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Insecticide resistance genes affect *Culex quinquefasciatus* vector competence for West Nile virus

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Insecticide resistance has been reported to impact the interactions between mosquitoes and the pathogens they transmit. However, the effect on vector competence for arboviruses still remained to be investigated. We examined the influence of two insecticide resistance mechanisms on vector competence of the mosquito *Culex quinquefasciatus* for two arboviruses, Rift Valley Fever virus (RVFV) and West Nile virus (WNV). Three Cx. quinquefasciatus lines sharing a common genetic background were used: two insecticide-resistant lines, one homozygous for amplification of the Ester² locus (SA2), the other homozygous for the acetylcholinesterase ace-1 G119S mutation (SR) and the insecticide-susceptible reference line Slab. Statistical analyses revealed no significant effect of insecticide-resistant mechanisms on vector competence for RVFV. However, both insecticide resistance mechanisms significantly influenced the outcome of WNV infections by increasing the dissemination of WNV in the mosquito body therefore leading to an increase in transmission efficiency by resistant mosquitoes. These results showed that insecticide resistance mechanisms enhanced vector competence for WNV and may have a significant impact on transmission dynamics of arboviruses. Our findings highlight the importance of understanding the impacts of insecticide resistance on the vectorial capacity parameters to assess the overall consequence on transmission.

1. Introduction

Over the last decades, arthropod-borne viruses (arboviruses) have taken the centre stage due to reemergence in endemic regions and new epidemic outbreaks in naive countries. There are numerous arboviruses spanning different viral families and genera such as Dengue, West Nile and Zika viruses (family Flaviviridae, genus *Flavivirus*), Chikungunya virus (family Togaviridae, genus *Alphavirus*) and Rift Valley Fever virus (RVFV) (family Phenuiviridae; genus *Phlebovirus*) that affect human health worldwide [1]. In the absence of vaccines and specific treatments, the control of mosquito populations is the only affordable measure to disrupt the transmission of arboviruses. For this concern, insecticide treatments have been and are still highly used to control mosquito populations. However, the overuse of these insecticides for public health and agricultural concerns increases selective pressures, leading to the selection and spread of resistance genes in mosquito populations [2–4].

Two main mechanisms are responsible for high level of resistance to insecticides in mosquitoes: overproduction of metabolic enzymes (i.e. metabolic resistance) and the modification of the insecticide target (i.e. target-site

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64 resistance) [5]. Metabolic resistance regroups the various 65 defense mechanisms against xenobiotics that sequestrate 66 and degrade the insecticide in less or non-toxic products, 67 thus decreasing the quantity of toxic molecules likely to 68 reach the target. Three major families of enzymes are 69 involved in this type of resistance: Glutathione S-transferases, 70 Cytochrome P450 monooxygenases and Carboxylesterases 71 [5]. Resistance by target-site modification is due to point 72 mutations in the gene coding of the insecticide target that 73 limits the insecticide binding. Three essential target proteins, 74 all of them being expressed in the nervous system, are the 75 target of insecticides of distinct families: the acetylcholinesterase 76 (target of carbamates and organophosphates), the γ-aminobuty-77 ric acid receptor (organochlorine) and the voltage-gated sodium 78 channels (pyrethroids and DDT [6-8]). The selection of one of 79 these mechanisms leads to increased vector survival in treated 80 environments and to a greater population size, which could 81 increase vectorial capacity.

82 Insecticide resistance genes are often associated with nega-83 tive pleiotropic effects that lead to fitness disadvantage or cost. 84 In insecticide-resistant Culex quinquefasciatus mosquitoes, 85 numerous life-history traits can be modified including 86 increased larval development time, reduced predation avoid-87 ance and reduced male reproductive success [9-14]. Such 88 negative impacts lead to the reduction of resistant allele fre-89 quency in the mosquito population when the insecticide 90 selective pressure is absent or very low [15,16]. Insecticide 91 resistance and their associated costs may interfere with the 92 development and the diversity of symbionts hosted by mos-93 quito vectors. In Cx. quinquefasciatus, the density of the 94 endosymbiotic bacteria Wolbachia was found to be significantly 95 higher in resistant mosquitoes compared to susceptible ones 96 [13,17], although this interaction is very dynamic [18]. Pyre-97 throid-resistant Anopheles gambiae carrying the kdr mutation 98 were shown to be more susceptible to infection by the fungi 99 Metharhizium anisopliae and Beauveria bassiana [19]. Lastly, a 100 recent study on Anopheles albimanus showed a higher bacterial 101 diversity in resistant compared with susceptible specimens 102 [20]. Insecticide resistance may also affect interactions between 103 mosquito vectors and pathogens they transmit, which may 104 have an impact on vectorial capacity. In Cx. quinquefasciatus, 105 insecticide-resistant mosquitoes with higher carboxylesterase 106 activity were less parasitized by the filaria parasite Wuchereria 107 bancrofti than their insecticide-susceptible counterparts [21]. In 108 An. gambiae, the main malaria vector, target-site mutations 109 responsible for insecticide resistance (ace-1 G119S and kdr 110 L1014F) increased the prevalence of Plasmodium falciparum 111 infections in the mosquito salivary glands, which could lead 112 to increased parasite transmission [22,23]. Consistently, pyrethroid-resistant An. gambiae from Tanzania (kdr-east, L1014S) 113 114 was found to be more competent for malaria than susceptible 115 vectors [24]. However, to our knowledge, there is no study 116 describing the potential effects of insecticide resistance on 117 arbovirus transmission.

118 Here, we aimed at characterizing the impact of the two 119 main organophosphate (OP) insecticide resistance mechan-120 isms (carboxylesterase overproduction and insensitive 121 acetylcholinesterase) on the vector competence of Cx. quinque-122 fasciatus mosquitoes for two arboviruses RVFV and West Nile 123 virus (WNV). For this purpose, four parameters (infection 124 rate (IR), dissemination rate (DR), transmission rate (TR) 125 and transmission efficiency (TE)) were compared between 126 resistant and susceptible mosquito lines sharing a common genetic background to determine the influence of insecticide resistance allele. We determined the respective contributions of insecticide resistance mechanism, the arbovirus, the time from blood feeding and the interactions between these variables in the dissemination and transmission of RVFV and WNV.

2. Material and methods

(a) Mosquito lines

We used three isogenic lines of Cx. quinquefasciatus; one susceptible (named Slab) and two lines resistant to OP insecticides. The OP resistant lines were: SA2 homozygous for the amplification of the Ester² locus (leading to overproduction of carboxylesterase) [25] and SR homozygous for the ace-1 G119S mutation [26]. The two resistant lines share a common genetic background with Slab. Each line was backcrossed for at least 14 generations with Slab with the recurrent selection with OP insecticides [9]. Eggs of the three mosquito lines were obtained from the Institut des Sciences de l'Evolution de Montpellier (ISEM) and set up to hatch under standard insectary conditions ($27 \pm 1^{\circ}C$, $70 \pm 8\%$ RH and 12 L:12 D photoperiod). Just after hatching, larvae were randomly seeded into plastic trays containing 11 of tap water at a constant density of about 500 individuals per tray. Larvae were fed ad libitum with a mixture of rabbit and fish food while adults were fed with 10% sucrose solution [w/v].

(b) Viral strains

We used the RVFV SH172805 strain from the lineage East/ Central Africa isolated from a human case in Mauritania in 2003 [27] and a WNV strain belonging to the lineage 1a and isolated from a horse in France (Camargue) in 2000 [28]. All virus stocks were produced on *Aedes albopictus* C6/36 cells, after four passages for RVFV and after three passages for WNV. For all virus stocks, supernatants were harvested and stored at -80° C until experimental infections.

(c) Oral infections of mosquitoes

Seven to 10-day-old females were fed on an infectious blood meal containing 1.4 ml of washed rabbit erythrocytes and 700 μ l of viral suspension supplemented with a phagostimulant (ATP) at a final concentration of 5 mM. The titres of infectious blood meals were 10⁷ PFU ml⁻¹ for both RVFV and WNV. Mosquitoes were allowed to feed for 1 h. Afterwards, fully engorged females were transferred in cardboard containers and maintained with 10% sucrose at 27 ± 1°C for 21 days. The three mosquito lines were infected once with the RVFV while three experimental infections were performed with the WNV (three with the Slab and SR lines and two with the SA2 line).

(d) Vector competence analysis

At 3, 7, 14 and 21 days post-infection (dpi), saliva was collected from individual mosquitoes (15–51 per mosquito line and per experimental infection) by forced salivation as previously described [29]. Briefly, legs and wings of each mosquito were removed and the mosquito's proboscis was inserted into a micropipette tip containing 5 μ l of foetal bovine serum (FBS). After 45 min, the saliva-containing FBS was expelled into 45 μ l of Dulbecco's MEM (DMEM). Following salivation, the head and the body of each mosquito were separated and individually homogenized in 300 μ l of DMEM that was supplemented with 2% FBS.

Vector competence was assessed based on four parameters: IR, DR, TR and TE. The IR corresponds to the proportion of mosquitoes with a body (abdomen and thorax) containing infectious

127 viral particles among fully engorged mosquitoes; the DR was calculated as the proportion of females with infected head tissues 128 (i.e. in which the virus successfully disseminated from the 129 midgut) among mosquitoes presenting infection in their bodies; 130 the TR represents the proportion of mosquitoes with infectious 131 saliva among mosquitoes able to disseminate the virus and the 132 TE corresponds to the proportion of mosquitoes whose saliva 133 contains infectious viral particles among all blood-fed 134 mosquitoes.

(e) Virus titration

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148 149 150 The detection of infectious viral particles in bodies, heads and saliva extracts was performed by titration on Vero cells. For this, six-well plates containing confluent monolayers of Vero cells were infected with serial 10-fold dilutions of body, head homogenates or saliva and incubated for 1 h at 37°C. Thereafter, cells were covered with an overlay consisting of DMEM, 2% FBS, 1% antibiotic-antimycotic mix (Invitrogen, Gibco) and 1% agarose and incubated at 37°C. Cells were incubated 4 days for samples infected with WNV or 5 days for those infected with RVFV. Lytic plaques were then counted after staining with a solution of crystal violet (0.2% in 10% formaldehyde and 20% ethanol).

(f) Statistical analyses

151 We analysed the RVFV and WNV infection outcome on Cx. quin-152 quefasciatus using four parameters as response variables: the IR, 153 the DR, the TR and the TE. To this aim, we examined the effects 154 of three explanatory variables: 'mosquito line' (a three-level categorical variable: Slab, SA2 and SR), 'arbovirus' (a two-level 155 categorical variable: RVFV and WNV) and 'dpi' the day post-156 infection (a numerical variable). All statistical analyses were 157 performed with R software 3.4.0 [30] using a generalized linear 158 model with a binomial error structure. Maximal models included 159 the variables 'mosquito line', 'arbovirus' and 'dpi' and all their 160 interactions. Significance of variables and selection of the mini-161 mal model has been assessed using the 'Anova' procedure 162 within the package 'car' [31], which performs a type III hypoth-163 esis. Estimates of each three parameters were computed and post 164 hoc tests (package 'lsmeans', [32]) were carried out to assess the 165 differences between estimates, and Bonferroni corrections were applied for multiple comparisons. For each mosquito tissue 166 (body, head and saliva), the viral loads were compared between 167 mosquito lines using Kruskal-Wallis test. 168

3. Results

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(a) Comparing vector competence for RVFV and WNV

We examined the effects of two insecticide resistance mech-174 anisms on the transmission of two arboviruses, RVFV and 175 WNV, by comparing vector competence of three Cx. quinque-176 fasciatus lines sharing a similar genetic background: two 177 insecticide-resistant lines (SA2 and SR) and the insecticide-178 susceptible reference line Slab. Overall 801 blood-fed females 179 (383 with RVFV-infected blood and 418 with WNV-infected 180 blood) were analysed to compare RVFV and WNV infection 181 dynamic in mosquitoes over time. This analysis includes 182 mosquitoes infected only once with one of the two viruses. 183

185 (i) Infection rate

The IR was significantly influenced by the *mosquito line* ($\chi^2 = 59.80$, p < 0.0001; table 1), by the *mosquito lin* × *arbovirus* interaction ($\chi^2 = 11.47$, p = 0.003; table 1) and by the *arbovirus* × *dpi* interaction ($\chi^2 = 5.45$, p = 0.019; table 1). Table 1. Statistical analyses of vector competence parameters after infections with Rift Valley Fever virus (RVFV) and West Nile virus lineage 1a (WNV). In these analyses, the influence of mosquito lines (Slab, SA2 and SR), arboviruses dpi) were tested. d.f. is the degree of freedom and χ^2 is the Chi-square value. Significance of variables was obtained after downward selection based on AIC (Akaike model are shown in hold minimal 14 and 21 imnact in the day post-infections (3, 7, information criterion) Variables with significant and WNV) and (RVFV

	infection r	ate		disseminat	ion rate		transmiss	ion rate		transmissio	n efficiency	
source	χ^{2}	d.f.	<i>p</i> -value	Х2	d.f.	<i>p</i> -value	χ^{2}	d.f.	<i>p</i> -value	χ^{2}	d.f.	<i>p</i> -value
mosquito line (ML)	59.8	2	<0.0001	6.32	2	0.042	0.04	-	0.843	3.04	2	0.219
arbovirus	0.53	-	0.467	4.13	1	0.042	2.37	-	0.124	2.87	-	0.09
days post-infection (dpi)	3.07	-	0.061	0.11	—	0.746	1.04	~	0.309	0.65	-	0.421
$ML \times arbovirus$	11.47	-	0.003	8.54	2	0.014	4.93	-	0.026	6.96	2	0.031
$\mathrm{ML} imes \mathrm{dpi}$				3.91	2	0.142	0.49	~	0.486	1.6	2	0.448
arbovirus $ imes$ dpi	5.45	~	0.019	27.83	-	<0.0001	3.55	~	0.059	15.92	~	<0.0001
${ m ML} imes$ arbovirus $ imes$ dpi		I		15.25	2	0.0005	4.20	-	0.040	11.37	2	0.003

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Slab

SA2

Figure 1. Vector competence parameters of the mosquito lines Slab, SA2 and SR infected with RVFV (a,c,e) and WNV (b,d,f). At 3, 7, 14 and 21 days after a blood Q3 meal containing RVFV or WNV (titre of 10^7 PFU ml⁻¹ for both RVFV and WNV), mosquitoes were examined for the presence of infectious viral particles detected by titration on Vero cells. The infection rate (a,b) corresponds to the proportion of mosquitoes whose bodies (thorax and abdomen) contain infectious viral particles among infected mosquitoes; the dissemination rate (c,d) is the proportion of females with infected head tissues among mosquitoes presenting infection in their bodies and the transmission efficiency (e,f) corresponds to the proportion of mosquitoes whose saliva contains infectious viral particles among all infected mosguitoes The number of mosquitoes analysed is indicated in brackets. Error bars represent the 95% confidence interval. Tests of significance were corrected for multiple testing using the Bonferroni procedure. Only tests with significant difference are represented. Asterisks indicate the significance level: *p < 0.05; ***p* < 0.01; ****p* < 0.001. These significant two-way interactions revealed that the effect

of insecticide resistance was different according to the tested arbovirus and that the kinetic of midgut infection was dis-tinct for RVFV and WNV. When mosquitoes were infected with RVFV, IRs ranged from 76% to 100% (figure 1a). Regard-less of dpi, a significant decrease in IR was observed in SR compared to Slab and SA2 (p < 0.0001 and p < 0.0001 for pairwise comparisons between SR/Slab and SR/SA2,

respectively) but no significant difference was observed between Slab and SA2 (p = 0.96). By contrast, the three mosquito lines showed similar IRs when challenged with the WNV (IRs > 95%, all p > 0.99; figure 1*b*).

(ii) Dissemination rate

We then investigated whether insecticide resistance mechanisms affect the viral dissemination beyond the midgut barrier

253 after infectious blood meals through the estimation of the DR. The effects of mosquito line and arbovirus were significant $(\chi^2 = 6.32, p = 0.042 \text{ and } \chi^2 = 4.13, p = 0.042, \text{ respectively};$ table 1). The mosquito line by arbovirus interaction ($\chi^2 = 8.54$, p = 0.014; table 1), the *arbovirus* by *dpi* interaction ($\chi^2 =$ 27.83, p < 0.0001; table 1) and the three-way interaction *mosquito line* × *arbovirus* × *dpi* ($\chi^2 = 15.25$, p = 0.0005; table 1) 260 influenced significantly the DR. This shows that insecticide resistance affected the level and the kinetic of dissemination of WNV compared to susceptible mosquitoes but no difference was observed between the resistant lines SR and SA2 (p = 0.0001, p = 0.0004 and p = 0.86 for pairwise comparisons between Slab/SA2, Slab/SR and SA2/SR, respectively; figure 1d). By contrast, there was no significant difference in the DR of RVFV between the three lines (pairwise 268 comparisons, all p > 0.84; figure 1*c*).

(iii) Transmission rate and transmission efficiency

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We then evaluated the TR and the TE. The statistical analysis 272 showed that both the TR and the TE were dependent on the 273 three-way interaction mosquito line \times arbovirus \times dpi ($\chi^2 =$ 274 4.2, p = 0.04 and $\chi^2 = 11.37$, p = 0.003; respectively, for TR 275 and TE; table 1). For TR, the mosquito line by arbovirus inter-276 action was also significant ($\chi^2 = 4.93$, p = 0.026; table 1). 277 Concerning TE, the mosquito line \times arbovirus ($\chi^2 = 6.96$, p =278 0.031; table 1) and *arbovirus* × *dpi* ($\chi^2 = 15.9$, *p* < 0.0001; 279 table 1) interactions were significant but not the mosquito 280 *line* \times *dpi* ($\chi^2 = 1.6$, p = 0.448; table 1). The significant mos-281 *quito* line \times arbovirus \times dpi interaction suggests that the 282 insecticide-resistant lines influenced viral transmission. The 283 significant interaction mosquito line × arbovirus showed that 284 insecticide resistance impacted the transmission of both arbo-285 viruses differently as observed in figure 1e,f. The significant 286 *arbovirus* \times *dpi* interaction indicated that the kinetic of viral 287 propagation was arbovirus-specific. 288

Very low TEs were observed with the RVFV regardless of 289 the dpi (all TEs less than 14%; figure 1e). Moreover, no signifi-290 cant difference was found between the three mosquito lines 291 (pairwise comparisons, all p > 0.94). By contrast, TEs of 292 WNV were very low at 3 and 7 dpi for the three mosquito 293 lines; and, a significant increase was observed from 14 to 294 21 dpi for both resistant lines: from 54% (± 0.07) to 94% 295 (± 0.04) in SA2 and from 60% (± 0.07) to 90% (± 0.04) in 296 SR. While for the Slab line, TE increased at day 14 and then 297 was found steady between 14 and 21 dpi with 27% (± 0.06) 298 at 14 dpi and 29% (± 0.07) at 21 dpi (figure 1f). Overall, 299 the insecticide-resistant lines SA2 and SR were significantly 300 more competent to transmit the WNV, but not RVFV, than 301 their susceptible counterpart (p = 0.01, p = 0.038 and p =302 0.9 for pairwise comparisons between Slab/SA2, Slab/SR 303 and SA2/SR respectively). 304

(b) Influence of insecticide resistance on WNV vector competence

To confirm the higher capacity of insecticide-resistant mos-310 quitoes to transmit WNV compared to their susceptible counterparts, we analysed data of three independent experimental assays where Slab, SA2 and SR were infected with the WNV and the mosquitoes examined only at 14 dpi. A total of 324 mosquitoes (124, 86 and 114 from the Slab, SA2 and SR lines, respectively) were examined and we determined the effects of mosquito line, experimental assay and the interactions between the two variables on IR, DR, TR and TE. On the four vector competence parameters examined, the experimental assay had a significant effect on three parameters ($\chi^2 = 91.85$, p < 0.0001; $\chi^2 = 17.47$, p = 0.00016and $\chi^2 = 12.31$, p = 0.002 for IR, DR and TE, respectively; table 2).

(i) Infection and dissemination rates

When analysing the IR, no significant difference was observed between the three mosquito lines (all p > 0.99; figure 2a) while for the DR, the main variable mosquito line had a significant influence ($\chi^2 = 18.38$, p = 0.0001; table 2). The two insecticide-resistant lines were more permissive for WNV dissemination than susceptible mosquitoes (p =0.0008, p = 0.022 and p = 0.63 for pairwise comparisons between Slab/SA2, Slab/SR and SA2/SR, respectively; figure 2b).

(ii) Transmission rate and transmission efficiency

Both the TR and the TE were dependent on the mosquito line by *experimental assay* interaction ($\chi^2 = 7.81$, p = 0.05 and $\chi^2 =$ 12.89, p = 0.005 for TR and TE, respectively; table 2) indicating that the difference between mosquito lines varied according to the experimental assay. In addition, a significant effect of *mosquito line* on TE was observed ($\chi^2 = 12.15$, p =0.0023; table 2). Overall, TEs of SA2 and SR lines were significantly higher than that of Slab (p = 0.03, p = 0.02 and p = 0.6for pairwise comparisons between Slab/SA2, Slab/SR and SA2/SR, respectively; figure 2c).

(iii) West Nile virus load

Finally, we compared the viral loads measured in bodies, heads and saliva of mosquitoes from Slab, SA2 and SR infected with WNV and examined at 14 dpi. Among the mosquito lines, Slab individuals had the lowest viral loads in their bodies (mean viral load of 5.09 ± 1.32 , 6.63 ± 0.97 and 6.02 \pm 1.10 log_{10} PFU for Slab, SA2 and SR, respectively; Kruskal–Wallis rank sum test = 23.91, p < 0.0001, figure 2d) and saliva (mean viral load of 2.90 ± 1.50 , 4.48 ± 0.72 and $3.64 \pm 1.36 \log_{10}$ PFU for Slab, SA2 and SR, respectively; Kruskal–Wallis rank sum test = 16.28, p =0.0003; figure 2f) compared to SA2 and SR. However, no significant difference of viral loads in heads was noted between the three mosquito lines (mean viral load of 5.57 ± 1.4 , 6.29 ± 1.23 and $5.52 \pm 1.46 \log_{10}$ PFU for Slab, SA2 and SR, respectively; Kruskal–Wallis rank sum test = 2.27, p = 0.32; figure 2*e*).

4. Discussion

Insecticide resistance has been shown to affect vector competence for pathogens such as the filarial parasite W. bancrofti [21] and the malaria parasite P. falciparum [22-24]. Here, we provide the first evidence of the impact of insecticide resistance mechanisms on the transmission of arboviruses. Using experimental infections, we compared four vector competence parameters (IR, DR, TR and TE) of insecticide-resistant (SA2 and SR) and -susceptible (Slab) Cx. quinquefasciatus lines for RVFV and WNV. These mosquito lines shared a common genetic background through introgression of the Slab genome and

sets are (three s χ^2 is the Chi-square value. Significance of variables was obtained after downward selection based on AIC. Variables with significant impact in the minimal model assay and experimental SR) and SA2 (Slab, line the effects of mosquito To this end, replications. experimental from different obtained infections with WNV after i of the experiment) were tested. d.f. is the degree of freedom and parameters competence analyses of vector Statistical shown in bold i, Table

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	infection ra	ate		disseminat	ion rate		transmiss	sion rate		transmissi	on efficiency	
source	χ,	d.f.	<i>p</i> -value	χ^2	d.f.	<i>p</i> -value	χ^2	d.f.	<i>p</i> -value	χ,	d.f.	<i>p</i> -value
mosquito line (ML)				18.38	2	0.0001	1.70	2	0.428	12.15	2	0.0023
experimental assay (Exp.)	91.85	2	<i>p</i> < 0.0001	17.47	2	0.00016	0.04	2	0.981	12.31	2	0.002
ML imes Exp.	I	I		I		1	7.81	S	0.05	12.89	æ	0.005

differed only by the insecticide selected loci which include the insecticide resistance alleles [9]. Therefore, any phenotypic changes between the insecticide-resistant and the susceptible specimens could be associated with the presence of insecticide resistance loci or with any linked loci hitchhiked during introgression. Moreover, Cx. quinquefasciatus is among the main vectors of WNV (reviewed in [33]) and can also transmit RVFV [34], both arboviruses with an increasing risk of emergence and extending geographical range [35]. The findings presented here show that insecticide resistance mechanisms did not affect vector competence for RVFV probably because the Cx. quinquefasciatus lines tested were poorly competent for this arbovirus [36]. However, both insecticide resistance mechanisms significantly impacted vector competence for WNV by increasing the DR, the TE and the viral loads in bodies and saliva of insecticide-resistant mosquitoes compared

to their susceptible counterparts. When infected with RVFV, very low transmission efficiencies were observed for the three mosquito lines (all values less than 14%) and no significant difference was found between the insecticide-resistant and -susceptible lines. To be transmitted by mosquitoes, arboviruses must overcome several tissue barriers associated with the midgut and the salivary glands [37]. So, we asked whether the observed low transmission of RVFV was the result of low IR and/or DR. Infection rates were high and quite similar between the three mosquito lines (all values greater than 76%). However, dissemination rates were very low (all values less than 17%) even for longer incubation periods (i.e. at 14 and 21 dpi). Thus, RVFV was able to infect and replicate in the midgut epithelial cells but showed low ability to disseminate in the mosquito general cavity and then, to infect salivary glands for subsequent transmission. The observed low dissemination and transmission of RVFV are consistent with previous investigations showing that the mosquito Cx. quinquefasciatus was less able to disseminate and to transmit RVFV compared to other mosquito species such as Aedes vexans [27,38,39]. Therefore, the presence of insecticide resistance mechanisms did not appear to change the interactions between the RVFV and *Cx. quinquefasciatus* mosquitoes in our conditions.

Unlike RVFV, WNV dissemination was significantly affected by insecticide resistance mechanisms. At days 14 and 21 post-infection, higher dissemination rates and transmission efficiencies were noted for SA2 and SR compared to Slab. Variations among the experimental replicates were observed highlighting the importance of performing several experimental replicates to better estimate the factors influencing arbovirus transmission. Collectively, insecticide-resistant mosquitoes showed higher transmission potentials due to a higher DR compared with their susceptible counterparts. In addition, viral loads in saliva and bodies of resistant individuals were also higher than in Slab individuals. Interestingly, both insecticide-resistant mechanisms (i.e. the esterase overproduction in SA2 and a modified acetylcholinesterase in SR) showed similar effects on WNV vector competence. Different insecticide resistance mechanisms were also found to increase the vector competence of mosquitoes for human and rodents malaria parasites [22,24,40] but not for avian malaria parasites vectored by Cx. quinquefasciatus and Culex pipiens [41,42].

Several non-exclusive mechanisms that could explain the observed impact of the carboxylesterases overproduction and the insensitive acetylcholinesterase on vector competence for



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Figure 2. Vector competence parameters and viral loads in bodies (thorax and abdomen), heads and saliva of mosquitoes from the Slab, SA2 and SR lines infected with the WNV (titre of 10^7 PFU ml⁻¹). Three different experiments were performed independently and infected mosquitoes were analysed at 14 days post-infection. The presence of infectious viral particles was detected by titration on Vero cells. (*a*) corresponds to the infection rate, (*b*) to the dissemination rate (*c*) to the transmission efficiency, (*d*) to viral load in the bodies, (*e*) to viral load in the heads and (*f*) to viral loads the saliva. The number of mosquitoes analysed is indicated in brackets. Error bars represent the 95% confidence interval. Tests of significance were corrected for multiple testing using the Bonferroni procedure. Asterisks indicate the significance level: **p* < 0.05; ***p* < 0.01; ****p* < 0.001. n.s.: no significant difference.

WNV were thus explored. There are no data indicating that these overproduced/mutated proteins could affect directly vector competence but it is likely that other loci in linkage disequilibrium could modulate directly vector competence, as demonstrated in pyrethroid-resistant An. gambiae [43]. Further work is ongoing to identify the resistance-linked loci and to characterize them functionally. Among these resistant mechanisms, indirect effects of insecticide resistance

loci (and/or linked loci) on (i) energetic resources, (ii) immune genes and (iii) microbiota may modulate the infection and dissemination of WNV. Concerning energetic resources, the overproduction of carboxylesterase enzymes in the SA2 line may deplete the energy reserves, thus reducing the resources available to cover other biological functions. The energetic resources hypothesis is consistent with a previous study in Cx. *quinquefasciatus* where

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442 insecticide-resistant mosquitoes carrying carboxylesterase 443 overproduction alleles have been found to contain less ener-444 getic reserves (lipids, glycogen and glucose) than their 445 susceptible counterparts [44]. This carboxylesterases overpro-446 duction may also lead to unbalanced redox equilibrium and 447 to oxidative stress, which could affect immunity [45,46]. Con-448 cerning immunity, gene expression analysis in insecticide-449 resistant and -susceptible An. gambiae revealed upregulation 450 of Defensin and Cecropin genes [47,48], two anti-microbial 451 peptides involved in the anti-Plasmodium [49] and antiviral 452 [50] immune responses. Ultimately, the higher competence 453 of SA2 and SR to transmit the WNV compared with Slab 454 could be the difference in the composition of their microbiota. 455 Indeed, there is an important bacterial diversity in mosquito 456 midgut that can modulate vector competence (reviewed in 457 [51]). Moreover, in the mosquito An. albimanus, insecticide-458 resistant specimens were found to harbour lower bacterial 459 diversity compared with susceptible mosquitoes [20]. 460

In conclusion, we showed that the two main insecticide resistance mechanisms affect the vector competence of *Cx. quinquefasciatus* for WNV. The selection of resistance mechanisms resulting from the widespread use of insecticides (in vector and pest control) may thus influence the epidemiology of arboviruses. Such information is crucial because it can help evaluating the impact of insecticide resistance and vector

control on the risk of emergence and on the spread of arboviruses. Further studies using diverse mosquito field populations should help understanding the effects of insecticide resistance on vector competence under different environmental contexts.

Ethics. Animals were housed in the Institut Pasteur animal facilities accredited by the French Ministry of Agriculture for performing experiments on live rodents. Work on animals was performed in compliance with French and European regulations on care and protection of laboratory animals (EC Directive 2010/63, French Law 2013–118, 6 February 2013). All experiments were approved by the Ethics Committee #89 and registered under the reference APAFIS#6427-2016061411435359 vI.

Data accessibility. This article has no additional data.

Authors' contributions. C.M.A., M.W. and A.-B.F. conceived and designed the experiments; C.M.A., L.M. and M.V. performed the experiments; H.A. carried out the statistical analyses; C.M.A., H.A., M.W., M.D. and A.-B.F. wrote the paper. All authors gave final approval for publication.

Competing interests. We have no competing interests.

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