

Putative Pleiotropic Effects of the Knock-Down Resistance (L1014F) Allele on the Life-History Traits in *Anopheles Gambiae*.

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Abstract

Background

Existing mechanisms of insecticide resistance have been known to help the survival of mosquitoes following contact with chemical compounds, even though they could negatively affect the life-history traits of resistant malaria vectors. In West Africa, the knock-down resistance mechanism, *kdr^R* (L1014F) is the most common. However, little knowledge is available on its effects on mosquito life traits. We investigated the fitness effects associated with this knock-down resistance allele in *Anopheles gambiae sensu stricto* (s.s.).

Methods

Two laboratory reference strains of *An. gambiae* s.s., Kisumu (susceptible) and KisKdr (*kdr* resistant) were used. Female mosquitoes were fed and allowed to lay eggs. Fecundity and fertility were assessed by examining the number of eggs per mosquito and larval hatching rates. Larval survivorship and pupation rates were also measured. Female mosquitoes of both strains were fed through membrane feeding assays, then the blood feeding success, blood volume and adult survivorship were monitored.

Results

An. gambiae carrying *kdr^R* allele (KisKdr) showed a lower ability to lay eggs. The mean number of larvae in the susceptible strain Kisumu was overall threefold higher than that seen in KisKdr strain with significant difference in the hatching rates (81.89% in Kisumu versus 72.89% in KisKdr, $p= 0.003$). KisKdr larvae had a significant higher larval survivorship than Kisumu. The blood feeding success was significantly higher ($p= 2.2 \cdot 10^{-16}$) in the resistant mosquitoes (84%) than that in the susceptible ones (34.75%). However, the mean blood volume was 1.36 $\mu\text{L}/\text{mg}$, 1.45 $\mu\text{L}/\text{mg}$ and 1.68 $\mu\text{L}/\text{mg}$ in Kisumu, homozygote and heterozygote KisKdr respectively. After blood feeding, the heterozygote KisKdr displayed highest survivorship when compared to that of Kisumu.

Conclusions

Our findings provide novel insights on fitness effects of the *kdr^R* (L1014F) allele in *An. gambiae*. The presence of this resistance allele tends to have an impact on mosquito life-history traits such as fecundity, fertility, larval survivorship and blood feeding behaviour. These data could help to guide the implementation of more reliable strategies for the control of malaria vectors.

Background

Malaria is still one of the most devastating parasitic diseases, especially in tropical regions worldwide. Indeed, this disease still spreads in 96 countries in America, many in parts of Asia and most of Africa [1]. Malaria is transmitted through the bite of infected female of *Anopheles* mosquito, which carry the

infection by protozoan parasites *Plasmodium* species to humans [2]. In most regions in sub-Saharan Africa, mosquito species including *Anopheles gambiae s.s.*, *Anopheles arabiensis*, *Anopheles coluzzii* and *Anopheles funestus* are the main vectors that transmit malaria parasites [3–5]. Since an effective malaria vaccine is yet to become available, vector control still remains the main control measure for the prevention of malaria transmission [6]. Indeed, Long-Lasting Insecticide-treated Nets (LLINs) and Indoor Residual Spraying (IRS) remain the backbone of malaria vector control and have been shown to contribute to malaria control through the reduction of human-vector contact [7]. Unfortunately, insecticide resistance to pyrethroids (permethrin, deltamethrin) and to other classes of insecticides has been reported in *Anopheles gambiae*, the main malaria vectors in several African countries [8–14]. The major insecticide resistance mechanisms in *An. gambiae* consist of target sites insensitivity (*ace-1^R*, *kdr^R*) and increased metabolic activity of detoxifying enzymes [15–20]. In *Anopheles gambiae s.s.*, mutations related to pyrethroids and dichlorodiphenyltrichloroethane (DDT) resistance are located mainly at codon 1014 within transmembrane segment 6 of domain II in the voltage-gated sodium channel (*Vgsc*). These mutations lead to a change of Leucine to either Phenylalanine (L1014F) or Serine (L1014S) [21, 22]. Further, additional mutation at position 1575 of the linker between domains III-IV in the *Vgsc* resulting in Asparagine-to-Tyrosine substitution (N1575Y) has been found occurring solely on a 1014F-bearing haplotype [23]. Recent studies carried out in Benin [24], Ivory Cost [25] and Burkina Faso [26] have shown that the L1014F allele frequency is still higher in the wild *An. gambiae* mosquitoes. However, little is known about the fitness effects induced by this resistance allele in malaria vector *Anopheles gambiae* at homozygote state.

Although the resistance alleles confer the potential of surviving particular insecticide exposures to mosquitoes, it is often assumed that they may also influence various fitness-related traits of mosquitoes (e.g. trophic behaviour, fecundity, fertility, parasite transmission, longevity and larval survivorship) in presence or absence of insecticide selection pressure [27]. Therefore, better understanding the effects of resistance alleles on the most important life-history traits of mosquitoes appears crucial to improve malaria vector control interventions.

Several studies have shown that insecticide resistance mechanisms can confer detrimental effects on reproductive fitness, host seeking, feeding and mating behaviours in *Anopheles* mosquitoes [28–30] as well as some *Aedes* [31–33] and *Culex* mosquitoes [34–36]. Decreased longevity and increased larval survivorship have also been observed in insecticide resistant strains of *Aedes aegypti*, *Culex pipiens* and *Anopheles gambiae* [31, 37–40]. A study carried out by Platt *et al.* [30] revealed that *kdr^R* heterozygous males of *An. coluzzii* were more likely to successfully mate than homozygote resistant ones illustrating a deleterious effect of homozygote resistant *kdr^R* allele on *An. coluzzii* parternity success. Also, they were more competitive compared to homozygote susceptible mosquitoes indicating a heterozygous fitness advantage [30]. Furthermore, it was demonstrated that pupae of *An. gambiae* homozygous for *ace-1^R* (G119S) allele were more likely to die during pupation stage than those of the susceptible strain [40]. All these studies throw light on the variability of mosquito life-history traits according to the species and the effects of specific insecticide resistance mechanism on these traits.

Herein we evaluated the relative effects of *kdr^R* (L1014F) allele on reproductive success, larval survivorship, blood feeding behaviour, and adult survivorship post blood meal in *Anopheles gambiae s.s.*

Methods

Mosquito strains and rearing

Two reference laboratory strains of *An. gambiae s.s.* were used. The insecticide susceptible reference strain, Kisumu, sampled from Kenya the early 1950s and maintained at insectary [41]. The KisKdr strain which is homozygous [*kdr^{RR}*] for the L1014F allele and resistant to both pyrethroids and organochlorines was obtained by introgression of the *kdr^R* (L1014F) allele into the Kisumu genome [42]. This strain has the same genetic background as Kisumu [*kdr^{SS}*] and was free of metabolic resistance.

In order to investigate the role of *kdr^R* (L1014F) allele in *An. gambiae s.s.* blood feeding behaviour, heterozygote [*kdr^{RS}*] resistant mosquitoes were obtained by crossing Kisumu females [*kdr^{SS}*] with KisKdr males [*kdr^{RR}*] and Kisumu males with KisKdr females encoded F1-1 (♂Kisumu X ♀KisKdr) and F1-2 (♀Kisumu X ♂KisKdr) respectively. Mosquitoes were therefore reared in Laboratory of Infectious Vector-Borne Diseases of Regional Institute of Public Health, Benin under the same soft insectary conditions (insecticide-free environment, 27 ± 2 °C ambient temperature, $70 \pm 8\%$ relative humidity and 12:12 light and dark period). Larvae were fed *ad libitum* with TetraMin Baby fish food and generated adults were fed *ad libitum* on 10% honey solution until they were ready to be used for further assays.

Female reproductive success assessment

At least three days after emergence, Kisumu and KisKdr females were blood-fed on a rabbit. The gravid mosquitoes of each strain were individually transferred into plastic cups containing wet Whatman filter paper for oviposition. They were allowed to feed on 10% honey solution until egg laying. The number of females laying eggs was recorded and eggs laid were counted using stereomicroscope (Leica Microsystems EZ4HD). Egg batches (from individual females) were transferred in separate plastic trays filled with dechlorinated water and the number of hatched larvae were recorded. Two biological replicates were performed.

Larval survival assessment

To assess the larval mortality associated with *kdr^R* (L1014F) allele, assays were performed as described by Yahouédo *et al.* [43]. Female oviposition was synchronized for the two strains. Every day, each of larvae was fed with 20 µg of TetraMin Baby fish food. Water was changed every two days to reduce the effect of pollution. Petri dishes containing larvae were inspected once daily and dead pupae or larvae were recorded and removed. Daily mortality of larvae was monitored until the last one reached pupal stage. Three biological replicates were run for each strain.

Assessment of blood feeding behaviour

Mosquito blood feeding was performed using the membrane feeding assays (MFAs) previously described by Kristan *et al.* [44]. Three to five days old females of Kisumu, KisKdr and those from the crossings namely F1-1 and F1-2 were used in three different experiments. Twenty-four (24) hours starved mosquitoes were exposed to feeders containing the blood sample pre-heated following procedures previously described [45] for 30 minutes. The fully blood-fed mosquitoes were scored 24 hours later and were kept for the evaluation of survivorship.

The blood meal size was assessed in the blood-fed mosquitoes from other mosquito batches using a spectrophotometer (MULTISCAN GO, Thermo Scientific) as previously described [46]. Three biological replicates of at least 30 individuals per strain group were run.

Mosquito longevity post-blood meal

The blood-fed mosquitoes Kisumu and KisKdr as well as the offspring F1-1 and F1-2 from feeding experiments were transferred into clean paper cups and allowed to feed on 10% honey solution. The mortality was recorded daily until the death of the last mosquito.

Data analysis

Data were recorded in appropriate designed forms, entered into Microsoft excel for data cleaning and exported to R version 3.4.4 [47] and GraphPad Prism 8.0.2 software (San Diego, California USA) for analysis. The normality of data distribution was checked using Shapiro Wilk test [48].

Fecundity of each strain was assessed as the total number of eggs over the total number of females that contributed to oviposition. A correlation between kdr^R genotype and fecundity was calculated using negative binomial model (NBM) defined as follow: $\log(Ov) = \text{Genotype} + \varepsilon$ where Ov is the number of eggs/female, Genotype is the two-level factor corresponding to the different genotypes tested, ε is the error parameter which follows a negative binomial distribution. Fertility for each strain as percentage of hatched larvae was evaluated by dividing the total number of larvae L1 over the total number of eggs. A correlation between kdr^R genotype and fertility was calculated using negative binomial model (NBM) defined as follow: $\log(Ha) = \text{Genotype} + \varepsilon$ where Ha is the percentage of larvae/female eggs batch. Descriptive statistics were used to calculate pupation percentage (number of pupae/number of L1 larvae), blood-fed mosquito percentage (number of blood-fed mosquitoes/number of exposed mosquitoes). Chi-square independence test was performed to compare proportions using the R software package "stats" [47]. Mann-Whitney procedure was used to compare the means between mosquito strains. Larval and blood-fed female survivorships were analysed using Kaplan–Meier survival curves. The Log-rank test was performed to evaluate the difference in survival between the strains. The larval survivorship and adult mortality differences between the two genotypes were tested using Cox proportional hazards regression model (Cox model) with a binomial error distribution. The models were calculated as follows: $\text{Survival} = \text{Genotype} + \varepsilon$, where Survival is a proportion of dead larvae or adult, Genotype is the two-level factor corresponding to the different genotypes tested and ε is the error parameter which follows a binomial distribution. Pupae were censored in the larval survivorship analysis.

The significance of differences in blood feeding rates between genotypes was assessed with the following generalized linear models (GLM): $Fed = Genotype + \varepsilon$, where fed is the blood-fed status, Genotype is a three-level factor corresponding to the different genotypes tested ($[kdr^{SS}]$, $[kdr^{RS}]$ and $[kdr^{RR}]$) and ε is the error parameter which follows a binomial distribution. All these analyses were set at significance threshold of $p \leq 0.05$.

Results

Reproductive success

One hundred and eighty (180) homozygote *An. gambiae* females of both KisKdr (n = 90) and Kisumu (n = 90) strains were used to assess the effect of kdr^R allele on their reproductive success (fecundity and fertility). The mean number of eggs laid per female (fecundity) and the average larval hatching rate (fertility) were significantly different between the two strains (30.72 ± 19.92 eggs/KisKdr female versus 87.98 ± 44.51 eggs/Kisumu female, $p = 1.074 \cdot 10^{-10}$; Fig. 1) and ($72.89 \pm 15.7\%$ hatched larvae/KisKdr female versus $81.89 \pm 12.4\%$ for Kisumu female, $p = 0.002$; Fig. 2). Moreover, KisKdr female fecundity and fertility decreased by 1.05 (GLM.NB: $F = 58.21$, $\Delta df = 1$, $p = 8.71 \cdot 10^{-12}$) and 0.12 (GLM.NB: $\chi^2 = 1062$, $\Delta df = 1$, $p = 0.0011$) respectively compared to those of Kisumu females. Overall, the reproductive success of KisKdr $[kdr^{RR}]$ females was significantly lower than that of Kisumu $[kdr^{SS}]$ females.

Larval survivorship

The fitness effect associated with the kdr^R allele during mosquito aquatic developmental stage was measured by comparing larval survival between KisKdr and Kisumu strains. A total of 1440 larvae of each strain were followed from egg hatching to pupation. The number of dead larvae was recorded to assess differences between the strains in overall survivorship. As shown in Fig. 3A, KisKdr mosquito larvae displayed a significantly higher survivorship than that of Kisumu mosquito larvae (Log-rank test: $\chi^2 = 110$, $\Delta df = 1$, $p = 2 \cdot 10^{-16}$). The risk of death of individual larvae when bearing kdr^R allele at homozygote state $[kdr^{RR}]$ is reduced by a factor of 59% compared to that when the larvae are homozygote susceptible $[kdr^{SS}]$ (Cox model: likelihood ratio test [LRT]: $\chi^2 = 114.7$, $\Delta df = 1$, $p = 2 \cdot 10^{-16}$). Consequently, pupation rate was significantly higher in KisKdr strain (85.84%, $CI_{95\%} = [84.12-87.75]$) than that of Kisumu (54.05%, $CI_{95\%} = [51.34-56.74]$) (Fig. 3B).

Blood feeding success

A total of the 200 females of KisKdr and 495 females of Kisumu mosquitoes were allowed to feed on blood. Respectively 84% (168/200) and 34.75% (172/495) were successfully fed as shown in Fig. 4A. The KisKdr females showed a significantly higher blood feeding rate than that of Kisumu ($p = 2.2 \cdot 10^{-16}$). Interestingly, the offspring heterozygote $[kdr^{RS}]$ females F1-1 and F1-2 displayed also consistently higher percent of blood-fed individuals (respectively, 74.74% (71/95) and 85.71% (90/105)) than that of Kisumu

[*kdr^{SS}*] individuals ($p = 2.2 \cdot 10^{-16}$) (Fig. 4A). Therefore, mosquitoes harbouring *kdr^R* allele at resistant homozygote showed high blood feeding ability compared to that of the susceptible homozygote Kisumu strain (GLM: [RLT]: $\chi^2 = 215.28$, $\Delta df = 2$, $p = 2.2 \cdot 10^{-16}$).

When using other batches of mosquito females for the same blood feeding assays, the average blood volume ingested by KisKdr individuals was similar to that of Kisumu specimens ($p = 0.22$) while the average amount of blood ingested by the heterozygous offspring (1.68 $\mu\text{L}/\text{mg}$) was significantly higher than that of Kisumu mosquitoes (1.36 $\mu\text{L}/\text{mg}$) ($p = 8 \cdot 10^{-4}$) as shown in Fig. 4B.

Adult female survivorships post-blood feeding

After the blood feeding assays, successful blood-fed females from Kisumu ($n = 172$), KisKdr ($n = 168$), F1-1 ($n = 71$) and F1-2 ($n = 90$) were followed up until the death of the last mosquito. The lifespan of homozygote resistant mosquitoes (KisKdr) was 15 days compared to homozygote susceptible ones (Kisumu) which still alive until 17 days (Fig. 5A). However, no difference was observed between the survivorship of the two strains (Log-rank test: $\chi^2 = 0.6$, $\Delta df = 1$, $p = 0.4$).

Moreover, offspring [*kdr^{RS}*] displayed a longer lifespan (24 days) than that of their parents (15 days for KisKdr; Log-rank test: $\chi^2 = 48$, $\Delta df = 2$, $p = 4 \cdot 10^{-11}$ and 17 days for Kisumu; Log-rank test: $\chi^2 = 54.9$, $\Delta df = 2$, $p = 10^{-12}$). In addition, these offspring displayed higher survival rate compared to KisKdr (hazard ratio = 0.44; Cox model: [LRT]: $\chi^2 = 38.12$, $\Delta df = 1$, $p = 7 \cdot 10^{-10}$) and Kisumu specimens (hazard ratio = 0.41; Cox model: [LRT]: $\chi^2 = 44.93$, $\Delta df = 1$, $p = 2 \cdot 10^{-11}$) as shown in Figs. 5A, 5B.

Discussion

An effective strategy for malaria vector control should neither be based only on the application of insecticide components nor focused on the existing resistance mechanisms developed by these vectors. Insecticide pressure contributes to the selection of mosquitoes carrying resistance alleles which are vertically transferred to the offspring [49]. It was demonstrated that alleles of genes conferring resistance in mosquito populations are usually associated with the disruption of some vector history traits of life [30, 31, 50]. The present study investigated the fitness effects associated with the presence of knock-down resistance allele in major malaria vectors *An. gambiae s.s.* carrying *kdr^R* allele.

Although all mosquito females subjected to oviposition assay were blood-fed, fecundity was significantly lower in homozygous KisKdr individuals compared to that of susceptible specimens. This result suggests that a physiological cost could be associated with the presence of *kdr^R* allele in pyrethroid resistant mosquitoes when they are in an insecticide-free environment. Similar pattern was observed in the main dengue vectors *Aedes aegypti*. Indeed, Brito and collaborators reported that a few number of pyrethroid resistant *Aedes aegypti* mosquitoes was able to lay eggs and a smaller amount of eggs was produced [51]. The KisKdr females displayed significantly lower fertility compared to that of their counterpart, Kisumu. This finding suggests that *kdr^R* allele could reduce hatchability of eggs in homozygote [*kdr^{RR}*] *An.*

gambiae mosquitoes. Under laboratory conditions, three hypotheses could support these observations in mosquitoes: i) a decreasing egg maturation, ii) a reduced egg laying ability and iii) an unsuccessful mating associated with lower mating success which has been reported in resistant mosquitoes [30, 34, 52]. The significant difference in fecundity and fertility between resistant and susceptible mosquito strains suggests yet other likely interesting pathways that could be targeted for disruption in malaria transmitting vectors. However, in field settings, there are other resistance mechanisms which were probably co-selected with *kdr^R* allele in *Anopheles* mosquitoes. Also, the wild mosquitoes are continuously under insecticide pressure [53] and consequently, differences in physiological responses in resistant individuals are important factors to be taken into consideration. Further molecular analyses could be considered in order to understand the differentially expressed genes between KisKdr female mosquitoes that have laid eggs and those that have not. This will pave the way to the identification of new genetic targets as alternative tools for vector control in the face of insecticide-resistance.

Larval survivorship and pupation rate were also assessed. The results showed that while 50% of Kisumu larvae were died at day 10, more than 50% KisKdr larvae were still alive or had reached pupation. The KisKdr larvae had a lower risk of death compared to their counterpart Kisumu suggesting that this fitness advantage might be associated with the *kdr* resistant [*kdr^{RR}*] genotype. Further, we reported a significant pupation rate in KisKdr compared to Kisumu mosquitoes. Unfortunately, this is evidence that the strong survivorship during the aquatic phase and the high pupation rate in KisKdr resistant mosquitoes will threaten current vector control interventions. This information could help for designing a most effective tools for insecticide resistance management. However, present findings were obtained with standardized larval density and controlled diet which is not the case in natural settings where mosquito larval development could also be affected by density-dependent competition and mortality [55].

After emergence, adult mosquito females need to feed on blood in order to have the crucial protein source required for egg development [56]. The present study provides the first evidence of blood feeding advantage associated with the knock-down resistance using *An. gambiae s.s.* mosquitoes carrying only *kdr^R* (L1014F) allele. Noteworthy, our results indicate a significant association between *kdr^R* allele and the high blood feeding success in *An. gambiae s.s.* The blood feeding success in mosquitoes depends on their craving for blood. Further, the presence of *kdr^R* allele could enhance the mosquito avidity for blood feeding. The significant blood feeding success in both [*kdr^{RR}*] and [*kdr^{RS}*] KisKdr mosquitoes with respect to the susceptible genotype [*kdr^{SS}*] could suggest a linkage disequilibrium (LD) at the loci of the para voltage-gated sodium channel (*Vgsc*) gene and the gene encoding the craving for blood meal taking. The benefit of this knock-down resistance to strengthen blood feeding ability might increase the human biting rate index and consequently the expected number of malaria infected individuals according to the Ross-MacDonald model [57]. An important parameter susceptible to favour the blood feeding success is the host seeking-behaviour which is likely reinforced by the interactions with *kdr^R* allele. Indeed, it was previously reported that the host-seeking performance in presence or absence of insecticide was increased in *kdr* heterozygous [*kdr^{RS}*] *An. gambiae* [29].

Furthermore, no significant difference was noticed in blood meal volume ingested by homozygote resistant [kdr^{RR}] mosquitoes compared to homozygote susceptible [kdr^{SS}] individuals. However, heterozygous KisKdr F1-1 and F1-2 mosquitoes ingested a higher blood volume compared to Kisumu specimens. A high blood meal size could lead to the ingestion of a high gametocyte density if the heterozygote kdr resistant females feed on an infected host. Moreover, it has been demonstrated that the heat shock protein 70 (Hsp70) protects *An. gambiae* mosquitoes against the high temperature stress through thermoregulation process during the blood meal on a warm-blooded host [58]. Accordingly, we hypothesize that this thermoregulation could strengthen the differentiation of ingested gametocytes into ookinetes in the midgut lumen of heterozygous [kdr^{RS}] KisKdr female since the parasite exflagellation could be initiated by fast fall of temperature within mosquito midgut [59]. Therefore, the risk of malaria transmission might remain very huge in regions where there is a high heterozygote kdr^R genotype frequency. Indeed, it was demonstrated that kdr homozygote resistant *An. gambiae* mosquitoes are more susceptible to the *P. falciparum* infection than the susceptible Kisumu [42] and consequently, this fact could be more striking in heterozygote mosquitoes.

Gametocyte-infected mosquitoes must survive long enough to become infectious and transmit the sporozoites to a new host [60]. So, one of the key factors modulating malaria transmission is the vector longevity post blood feeding. This study demonstrates that, the presence of kdr^R allele seems to increase the longevity of heterozygote KisKdr specimens while no survival advantage was observed in homozygous individuals compared to the susceptible strain Kisumu. This benefit in heterozygote [kdr^{RS}] over homozygote [kdr^{RR}], makes kdr an overdominance gene for this specific trait. We observed that heterozygote mosquitoes survive until 24 days. Thus, these specimens have sufficient lifespan to allow extrinsic incubation period of the *Plasmodium* parasites if they ingest gametocyte-infected blood. However, further investigations are needed to evaluate the cost of *Plasmodium* infection on heterozygote resistant KisKdr mosquito's survivorship. In fact, the intensity of infection (i.e. oocyst burden) affecting the vector immune responses [45] could relatively lessen the fitness of mosquitoes regarding their survival and fecundity [61].

Conclusion

In order to generate useful predictions of malaria transmission, the impact of resistance mechanisms on the vector life-history traits needs to be taken into consideration. The data presented here indicate that kdr^R allele induce a cost on fecundity and fertility in adult *An. gambiae*. Remarkably, this allele positively affects the larval survivorship, pupation rate, blood feeding success in homozygote resistant mosquitoes and increase the post blood feeding survivorship especially in heterozygote individuals. Further, this advantage conveyed by kdr^R allele could lead to increased vector competence in resistant mosquitoes and thus, in malaria transmission. It would be interesting to further characterize the fitness effects of kdr^R allele in the wild mosquitoes and identify the potential synergist genes.

Abbreviations

Kdr^R: Resistant allele of knock-down resistance; **Kdr^S**: Susceptible allele of knock-down resistance; **ace-1^R**: Resistant allele of insecticide-insensitive Acetylcholinesterase-1; **s.s.**: *Sensu stricto*; **L1014F**: Leucine substitution by Phenylalanine at codon 1014; **L1014S**: Leucine substitution by Serine at codon 1014; **N1575Y**: Asparagine-to-Tyrosine substitution at codon 1575; **LLINs**: Long-Lasting Insecticide-treated Nets; **IRS**: Indoor residual spraying; **G119S**: Glycine substitution by Serine at codon 119; **Vgsc**: voltage-gated sodium channel; **USA**: United States of America; **LD**: linkage disequilibrium; **GLM**: Generalized linear models; **NBM**: negative binomial model; **Hsp70**: Heat shock protein 70.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors have read and approved the final version of manuscript and consent to its publication.

Availability of data and materials

The datasets are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests for this study.

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Author's contributions

Conception and design of the work: LSD and AAM. Acquisition of data: AAM, EA, RMK, EGS, EBJs, LD, OYD and RBA. Analysis and interpretation of data: AAM, OYD and RBA. Drafting and substantial revision of the manuscript: LSD, AAM, OYD, RBA and AB. All authors read and approved the final version of manuscript.

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Figures

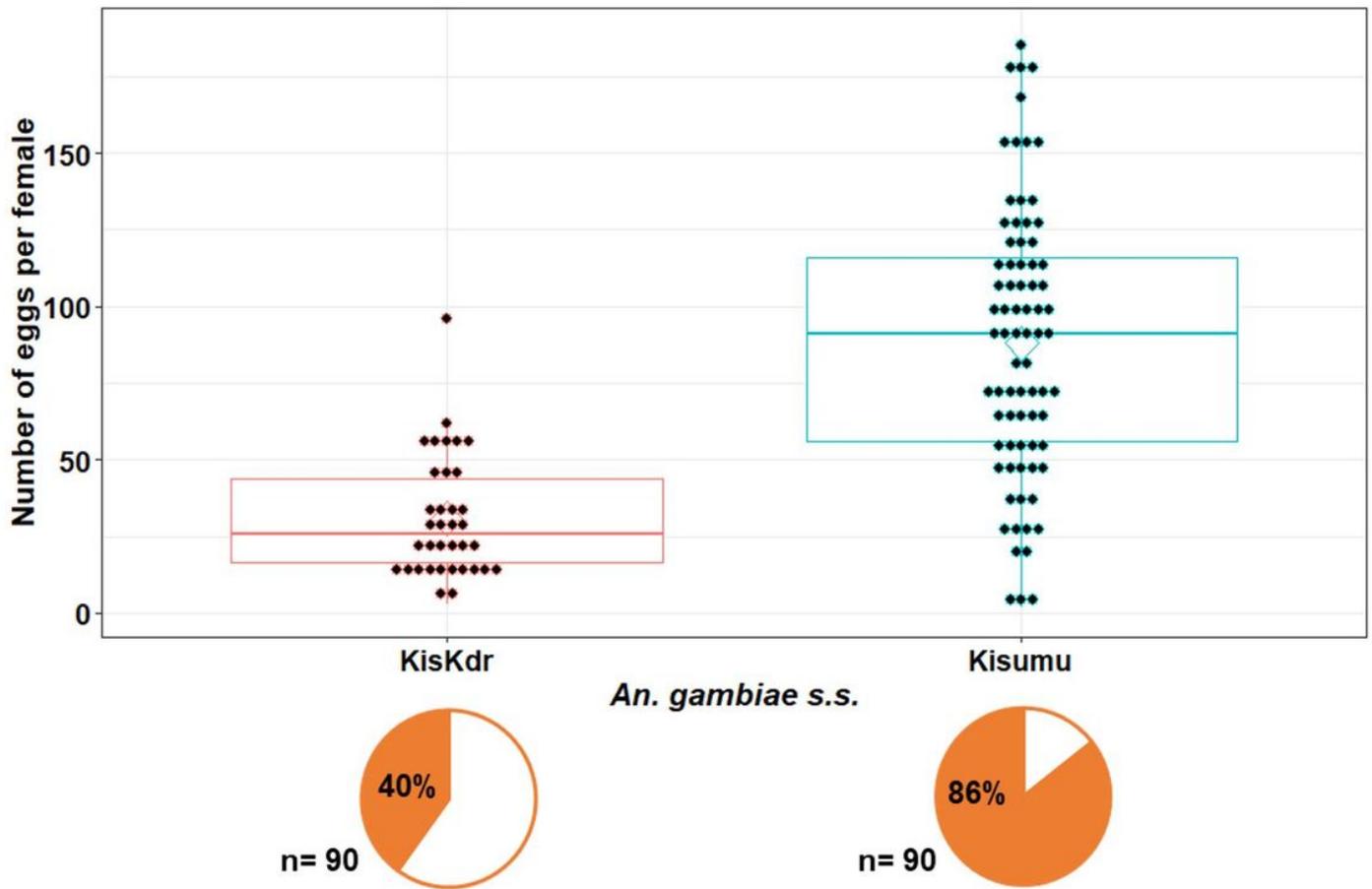


Figure 1

Fecundity in KisKdr and Kisumu strains. Each dot denotes the number of eggs laid by each female in each strain. Only females that laid at least one egg were included. Diamond point represents the mean number of eggs in each strain. Pie charts represent percentages of mosquito females that laid eggs in each strain. (n) indicate the total number of mosquito females subjected to the oviposition. Significant difference was observed in fecundity between both Kisumu and KisKdr mosquito females ($p= 1.074.10^{-10}$).

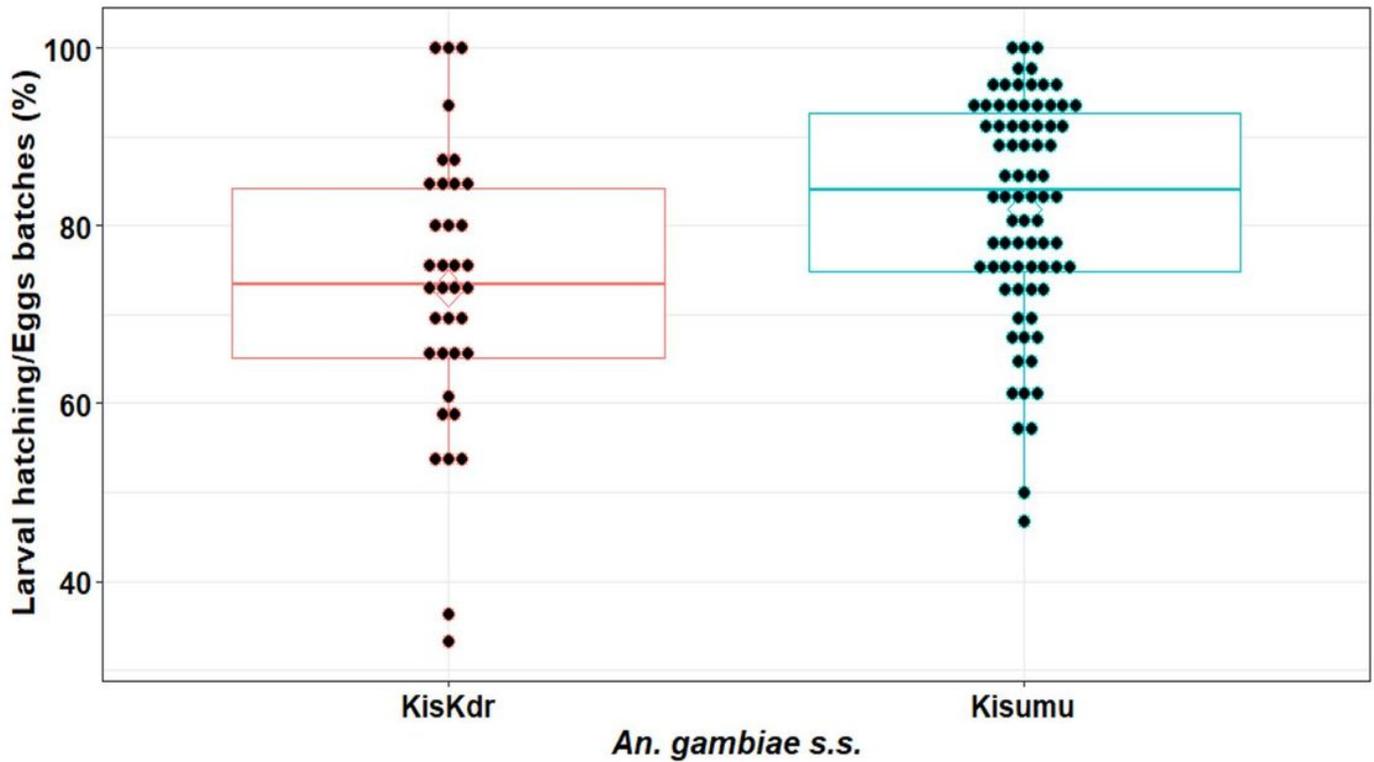


Figure 2

Kisumu and Kiskdr larval hatching percentages. Each dot denotes the percentage of larvae hatched from individual female egg batch within each strain. Diamond point represents the mean percentage of larvae hatched from each strain. Significant difference was observed in hatching rates between both Kisumu and KisKdr females ($p= 0.002$).

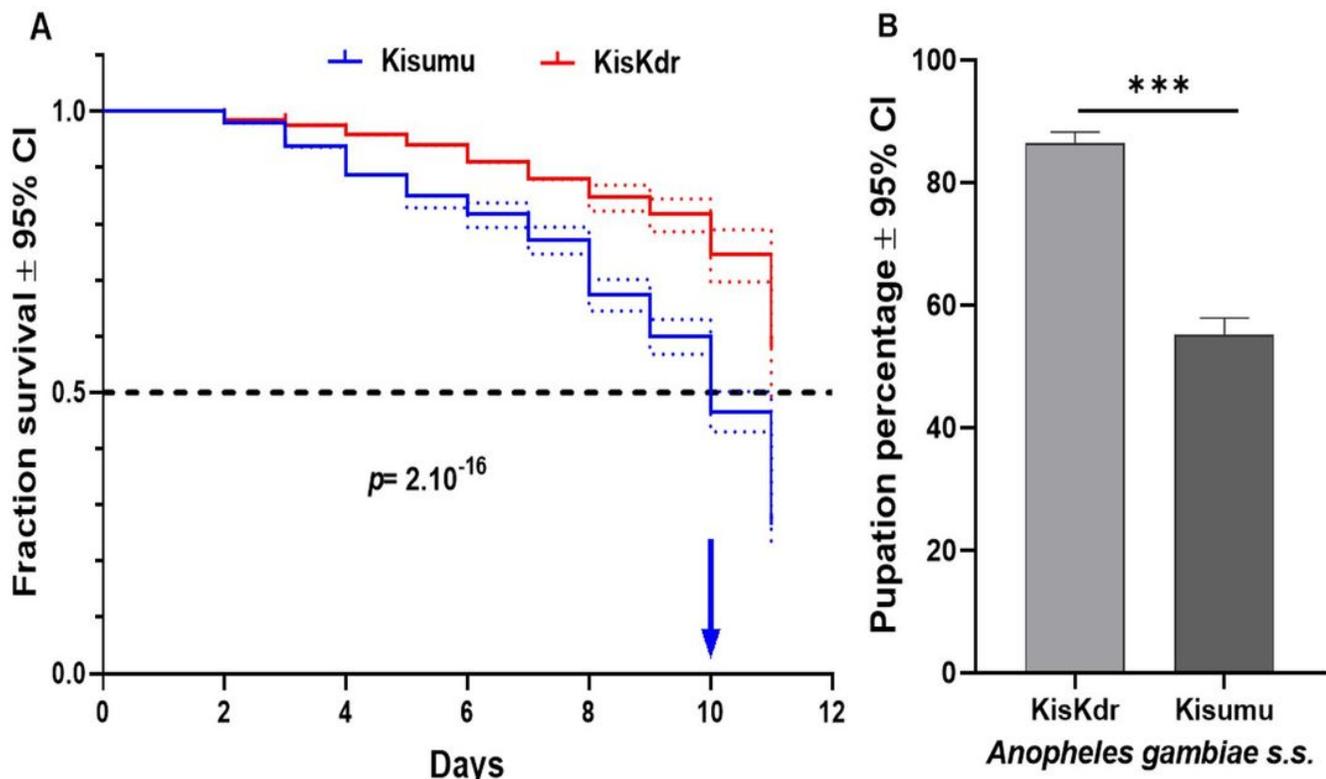


Figure 3

KisKdr and Kisumu larvae longevity (panel A) and pupation percentages (panel B). A: Dotted lines are 95% confidence intervals (CIs) around the respective survival curve. Arrow indicates the median survival time. B: Error bars are 95% confidence intervals (CIs) around each percentage. *** indicate $p < 0.0001$.

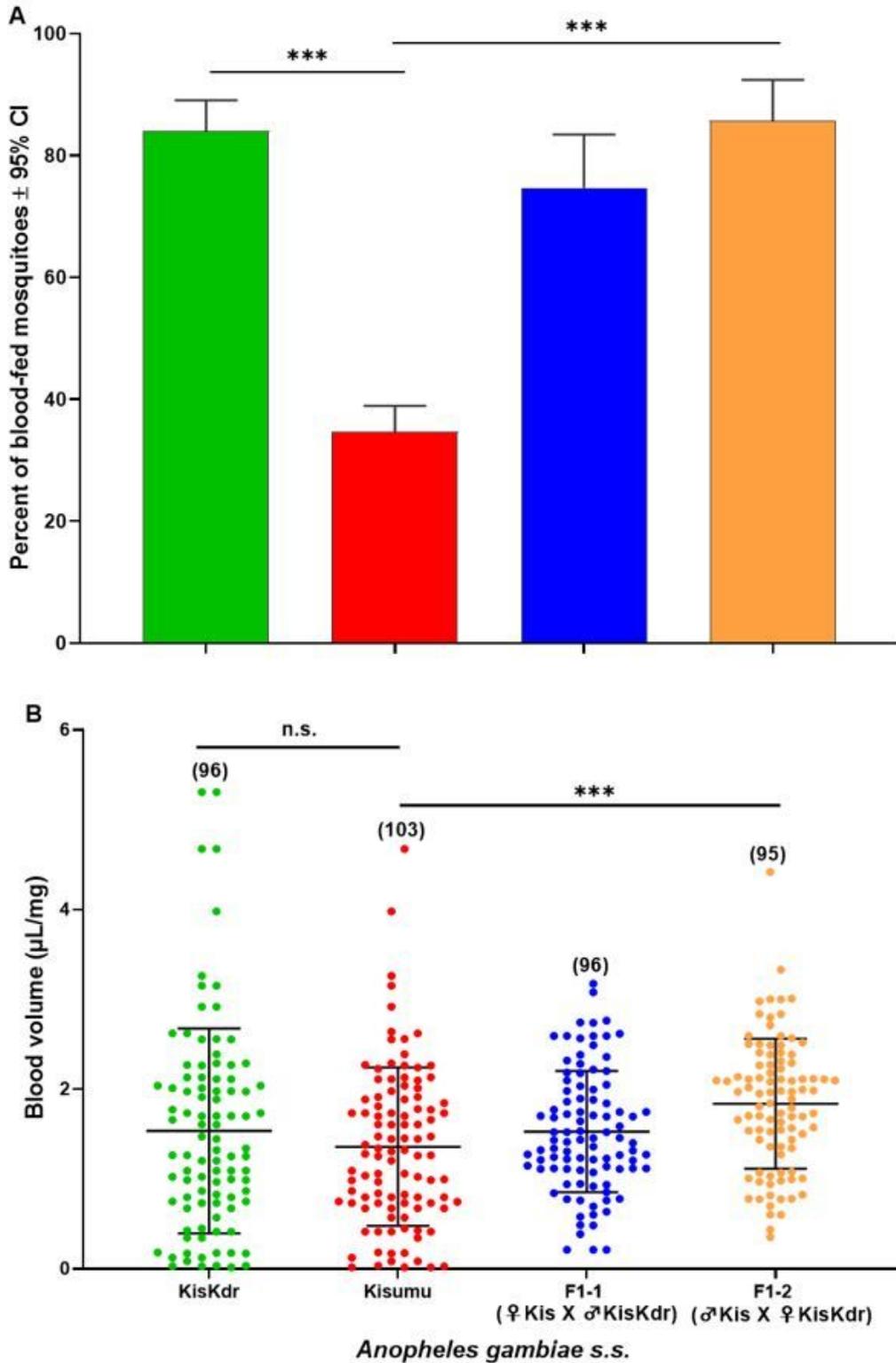


Figure 4

Percentages of blood-fed females (A) and blood meal size (B) in *kdrR(1014F)* resistant *An. gambiae*. *Kiskdr* and *Kisumu* are respectively the homozygous *kdr* resistant and susceptible parents. F1-1 ($\text{♀ Kis} \times \text{♂ KisKdr}$) and F1-2 ($\text{♂ Kis} \times \text{♀ KisKdr}$) represent the first-generation heterozygous offspring. In panel A, *** and n.s. indicate respectively $p = 2.2 \cdot 10^{-16}$ and not significant. In panel B, *** indicate $p = 8 \cdot 10^{-4}$. The sample sizes were showed in brackets on the top of scatter dots.

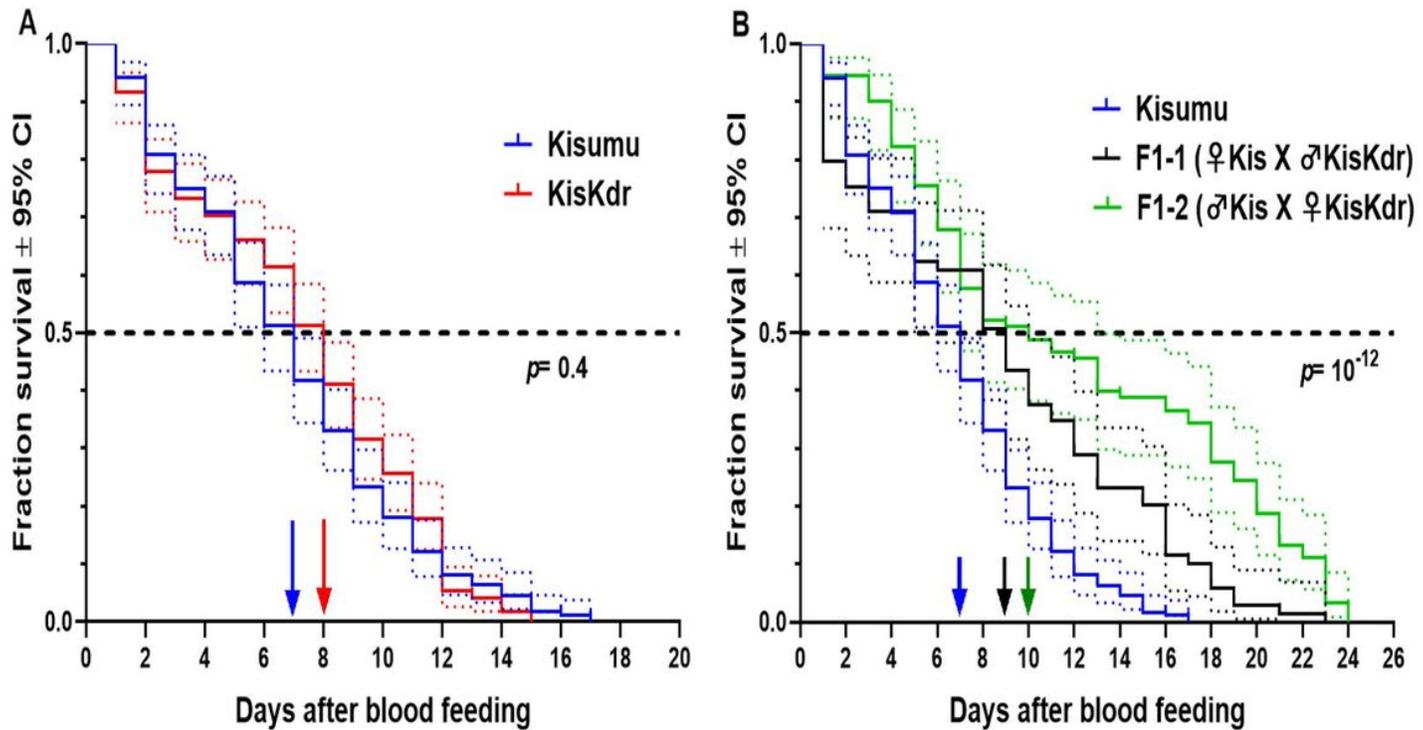


Figure 5

Parents and first generation female longevity after blood feeding. Dotted lines are 95% confidence intervals (CIs) around the respective survival curve. Arrows indicate the median survival time.