. All protein coding genes showing a

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For Summary 2003

1730 pairwise Wilcoxon test with Bonferroni-Holm correction, N=5, p ≤ 0.05).

1731 **Supplementary file 2 (.xlsx file). RNA-seq and CNV data sets.** All protein coding genes showing a significant diffe

50 **Supplementary file 2 (.xlsx file). RNA-seq and CNV data sets.** All protein

732 significant differential transcription (sheet 'RNA-seq') or a significant diff

733 (sheet 'CNV') are shown. See methods for filtering con

Supplementary file 2 (.xisx file). RNA-seq and CNV data sets. All protein coding genes showing a

1732 significant differential transcription (sheet 'RNA-seq') or a significant differential exonic coverage

1735 Supplemen The intertion of the extint of the transcriptions of the transcriptions

733 Supplementary File 3 (tiff image). Genomic architecture of the *kdr* locus in the IROF resistant lir

735 Supplementary File 3 (tiff image). Gen 334 beginning with a # were manually inferred from Blast X result.

35 **Supplementary File 3 (.tiff image). Genomic architecture of the** *kdr* **locus in the IROF resistant li

35 On chromosome 3, the 3' part of the VGSC gen** 735 **Supplementary File 3 (.tiff image). Genomic architecture of th**

736 On chromosome 3, the 3' part of the VGSC gene, including the

737 indicate the position of the coding exons. The duplication is 12

739 and right (

335 Supplementary File 3 (.tiff image). Senomic architecture of the kur locus in the IROF resistant line.

236 On chromosome 3, the 3' part of the VGSC gene, including the 21 last exons, is duplicated. Black bars

237 indi

1737 Indicate the position of the coding exons. The duplication is 125 Kb long, and is imperfect: the two

1738 copies, R and R' mostly differing in their intronic sequences. The duplication breakpoints, left (LB)

1739 an ration opies, R and R' mostly differing in their intronic sequences. The duplication breakpoints, left (LB)

and right (RB) are located at 315,905,811 bp and 316,030,250 bp on the reference genome AaegL5

The 1011Met codo 239 and right (RB) are located at 315,905,811 bp and 316,030,250 bp on the reference genome AaegL!

2740 The 1011Met codon is located on the upstream copy (R) and followed by a B type intron. The 1011

2741 codon is on the and right (RB) are located at 315,905,811 bp and 316,030,250 bp on the reference genome AaegL5.

The 1011Met codon is located on the upstream copy (R) and followed by a B type intron. The 1011lle

codon is on the downstrea

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The 1011 The 1011 The 1011Meta of 1011Meta on the 1011Meta of 1011Meta 1012
1742 data supported the sole expression of the 1011Met allele.
1743 Supplementary file 4 (xlsx file). Long reads overlapping the P450 duplication data supported the sole expression of the 1011Met allele.
743 Supplementary file 4 (.xlsx file). Long reads overlapping the P450 duplication breakpoints.
744 Supplementary file 5 (.docx file). Data supporting the identific 743 Supplementary file 4 (.xlsx file). Long reads overlapping the supplementary file 5 (.docx file). Data supporting the ider
745 flanking the P450 duplication.
746 Supplementary file 6 (.docx file). RNA interference exper Supplementary file 4 (.xlsx file). Long reads overlapping the P450 duplication breakpoints.
744 Supplementary file 5 (.docx file). Data supporting the identification of the transposable ele
745 Supplementary file 6 (.docx

Supplementary file 5 (100cx file). Data supporting the identification of the transposable elements

745 Supplementary file 6 (.docx file). RNA interference experiment. This document includes detailed

747 Supplementary fil 745 Flanking the P450 duplication.
746 Supplementary file 6 (.docx file
747 Fusults and methods.
748 Supplementary file 7 (.tiff image architecture. Theoretical struct
750 The hAt-like and PiggyBac-like t Supplementary file 6 (.docx file). RNA interference experiment. This document includes detailed

results and methods.

Supplementary file 7 (.tiff image). Proposed evolutionary steps leading to the observed duplication

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748 Supplementary file 7
749 architecture. Theoret
750 The hAt-like and Piggy
751 events are shown as complete copy of th

349 Supplementary file 7 (third mage). Proposed evolutionary steps leading to the observed duplication
750 architecture. Theoretical structures and those inferred from short and long reads data are indicated.
751 events ar The hAt-like and PiggyBac-like transposons are shown in purple and green respectively. Transposition

events are shown as dashed lines. The second hAt-like transposition event would have created an

incomplete copy of the The matrix and provide and PiggyBac-like and Piggy Bac-like and Piggy Bac-like and Piggy Bac-like and Piggy Bac-
1750 The hAT transposon, either directly or through a posterior deletion.
1753 Recombination events are shown incomplete copy of the hAT transposon, either directly or through a posterior deletion.

753 Recombination events are shown as blue crossed lines. Stars indicates junctions that were confirm

754 by short reads data.

755 753 Recombination events are shown as blue crossed lines. Stars indicates junctions that we
754 by short reads data.
755 **Supplementary File 8 (.docx file). Digital droplet PCR assays used to quantify the P450
756 and the**

254 by short reads data.
753 **Supplementary File 8 (.docx file). Digital droplet PCR assays used to quantify the P450 duplication**
756 **and the frequency of the Ile1011Met kdr mutation.** This document provides all technica Frame and the frequency of
755 **Supplementary File**
757 related these ddPCR
758 interpretation. 353 Supplementary File 8 (100ck file). Digital droplet PCR assays used to quantify the P450 duplication
and the frequency of the Ile1011Met kdr mutation. This document provides all technical details
related these ddPCR ass 256 and the frequency of the field lines kar matation. This document provides an technical details

T57 related these ddPCR assays including amplification primers, TaqMan probes, PCR conditions and interpretation. 758 interpretation primers, TaqMan primers, TaqMan primers, TaqMan primers, TaqMan probes, PCR conditions and data amplification primers, TaqMan primers, TaqMan primers, PCR conditions and data and data and data and dat

758 interpretation.

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