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Fitness costs associated with a GABA receptor mutation conferring dieldrin resistance in *Aedes albopictus*

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Understanding the dynamics of insecticide resistance genes in mosquito populations is pivotal for a sustainable use of insecticides. Dieldrin resistance in *Aedes albopictus* is conferred by the alanine to serine substitution (A302S or *RdI^R* allele) in the γ-aminobutyric acid (GABA) receptor encoded by the *RdI* gene. On Reunion Island, dieldrin resistance was initially reported in natural *Ae. albopictus* populations sampled in 2008 despite the ban of dieldrin since 1994. To monitor insecticide resistance in *Ae. albopictus* on the island and to identify its drivers, we measured (i) the frequency of resistance alleles in 19 distinct natural populations collected between 2016 and 2017, (ii) fitness costs associated with dieldrin resistance in laboratory-controlled experiments, and (iii) the resistance conferred by *RdI^R* to fipronil, an insecticide widely used on the island and reported to cross-react with *RdI^R*. The results show a persistence of *RdI^R* in *Ae. albopictus* natural populations at low frequencies. Among the measured life history traits, mortality in pre-imaginal stages, adults' survival as well as the proportion of egg-laying females were significantly affected in resistant mosquitoes. Finally, bioassays revealed resistance of *RdI^R* mosquitoes to fipronil, suggesting that the use of fipronil *in natura* could select for the *RdI^R* allele. This study shows that dieldrin resistance is persistent in natural mosquito populations likely as a result of combined effects between fitness costs associated with *RdI^R* and selection exerted by cross-reacting environmental insecticides such as fipronil.

INTRODUCTION
Given the medical, veterinary and agricultural impacts of insect pests and vectors, a large range of chemical insecticides including carbamates, organochlorines, organophosphates and pyrethroids have been extensively used for their control. The massive use of these insecticides has led to the rapid selection and spread of resistance mutations in insects' natural populations (Hemingway and Ranson 2000; Raymond et al. 2001). Mechanisms of insecticide resistance that jeopardize many vector control methods include overproduction of metabolic enzymes (i.e. metabolic resistance) that sequestrate and degrade insecticides into less- or non-toxic products, and the modification of the insecticide targets (i.e. target-site resistance) conferred by point mutations reducing the affinity of the insecticide with its target protein (Labbé et al. 2017). These latter mutations have been documented in three groups of proteins involved in the transmission of nervous influx, namely the voltage-gated sodium channels (VGSC) (target of pyrethroids and dichlorodiphenyltrichloroethane or DDT), the acetylcholinesterase (AChE) (target of carbamates and organophosphates) and the γ-aminobutyric acid (GABA) receptor (target of organochlorines) (Labbé et al. 2017). These mechanisms have been reported in mosquito species belonging to *Culex*, *Anopheles* and *Aedes* genera, including the Asian tiger mosquito *Aedes albopictus* species (Hemingway et al. 2004; Labbé et al. 2017; Moyes et al. 2017).

Aedes albopictus is native of Southeast Asia and is currently considered among the most invasive mosquito species in the world (Bonizzoni et al. 2013; Kraemer et al. 2015). Its ecological plasticity of different traits, including egg diapause and the ability to use natural or urban larval breeding sites as well as its opportunistic feeding behaviour may have facilitated dispersal and adaptation to several newly colonized environments displaying a wide range of climatic conditions (Bonizzoni et al. 2013). In addition to an aggressive diurnal human-biting behaviour, *Ae. albopictus* is of significant concern to public health due to its involvement in the transmission of numerous arboviruses such as Chikungunya, Dengue or Zika (Gratz 2004; Tsetsarkin et al. 2007; Paupy et al. 2009; McKenzie et al. 2019). In the absence of vaccines and of effective treatments against such viruses, the control of *Ae. albopictus* populations with insecticides remains the only affordable measure to disrupt arboviral transmission. However, as observed for other mosquito species (Hemingway et al. 2004; Labbé et al. 2017), the use of insecticides had led to the rapid selection of distinct insecticide resistance mechanisms in *Ae. albopictus* (Ranson et al. 2010; Vontas et al. 2012; Moyes et al. 2017; Li et al. 2018). Increased activities of insecticide detoxification enzymes (cytochrome P450 monooxygenases, glutathione S-transferases and carboxylesterases) have been observed in *Ae. albopictus* populations from China and Malaysia (Ishak et al. 2016;
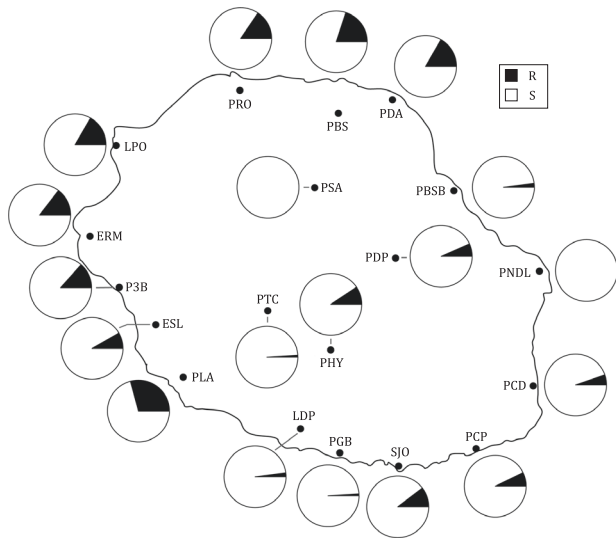
Fig. 1 Allelic frequencies of *Rdl* gene (S: susceptible and R: resistant) in 19 *Aedes albopictus* populations on Reunion Island. See Table S1 for details on sites and mosquito populations.

Li et al. 2018). Similarly, an up-regulation of the carboxylesterase *CCEae3a* gene, related to temephos resistance, was reported in *Ae. albopictus* populations from Greece (Grigoraki et al. 2015). Lastly, target-site resistance has been reported in VGSC and GABA receptor encoding genes of *Ae. albopictus* (Tantely et al. 2010; Kasai et al. 2011; Low et al. 2015; Chen et al. 2016; Xu et al. 2016; Li et al. 2018; Auteri et al. 2018; Su et al. 2019; Zhou et al. 2019; Ahmad et al. 2020).

The GABA receptor is encoded by the *Rdl* (*Resistance to dieldrin*) gene and is made up of five subunits. Each subunit is composed of an extracellular cysteine loop and of four transmembrane segments (TM1 to TM4). Resistance to the organochlorine insecticide dieldrin results from point mutations in the second transmembrane domain of the GABA receptor (ffrench-Constant et al. 1993). In insects, reported mutations correspond to substitution of an alanine in homologous positions 296 or 302 to a glycine (A296G or A302G) or a serine (A296S or A302S) (ffrench-Constant et al. 2000; Du et al. 2005; Tantely et al. 2010; Wondji et al. 2011). In mosquitoes, the A296G substitution has been detected in *Anopheles coluzzii* and *Anopheles gambiae* (Du et al. 2005; Grau-Bové et al. 2020). The A296S substitution has been reported in *Anopheles arabiensis*, *An. coluzzii*, *Anopheles funestus* and *Anopheles sinensis* (Du et al. 2005; Wondji et al. 2011; Yang et al. 2017; Grau-Bové et al. 2020). The homologous A302S substitution has been reported in *Aedes aegypti*, *Ae. albopictus*, *Anopheles stephensi*, *Culex pipiens* and *Culex quinquefasciatus* (Thompson et al. 1993; Andreasen and ffrench-Constant 2002; Tantely et al. 2010; Low et al. 2015; Taskin et al. 2016). For *Ae. albopictus*, the A302S mutation was initially reported in natural mosquito on Reunion Island (Tantely et al. 2010) where the species is recognized as the main vector of Chikungunya and Dengue viruses (Renault et al. 2007; Delatte et al. 2008).

In this study, we performed extensive genotyping of *Ae. albopictus* populations sampled in 19 sites across Reunion Island to monitor the persistence of dieldrin resistant specimens in the field and to assess whether the *Rdl^R* allele is homogeneously distributed on the island. Then, we examined fitness costs associated with *Rdl^R* in *Ae. albopictus* by measuring survival of pre-imaginal instars, larval development time, adult survival, sex ratio, females' fecundity and fertility in resistant and susceptible mosquitoes. Finally, we performed bioassays using fipronil to evaluate cross-resistance conferred by *Rdl^R* to fipronil.

MATERIALS AND METHODS
Mosquito field samples and molecular genotyping
Aedes albopictus specimens were collected as eggs in 19 sites across Reunion Island between November 2016 and March 2017 and were reared to adulthood in the laboratory (see Latreille et al. 2019) (Table S1 and Fig. 1). Total DNA was individually extracted from adult mosquitoes and stored at −80 °C until used, as described in Latreille et al. (2019). The detection of the A302S mutation in the different *Ae. albopictus* specimens was performed using a previously described PCR-RFLP test (Tantely et al. 2010).

Mosquito laboratory strains
Two *Ae. albopictus* laboratory strains were used: a dieldrin susceptible S-Run strain, homozygous for the susceptible *Rdl^S* allele (SS) and a dieldrin resistant R-Run strain homozygous for resistant *Rdl^R* allele (RR). These strains have been previously constructed using both dieldrin selection and a molecular test targeting the A302S mutation (see Lebon et al. 2018). To homogenize the genetic background of the two laboratory strains, nine successive backcrosses were performed between S-Run and R-Run. We used the protocol previously used to construct backcrossed resistant strains in mosquitoes (see Berticat et al. 2002; Djogbénou et al. 2007; Assogba et al. 2015). For this, S-Run females were first crossed with R-Run males. Then males from the heterozygous (RS) progeny were crossed with S-Run (SS) females. After each backcross, resistant (RS) larvae were selected with 0.1 mg.L^{-1} dieldrin as described by Lebon et al. (2018). Then, the surviving heterozygous males were crossed with S-Run (SS) females. After the last backcross, males and females from the derivative strain were crossed and the resulting progeny was submitted to 0.1 mg.L^{-1} dieldrin selection for two generations in order to increase *Rdl^R* frequency and hence the number of homozygous resistant (RR) specimens. For strain purification, forty males were individually isolated and each male was crossed with four females. After mating, males were genotyped using the PCR-RFLP procedure previously described (Tantely et al. 2010), and only cages with homozygous (RR) males were kept. After blood-feeding, females were isolated to lay their eggs and then genotyped. Offspring from the crosses between homozygous (RR) males and females were used to obtain a resistant backcrossed R-Run strain. All subsequent experiments with laboratory strains were carried out using backcrossed R-Run and the S-Run strains, which are expected to share over 99% common nuclear background following the nine successive backcrosses (Berticat et al. 2002). Mosquitoes were maintained in laboratory standard conditions at 26 ± 1 °C and 80% relative humidity (RH) with 12 h light/12 h dark photoperiod. Larvae were supplied every two days with yeast tablets and adults were fed with 10% sucrose solution. To get eggs and assure the maintenance of mosquito strains, females were blood-fed using a Hemotek feeding system (Hemotek Limited, Great Harwood, UK) with defibrinated cow blood.

Fitness cost parameters
We measured fitness cost associated with *Rdl^R* by comparing the pre-imaginal mortality, larval development time, adult survival, sex ratio, female fecundity and fertility between the dieldrin susceptible (S-Run) and the resistant (R-Run) strains.

Pre-imaginal mortality, larval development time, sex ratio and adult survival
To determine the larval development time, pre-imaginal mortality, sex ratio and adult survival, mosquitoes were processed following previously described procedures (Agnew et al. 2004). Briefly, egg hatching was synchronized for both S-Run and R-Run and 500 first-instar larvae from each strain were individually transferred into vials containing 5 mL of a TetraMin® Baby fish food solution (Tetra GmbH, Melle, Germany) at 2 g.L^{-1} in mineral water. No additional food was provided to larvae until emergence. All vials were placed under the same room conditions at 26 ± 1 °C and 80% RH with 12 h light/12 h dark photoperiod. Five racks were used for each mosquito strain and all racks were moved every day to avoid positional effects. Dead larvae and pupae were daily recorded. At emergence, duration of larval developmental time as well as sex were recorded. Adults were kept in their respective vials and no sucrose provided. Therefore, we measured their ability to survive by metabolizing nutritional reserves accumulated during the larval development. Dead adults were daily recorded. This protocol was triplicated.

Female fecundity and fertility

To measure fecundity and fertility of S-Run and R-Run females, crosses were performed in $30 \times 30 \times 30$ cm cages using one-day-old virgin females ($n = 300$) and males ($n = 300$) from the same strain. Five days after caging, a blood meal was provided to females and 100 gravid females from each strain were individually isolated for oviposition. The number of eggs per female was recorded, then eggs were allowed to hatch to assess the hatching rate. This experiment was triplicated.

Bioassays

Bioassays were performed to assess resistance levels of S-Run and R-Run strains to dieldrin and fipronil insecticides. Dieldrin (Dr. Ehrenstorfer, Germany) and fipronil (Dr. Ehrenstorfer, Germany) stock solutions were prepared at 1 g.L^{-1} by dissolving the insecticides in absolute ethanol and acetone, respectively, and were stored at $4\,°C$. Then, sets of 20 late third- and early four-instar larvae from each mosquito strain were placed in plastic cups containing 99 mL of tap water and 1 mL of insecticide with final concentrations ranging from 0 to 10 mg.L^{-1} for dieldrin and 0 to 5 mg.L^{-1} for fipronil. Larval mortality was recorded 24 h after exposure for each condition and was triplicated using larvae reared in different trays. Data were analysed with the BioRssay version 6.2 (Milesi et al. 2013) in R software version 3.6.2 (R Core Team 2019).

Statistical analyses

The Hardy–Weinberg equilibrium test was performed using GenAlEx v.6.5 (Peakall and Smouse 2012) to examine the departure of SS (homozygous susceptible), RR (homozygous resistant) and RS (heterozygous) genotypes from panmixia in each collection site and at the island scale. Differences of RdI^R allele frequency between sample sites were tested using the Fisher exact test and the Bonferroni correction was applied for multiple comparisons.

Pre-imaginal mortality, larval development time and adult survival were analysed using Cox proportional hazards models with the *coxph* function from the *survival* R package (Therneau 2015). Mosquitoes dead at emergence were excluded from the analyses of adult survival. For larval development time and adult survival, the effects of two explanatory variables and their interaction were analysed: *mosquito strain* (S-Run or R-Run) and *sex* (male or female). For the pre-imaginal mortality, only the effect of the *mosquito strain* was tested. In each Cox model, we added the *replicate* variable (three-level factors: A, B and C) as a strata term to consider the experimental variability. The selection of the minimal models was performed based on the Akaike Information Criterion (AIC) and the significance of each variable was assessed with the function *Anova* from the *car* R package (Fox and Weisberg 2019).

Sex ratio, fecundity (the proportion of females laying eggs and the number of laid eggs for each female) and fertility (hatching rate) were analysed by using generalized linear models (GLMs). In each GLM, the effects of *mosquito strain* (S-Run or R-Run), *replicate* (three-level factors: A', B' and C') and their interactions were examined. A binomial or quasibinomial error distribution was used for the GLMs on the sex ratio, the number of females laying eggs and the hatching rate. A quasipoisson error distribution was used for the number of eggs by female. The selection of the best models was performed with AIC or using the likelihood ratio test (function *anova* from *stats* R package). The significance of each variable was assessed using the *Anova* function from the *car* R package, which performs a type III hypothesis test (Fox and Weisberg 2019). Then, based on the best GLM, we used the *emmeans* R package (Russell 2021) to estimate the means and standard errors of the sex ratio, fecundity and fertility parameters. All statistical analyses were done with R software version 3.6.2 (R Core Team 2019).

RESULTS

Low RdI^R frequencies in Reunion *Aedes albopictus* populations

Overall, 860 *Ae. albopictus* mosquitoes from 19 sites (24 to 48 mosquitoes per site, with equal proportion of females and males except for PLA and PSA sites) were genotyped for the RdI^R allele (A302S mutation) (Table S1). The RdI^R allele was observed in most of the sites (17 out of 19 sites), with a frequency ranging from 1% (95% Confidence Interval [95% CI]: −2–4%) in PTC and PGB sites to 29% (95% CI: 16–42%) at PLA site (Fig. 1 and Table S1). Of note, all specimens were homozygous susceptible at PNDL and PSA sites

(located in the East and the Centre of the island respectively, see Fig. 1). No significant difference in RdI^R frequencies was observed between males and females in all sites (Chi-squared and Fisher tests, all $P > 0.05$, Table S1). At the island scale, *Ae. albopictus* is a panmictic population with no significant departure from the Hardy–Weinberg equilibrium genotypic frequency (Chi-squared test, $\chi2 = 3.332$, $df = 1$, $P = 0.068$). When performing analyses for each population, almost all sampled populations were at genetic equilibrium as the analysis did not show a significant departure from the Hardy–Weinberg equilibrium (Chi-squared test, all $P > 0.05$). However, a significant difference was observed between the expected and observed genotypic frequencies at PLA site (Chi-squared test, $\chi2 = 8.138$, $df = 1$, $P = 0.004$), reflecting a departure from Hardy–Weinberg equilibrium of genotypic frequency. Homozygous resistant RR mosquitoes were absent from almost all sites except in LPO (West of the island) and PRO sites (North of the island) where one and two RR mosquitoes were detected, respectively (Table S1). Higher RdI^R allelic frequencies were observed in sites located in the North (PRO, PBS and PDA) and the West (LPO, ERM, P3B, ESL and PLA) as compared to those in the East (PBSB, PNDL and PCD), the South (PCP, SJO, PGB and LDP) and the Centre (PTC, PHY, PDP and PSA) of the island (Fig. 1). Pairwise comparisons of RdI^R allelic frequencies showed significant differences between sites (Fig. 1 and Table S2). For instance, PLA site (West) displayed a significant higher RdI^R frequency as compared to the following sites: PBSB, PCD and PNDL (East), LDP, PCP and PGB (South), PDP, PTC and PSA (Centre) and ESL (West) (Fisher tests, all $P < 0.05$).

Mosquito pre-imaginal mortality is impacted by the RdI^R allele

To examine the effects of the RdI^R allele on pre-imaginal mortality, the number of dead larvae and pupae was recorded daily from egg hatching to adult emergence and compared between S-Run ($n = 1492$) and R-Run ($n = 1497$) strains. A significant effect of mosquito strain was detected (Cox model, $\chi2 = 158.66$, $df = 1$, $P < 0.001$) (Fig. 2A and Fig. S1) with the R-Run strain displaying a higher pre-imaginal mortality (mean rate: 25.3%; 95% CI: 23.0–27.5%) as compared to S-Run (mean rate: 7.9%; 95% CI: 6.5–9.3%). This result was observed in each of the three replicates, but one replicate showed particularly higher mortality in resistant (47.7%; 95% CI: 43.3–52.1%) than in susceptible mosquitoes (10.3%; 95% CI: 7.6–13.0%) (Fig. S2).

No effect of RdI^R on larval development

The larval development time was examined by counting the number of days required for the first-instar larvae to reach adulthood ($n = 1373$ for S-Run and $n = 1117$ for R-Run). No significant interaction between the mosquito strain and sex was detected (Cox model, $\chi2 = 0.199$, $df = 1$, $P = 0.656$) and no significant effect of the mosquito strain was found with a median number of 8 days (95% CI: [8;8]) for larval development time for both R-Run and S-Run strains (Cox model, $\chi2 = 1.766$, $df = 1$, $P = 0.184$) (Fig. 2B and Fig. S1). As expected, a significant effect of sex was detected for both strains, with males emerging earlier (median: 8 days, 95% CI: [8;8]) than females (median: 9 days, 95% CI: [9;9]) (Cox model, $\chi2 = 213.660$, $df = 1$, $P < 0.001$).

RdI^R affects mosquito sex ratio

We also examined whether the RdI^R allele affects the sex ratio by counting males and females at adult emergence for both strains ($n = 1373$ for S-Run and $n = 1117$ for R-Run). The analyses showed that the sex ratio was significantly influenced by the mosquito strain (GLM, $\chi2 = 5.791$ $df = 1$, $P = 0.016$), the replicate ($\chi2 = 15.545$ $df = 2$, $P < 0.001$) and also by the interaction between mosquito strain and replicate ($\chi2 = 10.378$, $df = 2$, $P = 0.006$). Although the global proportion of females was significantly higher for R-Run strain (0.46 ± 0.02 [Standard Error: SE]) relatively to S-Run
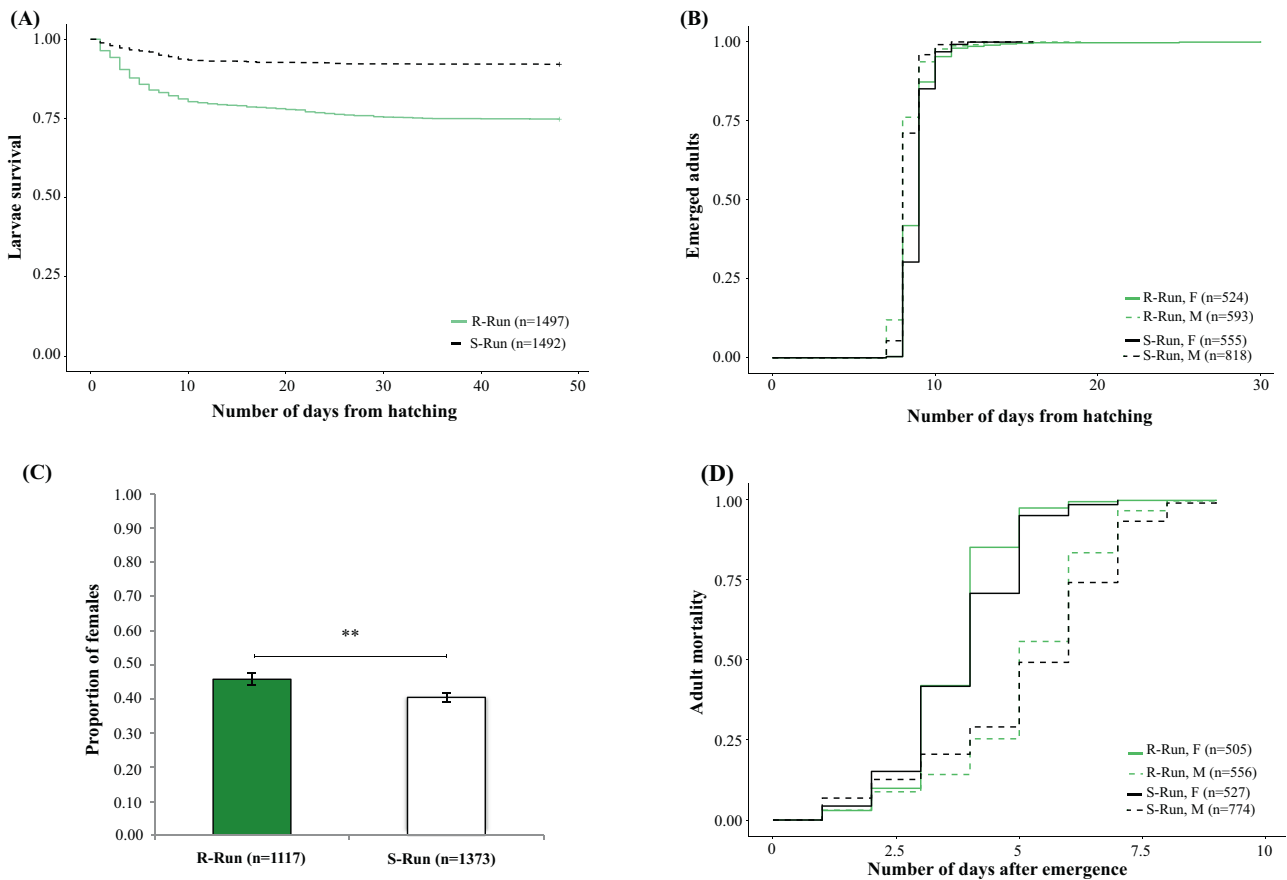
Fig. 2 Comparison of larvae survival, pre-imaginal development, sex ratio and adult mortality according to mosquito strain (R-Run and S-Run). Larvae survival (proportion of larva surviving each day) (**A**), pre-imaginal development time (proportion of adults emerging each day) (**B**) and sex ratio (**C**) according to mosquito strain. Adult mortality (proportion of adults dying each day) according to mosquito strain and sex (**D**). The error bars correspond to standard errors and asterisks indicate the level of significance of differences (**$P < 0.01$). The numbers in parentheses correspond to the number of specimens used for a given mosquito strain and/or sex (M: male or F: Female).

strain (0.40 ± 0.01) (Fig. 2C), a significant difference was not systematically detected in all three replicates (Fig. S3).

Shorter lifetime for dieldrin resistant adult mosquitoes
Adult survival was obtained by counting the number of days between emergence and death ($n = 1301$ for S-Run and $n = 1061$ for R-Run), with no sucrose solution provided. A significant effect of mosquito strain was detected with a lower survival for resistant (median: 4 days, 95% CI: [4;4]) than for susceptible mosquitoes (median: 5 days, 95% CI: [5;5]) (Cox model, $\chi2 = 36.041$, $df = 1$, $P < 0.001$) (Fig. 2D and Fig. S1). In addition, a significant effect of sex was detected with a global higher survival of males compared to females (median: 5 days, 95% CI: [5;5] for males vs. 4 days, 95% CI: [4;4] for females) (Cox model, $\chi2 = 761.965$, $df = 1$, $P < 0.001$). No significant interaction between the mosquito strain and sex on adult survival was detected (Cox model, $\chi2 = 1.797$, $df = 1$, $P = 0.180$). Hence, the difference between males and females was similar for each strain regardless of their genotype (R-Run males and females: 5 days [95% CI: [5;5]] and 4 days [95% CI: [4;4]], respectively; S-Run males and females: 5 (95% CI: [5;6]) and 4 days [95% CI: [4;4]], respectively).

A lower proportion of resistant females laying eggs
We evaluated the effects of Rdl^R on fecundity by measuring the proportion of egg-laying females and the number of eggs laid per female for both for R-Run and S-Run strains. A significant effect of mosquito strain was found (GLM, $\chi2 = 7.461$, $df = 1$, $P = 0.006$) on the proportion of egg-laying females, which was lower in R-Run

(0.85 ± 0.02 [SE], 230 out of 287) than in S-Run females (0.92 ± 0.02, 260 out of 300) (Fig. 3A). Although a significant effect of replicate was detected (GLM, $\chi2 = 102.531$, $df = 2$, $P < 0.001$), no significant mosquito strain by replicate interaction was detected (GLM, $\chi2 = 1.089$, $df = 2$, $P = 0.580$), indicating that the rate of R-Run females that laid eggs was significantly lower than that of S-Run females in each replicate (Fig. S4).

The number of eggs per female was not statistically different between mosquito strain (GLM, $\chi2 = 1.210$ $df = 1$, $P = 0.271$). However, the replicate (GLM, $\chi2 = 52.287$, $df = 2$, $P < 0.001$) and the interaction between mosquito strain and replicate ($\chi2 = 14.737$, $df = 2$, $P < 0.001$) influenced significantly the number of eggs. Indeed, S-Run females laid more eggs than R-Run females in two out of three replicates (Fig. 3B) but the overall difference was not significant. Similarly, the hatching rate was not influenced by the mosquito strain (GLM, $\chi2 = 1.135$, $df = 1$, $P = 0.287$) but was influenced by the replicate (GLM, $\chi2 = 192.636$, $df = 2$, $P < 0.001$) and the interaction between mosquito strain and replicate (GLM, $\chi2 = 16.344$, $df = 2$, $P < 0.001$) (Fig. 3C). A significant difference of hatching rates between R-Run and S-Run was not systematically detected through the three replicates.

The Rdl^R allele confers resistance to fipronil
The bioassay results showed that the R-Run strain displayed significantly higher resistance levels to dieldrin and to fipronil than the S-Run strain. Resistance Ratios ($RR_{50} = LD_{50R-Run} / LD_{50S-Run}$) of 70.0 and 3.41 were measured for dieldrin and fipronil, respectively (Fig. 4A, B).
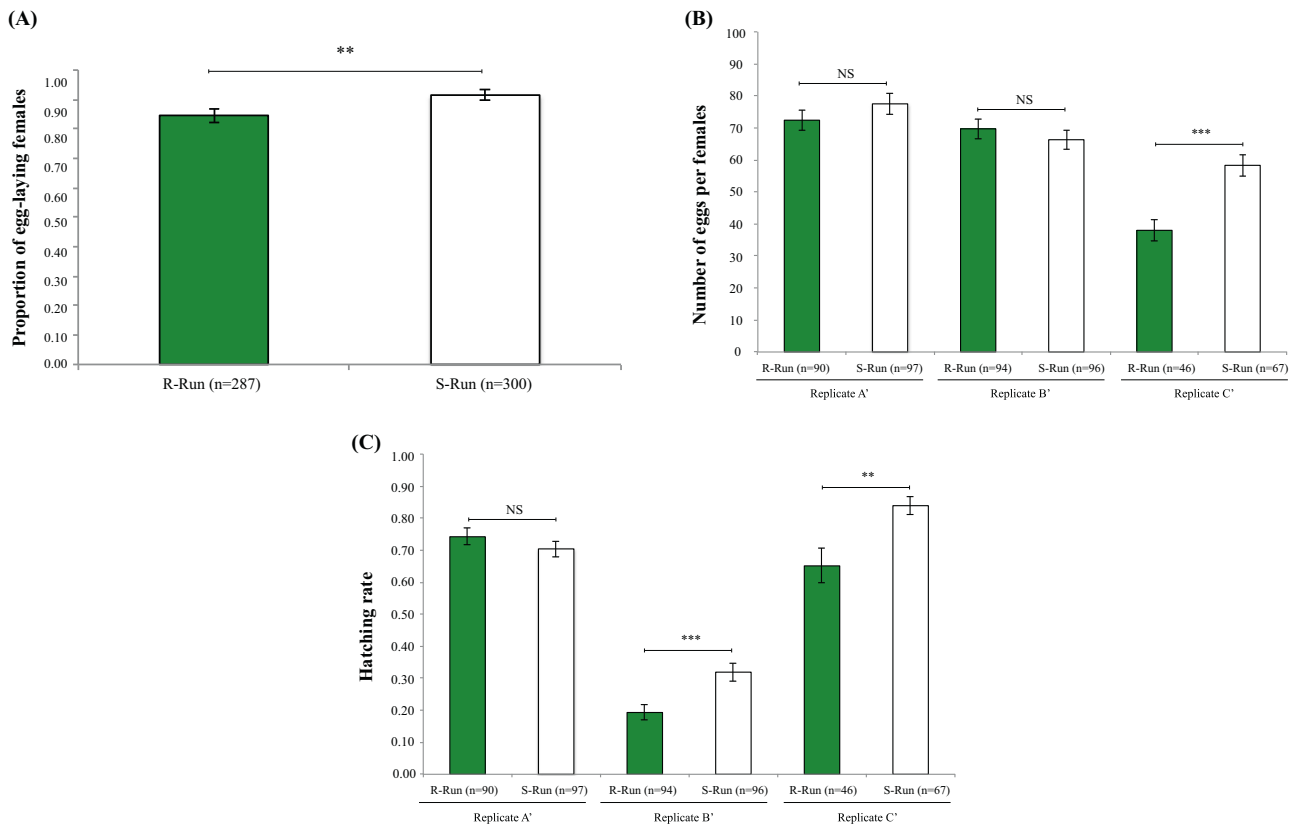
Fig. 3 Fecundity and fertility of females according to mosquito strain (R-Run and S-Run). Proportion of egg-laying females according to mosquito strain (**A**). Number of eggs per female (**B**) and hatching rate (**C**) according to mosquito strain and replicate. The errors bars correspond to standard errors and asterisks indicate the level of significance of differences (NS: No significant difference; **$P < 0.01$; ***$P < 0.001$). The numbers in parentheses correspond to the number of specimens used for a given mosquito strain.

DISCUSSION

To assess whether Rdl^R is maintained in natural mosquito populations of *Ae. albopictus* and to understand the drivers of its distribution, we first performed extensive genotyping of specimens collected between 2016 and 2017 in 19 sites across Reunion Island. Secondly, we evaluated under laboratory-controlled conditions the fitness costs associated with Rdl^R. As fitness associated with a resistance allele can vary according to the genetic background, the Rdl^R allele was backcrossed into the susceptible S-Run background. It is important to note that we cannot exclude the possibility of selecting for other resistance loci during successive backcrosses as dieldrin was used following each backcross to select for Rdl^R. However, when Rdl^R is genetically linked to the male locus through translocation, dieldrin resistance and male sex are almost perfectly co-transferred to the progeny, strongly suggesting that resistance is conferred by Rdl^R alone (Lebon et al. 2018). Nevertheless, sharing a common nuclear background between resistant and susceptible strains was helpful to describe fitness cost associated with the phenotype of dieldrin resistance in *Ae. albopictus* and to reduce any additional confounding effect.

The Rdl^R allele was detected in almost all sampled mosquito populations with low frequencies, ranging from 1 to 29%. Although almost all of our sampled populations were at genetic equilibrium, very few homozygous resistant RR mosquitoes (0.4%) were observed in the data set, homozygous susceptible SS (81.6%) and heterozygous RS (18.0%) mosquitoes being predominant (Table S1). In *Ae. albopictus* from Malaysia, Low et al. (2015) also observed a predominance of susceptible and heterozygous genotypes in a sample where the Rdl^R allelic frequency was 38%. The low Rdl^R frequencies observed in our study are consistent with the previous study of Tantely et al. (2010) reporting low Rdl^R frequencies in *Ae.*

albopictus collected in 2008 (10 and 30% in Ravine à Jacques and the city of Saint-Denis, respectively). Similarly, Rdl^R frequencies of 7.5–24.5% were observed in *Ae. albopictus* populations collected in 2011 and 2012 in Saint-Benoit, Saint-Denis and Saint-Paul localities (Lebon et al. 2022). As Rdl^R is still detected in *Ae. albopictus* more than eight years after its first report in 2008, our results confirm the long-term persistence of Rdl^R in *Ae. albopictus* field populations on Reunion Island despite the ban of dieldrin on the island since 1994.

It is well known that mutations conferring resistance to insecticides are often associated with measurable fitness cost that can lead to reduced performance in resistant mosquitoes (Bourguet et al. 2004; Berticat et al. 2004; Duron et al. 2006; Kliot and Ghanim 2012; Assogba et al. 2015, 2016; Freeman et al. 2021; Deng et al. 2021). Therefore, in the absence of insecticide pressure, susceptible individuals are expected to out-compete resistant individuals and thus drive resistance alleles to low frequencies (Raymond et al. 2001). For dieldrin, both reversion to susceptibility and maintenance of the resistance have been documented in literature. A reversion of insecticide resistance was reported in *Anopheles culicifacies* from India three years after stopping dieldrin spraying (Bhatia and Deobhankar 1963). In Northern Nigeria, six years after the withdrawal of dieldrin, Hamon and Garret-Jones (1962) did not observe a reversion to susceptibility in *An. gambiae* in the field and reported the presence of resistant RR and RS genotypes in untreated area of Sierra Leone, Ivory Coast, Liberia, Guinea and Burkina Faso. More recent studies also described the persistence of mutations conferring resistance to dieldrin in *Anopheles* populations in Africa (Wondji et al. 2011; Grau-Bové et al. 2020) and in *Ae. albopictus* from Malaysia (Low et al. 2015) despite discontinuation of dieldrin use. These studies thus demonstrated that the resistance to dieldrin persists in several mosquito species as described in this study in *Ae.*
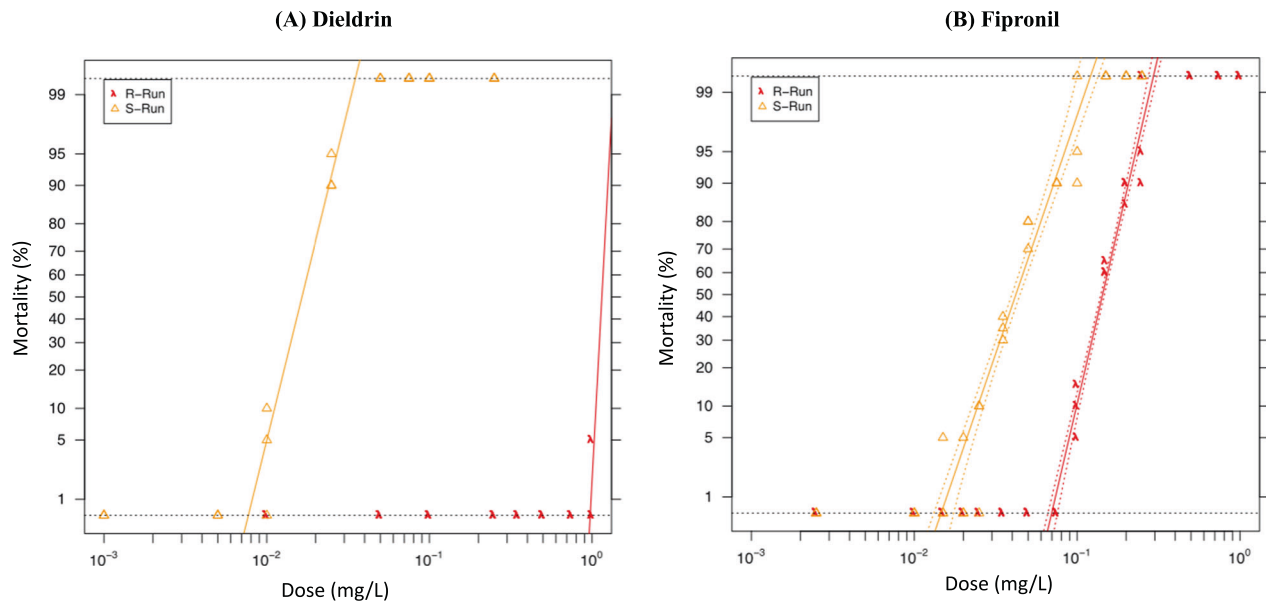
Fig. 4 Bioassay results. Results of treatments with dieldrin (**A**) and fipronil (**B**) on larvae of R-Run and S-Run mosquito strains.

albopictus populations from Reunion Island despite the ban on the use of dieldrin in Reunion Island over 25 years. Interestingly, the persistence of RdI^R is also observed in *Cx. quiquefasciatus* populations on the island with frequencies close to fixation in some sites (Lebon et al. 2022). Different non-exclusive hypotheses including fitness cost and/or cross-resistance could explain this persistence of the RdI^R allele.

Previous investigations on *Anopheles* mosquitoes have shown that dieldrin resistance affects life history traits including mating competitiveness, predator avoidance and response to oviposition (Rowland 1991a, 1991b; Platt et al. 2015) but not longevity nor its vectorial capacity (Hamon and Garret-Jones 1962). In the present study, the fitness cost of RdI^R was measured by comparing the pre-imaginal mortality, larval development time, adult survival, female fecundity and fertility between the dieldrin resistant R-Run and susceptible S-Run strains sharing a common genetic background. Among the measured parameters, a significant difference between resistant and susceptible mosquitoes was evidenced in mortality in pre-imaginal stages, adults' survival and the proportion of egg-laying females in our experimental conditions (backcrossed mosquito strains and laboratory-controlled environment). In summary, the pre-imaginal mortality of resistant mosquitoes was 3 times higher than in susceptible mosquitoes (25.3 and 7.9%, respectively). The adult survival was slightly shortened in resistant mosquitoes as compared to susceptible mosquitoes (4 and 5 days, respectively). Regarding the proportion of egg-laying females, a reduction of 7% was observed between resistant and susceptible mosquitoes. We also observed a reduction of fecundity in resistant females as compared to susceptible ones, although this reduction was not observed in all replicates. Despite the reduction of some life history traits in resistant individuals as compared to susceptible mosquitoes, these fitness costs were measured under laboratory conditions and may not reflect the fitness of resistant and susceptible mosquitoes in the field. It would be interesting in future investigations to compare life history traits between resistant and susceptible mosquitoes under field conditions. Such data could be used to model the dynamics of the RdI^R allele in nature. Besides modelling, population cage experiments could be also performed to examine the competitive advantage of susceptible over resistant mosquitoes in the absence of insecticide. It would be also interesting to examine the influence of temperature on fitness cost associated with resistance to dieldrin

in *Ae. albopictus* as it has been previously shown in *Drosophila melanogaster* that homozygous resistant flies carrying the RdI^R allele lose their locomotor ability when exposed to 38 °C temperature (ffrench-Constant et al. 1993).

The persistence of RdI^R in *Ae. albopictus* populations on Reunion Island could also result from the selective pressure exerted by other pesticides targeting GABA receptors (Buckingham et al. 2005; Tantely et al. 2010). Since fipronil is widely used on the island for the control of termites and ants, other arthropods, including mosquitoes, might be exposed to an insecticide previously reported as targeting the GABA receptor (Hosie et al. 1995; Buckingham et al. 2005; Taylor-Wells et al. 2015). Therefore, we performed bioassays with fipronil and observed a low resistance level conferred by the RdI^R allele in *Ae. Albopictus* ($RR_{50} = 3.41$). This confirms a cross-resistance conferred by RdI^R to dieldrin and fipronil as described in *Anopheles* mosquitoes (Brooke et al. 2000; Kolaczinski and Curtis 2001; Davari et al. 2007) and other insects such as *D. melanogaster* (Hosie et al. 1995). In addition to fipronil, pesticides used for agricultural and veterinary purposes such as endectocides and other chemicals could also display cross-resistance with dieldrin (Ozoe et al. 2013) on Reunion Island. Therefore, it will be interesting to investigate pesticide-use practices to identify additional factors explaining the persistence of RdI^R in *Ae. albopictus* on the island. In this perspective, analyses of soils could be conducted to evaluate the presence and long-term persistence of pesticides targeting the GABA receptor.

Lastly, we cannot exclude the possibility that the stability of dieldrin resistance in natural *Ae. albopictus* populations is an example of a balanced polymorphism independent of insecticide exposure, as previously documented for malathion resistance in *Lucilia cuprina* (Hartley et al. 2006). However, we must emphasize that in contrast with the situation reported for *L. cuprina*, we show that dieldrin resistance is associated with a fitness cost in laboratory conditions. In natural conditions, such a fitness cost should have reduced RdI^R frequency. Therefore, we hypothesized that cross-resistance to fipronil (or any other selective pressure on the island) may be at least in part responsible for the maintenance of RdI^R in *Ae. albopictus* natural populations.

CONCLUSION
In the present study, we confirmed the long-term persistence of RdI^R in *Ae. albopictus* populations on Reunion Island and report

measurable fitness costs associated with Rdl^R. In addition, we report a cross-resistance conferred by Rdl^R to fipronil. Therefore, we suggest that the long-term persistence of this resistance in Reunion *Ae. albopictus* natural populations may result of combined effects between fitness costs associated with Rdl^R and cross-resistance to fipronil or to any chemical cross-reacting with the GABA receptor. As insecticide resistance genes can affect other mosquito traits such as immunity and vector competence (Alout et al. 2013; Atyame et al. 2019; Deng et al. 2021), particular attention must be given to insecticide resistance in order to better understand the transmission patterns of mosquito-borne pathogens. This is of outmost importance for Reunion Island since *Ae. albopictus* is the main vector involved in a major Dengue epidemic still ongoing on the island.

DATA AVAILABILITY

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COMPETING INTERESTS
The authors declare no competing interests.

ADDITIONAL INFORMATION
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