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# Test for association between dieldrin resistance and 2La inversion polymorphism in *Anopheles coluzzii* from Lagos, Nigeria

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**Abstract.** The assortment of paracentric chromosomal inversion 2La is associated with the maintenance of dieldrin resistance in laboratory colonies of the malaria vector *Anopheles gambiae*. This association has not been tested in field populations. The aim of this study was to test the association between inversion 2La and dieldrin resistance in a field population of *An. coluzzii* in Nigeria. Field collected immature stages of *Anopheles* were raised to adults and exposed to 4% dieldrin according to WHO criteria. Knockdown was recorded at 10 min intervals for 1 hour and final mortality was recorded 24 hours post exposure. Species and inversion 2La diagnostic PCR assays were conducted on the resistant and susceptible mosquitoes. The mosquitoes were highly resistant to 4% dieldrin (17.1% knock down and 25.7% final mortality; $KDT_{50}$ and $KDT_{95}$ calculated as 170 and 1, 514 minutes respectively). Frequencies of 2La in both the resistant and susceptible cohorts assorted within Hardy-Weinberg estimates ($\chi^2$=1.32, p=0.8 for dead/susceptible mosquitoes and $\chi^2$=2.54, p=0.5 for survivors or resistant mosquitoes). However, a higher number of heterozygous mosquitoes were observed in the resistant cohort compared to the susceptible, with significant variation in karyotype frequencies ($\chi$2=11.08, DF=2, p<0.05) and a significantly higher frequency of the 2La inversion arrangement in the resistant cohort (Pearson's $\chi$2 = 4.58, p = 0.03.). These data are the first to associate paracentric chromosome inversion 2La and dieldrin resistance in field population of *An. coluzzii*. Dieldrin resistance shows a weak but significant association with 2La whose assortment is affected by positive heterosis. Variation in the assortment of 2La inversion arrangements between resistant and susceptible cohorts of this *An. coluzzii* population suggests that dieldrin resistance is at least partially linked to inversion 2La which may explain the persistence of dieldrin resistance in this population despite a significant absence of selection for resistance to this insecticide.

## INTRODUCTION

Members of the *Anopheles gambiae s.l.* and the *An. funestus* group, which account for majority of malaria transmission in Africa, have continued to develop resistance to insecticides used in public health. The mechanisms of resistance in these vectors include behavioural alteration (insecticide avoidance), integument modification, metabolic enzymatic detoxification and target site mutations (WHO, 2012). Aside these, adaptive genetic mechanisms associated with survival of mosquito populations are important when considering the operational impact of insecticide resistance. A major mechanism of adaptation to natural environments in *Anopheles* is the

occurrence of polymorphic chromosomal inversion which ensure the maintenance of co-adapted genes, often as polymorphisms within populations despite selection against deleterious alleles (Ayala *et al.*, 2014). This is because near inversion breakpoints, recombination of loci is highly reduced, especially in the heterozygous state, and therefore influences the assortment of those genes through successive generations (Calvete *et al.*, 2012). This basically amounts to a positive heterotic system which can prevent deleterious alleles, such as insecticide resistance genes, from drifting out of populations even in the absence of selection (Brooke *et al.*, 2002).

In the major malaria vector species *An. gambiae* and *An. coluzzii*, the bulk of inversions responsible for adaptation to natural environments are found on chromosome 2. Notable among these inversions is 2La located on the left arm of the chromosome. This inversion has been reported to associate with insecticide resistance (Brooke *et al.*, 2000). In Nigeria, reports of resistance to all the classes of insecticides in the major malaria vector populations is on the increase (NPIRMN, 2017-2020). While effective vector control interventions in Nigeria used dichlorodiphenyltrichloroethane (DDT) and dieldrin in the early 1950s, it did not take long for mosquitoes to develop resistance to dieldrin insecticide even in areas without intervention (Service and Davidson, 1964). Despite reports on the alarming spread of resistance to dieldrin in the 1970s, efforts have not been made to monitor resistance to this insecticide in mosquito populations in Nigeria. More recently, a strong association was established between dieldrin resistance and polymorphic inversion 2La in colonies of *Anopheles gambiae* Giles *sensu stricto* Ian P20 collected in Nigeria and maintained in the laboratory at the London School of Hygiene and Tropical Medicine for over 30 years (Brooke *et al.*, 2000). This report has not been followed up with data to associate the inversion with dieldrin resistance in wild populations. We therefore determined the level of resistance of wild populations of *Anopheles coluzzii* to dieldrin in Nigeria, and examined a possible association between this resistance, and the frequencies and assortment of polymorphic inversion 2La.

## MATERIALS AND METHOD

### Study area

The study samples were collected from Kosofe Local Government (Latitude 6°36' 6.16"N, Longitude 3°25' 26.95"E). Kosofe is one of the twenty Local Government Areas (LGAs) in Lagos state, South West Nigeria. The vegetation at Kosofe is swamp forest which has been encroached by construction of houses, markets and other infrastructure. Intensive malaria control interventions have been ongoing at Kosofe Local Government by the Lagos State Government. Apart from the distribution of Long Lasting Insecticide Nets (LLINs) by the state government, a 15-day Indoor Residual Spraying (IRS) exercise, covering about 136,526 households in three local government areas of the state including Kosofe, was conducted in 2011 by the State Ministry of Health. Another 19-day IRS exercise was also conducted in 2015 (PMI, 2016).

### Methods

*Anopheles* larvae were collected from Kosofe LGA in May, 2018, and reared to adults in the insectary at Lead City University, Ibadan. Adults were fed with 10% sugar soaked in cotton wool. Three to five day old adult females were exposed to 4% dieldrin impregnated papers in six replicates with an average of 25 mosquitoes per tube and two control replicates containing 25 mosquitoes per tube according to WHO criteria (WHO, 2013). Knock-down was recorded at 10 minute intervals for one hour and final mortality was scored after 24 hours. Survivors and dead mosquitoes were preserved singly in silica gel in 1.5ml Eppendorf tubes for PCR analysis. All the mosquitoes exposed were morphologically identified (Gillies and Coetzee, 1987). Furthermore, molecular identification of the mosquitoes was conducted using PCR and

enzyme digest (Scott *et al.*, 1993). Polymorphic inversion 2La was karyotyped using PCR (White *et al.*, 2007). 2La inversion frequency data for the survivors and dead mosquitoes were analysed separately using SPSS v23 by first calculating the allelic frequencies for 2La and 2La+ and then calculating the expected numbers for 2La/2La, 2La/2La+ and 2La+/2La+ and subjecting the result to chi square estimates $\chi^2 = \Sigma(\text{Observe-Expected})^2/\text{Expected}$. The resulting Hardy Weinberg estimates calculated for the survivors and dead mosquitoes were further compared using chi-quare analysis. In order to test for an association between the assortment of inversion 2La and resistance to dieldrin, chi-square analysis was also used to compare 2La karytotype and inversion frequencies between the resistant and susceptible cohorts following an adjustment to equalise sample sizes. A further analysis was conducted using likelihood ratio test on logistic model (R statistics with its associated lm) for inversions 2La/2La, 2La/2La+ and 2La+/2La+ to ascertain which of the inversion correlates more with resistance between resistant and susceptible cohorts.

## RESULTS

A total of 152 adult female mosquitoes were exposed to the 4% dieldrin insecticide. All the mosquitoes were molecularly identified as *An. coluzzii*. The knock down rates at 10 min intervals during the 1 hour exposure are shown in Figure 1. During this period, 26 (17.1%) mosquitoes were knocked down and $KDT_{50}$ and $KDT_{95}$ calculated as 170 and 1, 514 minutes respectively (Figure 1). The 24 hours mortality result of 24.7% showed that the mosquito population is highly resistant to dieldrin.

Inversion 2La karyotype data for resistant (n=113) and susceptible cohorts (n=39) are shown in Tables 1 and 2. A higher proportion of heterozygous mosquitoes were observed in the resistant cohort (54%) as compared with the susceptible population (43.5%). Frequencies of the inversion did not deviate significantly from Hardy-Weinberg estimates in both the resistant and susceptible populations (Tables 1 and 2). However, the results of the R statistics (p=0.402) indicate a positive residual of 15.833 for the 2La/2La+ heterokaryotype while negative values of -3.167 and -12.667

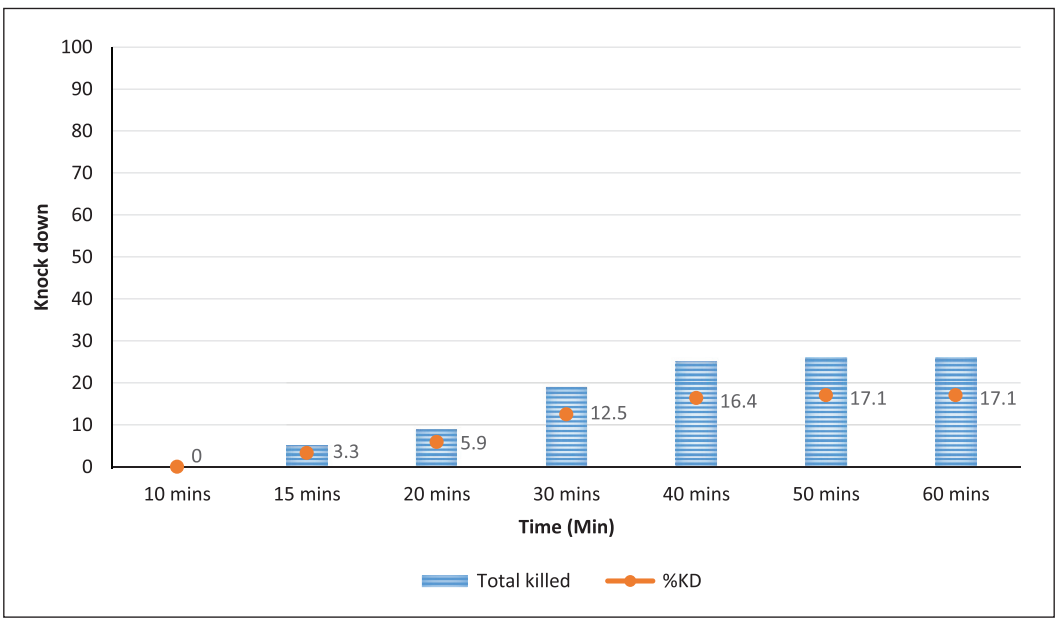


Figure 1. 1 hour knock down rates of field collected *Anopheles coluzzii* mosquitoes exposed to 4% dieldrin from Kosofe LGA, Lagos state.

Table 1. Observed and expected (Hardy-Weinberg, H-W) karyotype frequencies for polymorphic chromosome inversion 2La in the resistant cohorts of *Anopheles coluzzii* that survived on exposure to 4% dieldrin from Kosofe Local Government in Lagos

| Strain | *Anopheles coluzzii* (n=113) | | |
|---|---|---|---|
| Inversion karyotype | 2La/2La | 2La/2La+ | 2La+/2La+ |
| No. observed | 12 | 61 | 40 |
| Expected H-W | 15.933 | 52.997 | 43.957 |
| Chi square ($\chi^2$) | $\chi^2$ = 2.536 (NS) | | |

NS – non significant chi-square value.

Table 2. Observed and expected (Hardy-Weinberg, H-W) karyotype frequencies for polymorphic chromosome inversion 2La in susceptible cohorts of *Anopheles coluzzii* exposed to 4% dieldrin from Kosofe Local Government in Lagos

| Strain | *Anopheles coluzzii* (n=39) | | |
|---|---|---|---|
| Inversion karyotype | 2La/2La | 2La/2La+ | 2La+/2La+ |
| No. observed | 1 | 17 | 21 |
| Expected H-W | 2.340 | 14.391 | 22.308 |
| Chi square ($\chi^2$) | $\chi^2$ = 1.318 (NS) | | |

NS – non significant chi-square value.

Table 3. Comparison of karyotype frequencies for polymorphic chromosome inversion 2La between resistant and susceptible (adjusted) cohorts of *Anopheles coluzzii* exposed to 4% dieldrin. Samples were collected from Kosofe Local Government in Lagos

| Response to dieldrin exposure phenotype | Inversion karyotype | | | Inversion 2La frequency |
|---|---|---|---|---|
| | 2La/2La | 2La/2La+ | 2La+/2La+ | |
| Resistant (observed) | 12 | 61 | 40 | 0.38 |
| Susceptible (expected) | 3 | 49 | 61 | 0.24 |
| Chi square ($\chi^2$) | $\chi^2$ = 11.08, p = 0.004 | | | Pearson's $\chi^2$ = 4.58, p = 0.03 |

were calculated for the 2La/2La and 2La+/2La+ homokaryotypes respectively. This further indicate positive heterosis. There was a significant variation in 2La karyotype assortment between the survivor (observed) and susceptible (expected) mosquito cohorts based on a chi-square test in which the expected ratios (susceptible sample) were adjusted by a factor of 2.9 to match the sample size of the resistant cohort (Chi-square: $\chi^2$=11.08, DF=2, p<0.05) (Table 3). Given that the frequency of the 2La arrangement was 0.38 in the resistant cohort and 0.24 in the susceptible cohort, these data indicate a significant association between the assortment of inversion 2La and resistance to dieldrin. This is reinforced by a 2X2 contingency table indicating a significant increase in the frequency of the 2La arrangement in the resistant sample (Pearson's $\chi^2$ = 4.58, p = 0.03) (Table 3).

## DISCUSSION

The high level of resistance to dieldrin detected in this study can be attributed to two scenarios. First is the use of DDT and dieldrin

for insect pest control in agriculture where unacceptable quantities of these insecticides are found in top soils in Nigeria (Osesua *et al.*, 2017), possibly exerting continuous selection pressure on the mosquito populations at the immature stages. Second, a genetic linkage between DDT and dieldrin ($R^{dl-2}$ and $R^{DDT}$) resistances has previously been reported in *An. gambiae* (Haridi *et al.*, 1974). The high levels of DDT and pyrethroid resistance reported in populations of *Anopheles* in and round the collection area (Oduola *et al.*, 2010; 2012; Adeogun *et al.*, 2017; NPIRMN, 2017-2020) may therefore inadvertently lead to unexpectedly high levels of resistance to dieldrin in these populations. However, linkage to inversion 2La is also likely to be significant.

Inversion 2La is one of the major polymorphic inversions associated with adaptive characteristics in *An. gambiae* (Coluzzii *et al.*, 1979) and, by inference, the very closely related *An. coluzzii*. In this study, *An. coluzzii* mosquitoes that survived exposure to dieldrin showed an excess of heterozygotes for inversion 2La, with a significantly increased frequency of the 2La arrangement. This suggests a weak yet significant association between 2La inversion arrangement and resistance to dieldrin in this population, in which dieldrin-resistant individuals are more likely to carry either the 2La/2La or 2La/2La+ karyotypes than dieldrin-susceptible mosquitoes. In a laboratory strain of *An. gambiae* that were collected from Southern Nigeria (Ian P20, 1979) and established for over 20 years at the London School of Hygiene and Tropical Medicine, Brooke *et al.* (2000) reported a higher number of heterozygous individuals that survived exposure to dieldrin while the majority of knocked down individuals were homozygous for 2La. This report therefore partially aligns with the results of this study, suggesting that resistance to dieldrin in this population is at least partially maintained by linkage disequilibrium to 2La and the effect of positive heterosis associated with the assortment of this inversion. In this linkage system, heterokaryotypes are associated with increased fitness owing to the cumulative heterotic effect of heterozygous genotypes at those loci trapped near the inversion breakpoints, and positive heterozygosity is therefore maintained through generations. It has previously been shown that the *rdl* locus occurs within the breakpoints of inversion 2La (Brooke *et al.*, 2002) in *An. gambiae* and, by linkage disequilibrium, resistance to dieldrin via *rdl* is inadvertently maintained. This study also detected a high number of 2La+/2La+ homokaryotypes and only a small number of the alternative 2La/2La homokaryotype in the population. This is similar to the work of Coluzzii *et al.* (1979) who found that the frequency of the 2La+/2La+ homokaryotype in *An. gambiae* is high in Southwest Nigeria as compared with the 2La/2La counterpart but gradually reduces while the 2La/2La increases as one moves northwards.

## CONCLUSION

These data are the first to provide information on the association between the assortment of paracentric chromosome inversion 2La and dieldrin resistance in a field population of *An. coluzzii*. Dieldrin resistance shows a weak but significant association with 2La whose assortment is affected by positive heterosis, leading to the inadvertent maintenance of the resistance phenotype through successive generations.

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**Competing interests**
None declared.

**Authors' contributions**
AOA, BDB and DRO contributed equally to this work; AOA and BDB conceived the work;

AOA and DRO conducted the field work; ST, KA, TAO conducted the laboratory analysis; AKO did the statistical analysis. All authors contributed in the interpretation of the data and manuscript write-up, and approved the final manuscript.

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