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Shared and species-specific resistance-associated variants and genomic signatures of selection in sympatric Anopheles gambiae and Anopheles coluzzii from Mount Cameroon

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Abstract

Background

Insecticide resistance is eroding the efficacy of long-lasting insecticidal nets and indoor residual spraying, the main tool for malaria control across sub-Saharan Africa. Despite carrying a high transmission burden, data on the genomic architecture of *An. gambiae* complex needed to guide adaptive, evidence-based vector control decisions in southwestern Cameroon is lacking. The scale and mechanistic diversity of local resistance remains undefined.

Methods

We profiled the genomes *An. gambiae* complex populations across six localities spanning lower (18–197 m) and intermediate (371–584 m) altitudes on Mount Cameroon's slopes. The cross-sectionally study integrated WHO phenotypic bioassays (n = 1,250), with genetic diversity derived from whole-genome sequencing of 276 samples analysed on the MalariaGEN Vector Observatory platform and finally PCR-RFLP (n = 265). Genomic data were screened for target-site SNPs, copy number variants, genome-wide selection sweeps (H12), and haplotype-sharing networks across taxa.

Results and discussion

Bioassays confirmed resistance to deltamethrin (29–72% mortality) and bendiocarb (57–66%), with partial recovery following PBO pre-exposure. The *Kdr-West* (L995F) mutation was near-fixed across all taxa (88–95%), while *Kdr-East* (L995S) was documented for the first time in Southwest Cameroon. *Ace-1* (G280S) heterozygosity reached 78.1%, and *Cyp9k1* amplification (46–100%) was widespread. Novel H12 selection sweeps within carboxylesterase gene clusters and the first regional report of GABA/Rdl A296G point to previously undescribed resistance mechanisms actively under selection. Critically, haplotype sharing across taxa demonstrates active inter-taxon gene flow, a route by which resistance alleles can spread beyond their species of origin.

Conclusion

Southwest Cameroon harbours a genomically complex, multi-mechanistic resistance landscape that conventional single-locus surveillance substantially underrepresents. Ongoing gene flow between *An. gambiae* and *An. coluzzii* threatens to accelerate resistance spread across taxa and localities, underscoring the need to integrate whole-genome sequencing with phenotypic bioassays for genomics-informed vector surveillance and intervention planning across the region

Introduction

Malaria remains one of Africa's gravest public-health burdens (WHO, 2025), with the overwhelming majority of transmission across sub-Saharan Africa mediated by sibling species of the *An. gambiae* complex[1], [2], [3], [4]. This transmission is greatly influenced by the behaviour and competency of these sibling species (*An. gambiae* sensu stricto, *An. coluzzii, An. arabiensis* and to an extent *An. melas)*, while ecology and the anthropogenic activity continue to escalate the intensity of the disease transmitted [5], [6], [7].The persistence of these vectors, and thus their vectorial capacity to transmit *Plasmodium*, has been further entrenched by the widespread evolution of insecticide resistance [8], while transmission is modulated more subtly by the *Plasmodium* species circulating locally[9]. Such forces have together sustained the proliferation of the two most efficient and widely distributed African malaria vectors, *An. gambiae* and *An. coluzzii*, across urban, semi-urban and rural communities[10], [11].

Sustained deployment of LLINs, ITNs, indoor residual spraying and chemoprophylaxis[10], [14], [15] led to the substantial decline in malaria between 2000 and 2018 (WHO, 2025). That progress has since stalled, with recent finding suggesting that arthropod-borne diseases still account for more than 17% of the global infectious-disease burden, jeopardizing decades of hard-won gains[16]. The principal biological threat is the escalating insecticide resistance now present across the *An. gambiae* complex, which erodes the efficacy of every major insecticide class through two converging mechanisms: target-site insensitivity and metabolic detoxification[8], [17], [18]. In *An. gambiae* complex, target-site resistance arises from point mutations in the voltage-gated sodium channel (*Vgsc*), notably L1014F/S or L99F/S (*Kdr-West/East*), which reduces DDT and pyrethroid binding to the target protein[19], [20], [21]. A secondary substitution, N1570Y which is increasingly reported across contemporary vector-surveillance studies, arises on *Kdr* haplotypes to intensify the pyrethroid-resistance phenotype, extending the reach of the primary knockdown mutations[22]. Resistance to carbamates, together with organophosphates, follows the G119S substitution in the acetylcholinesterase (*Ace-1*) gene, has spread across different continents and is frequently reinforced by duplication of the locus[23], [24], [25], [26], [27]. A third target site, the GABA-gated chloride channel (*Rdl*), carries the A296S/G substitution conferring dieldrin resistance, so completing the recognized repertoire of target-site mechanisms[28]. Critically, these alleles recur across geographically distant populations, spreading between sibling species through adaptive introgression that accelerates their continent-wide dissemination[29]. Metabolic resistance, by contrast, proceeds through the over-expression of detoxifying enzymes. Cytochrome-P450s, especially the (*Cyp6aa/p, Cyp9k1, CYP6P3* and *CYP6M2*, which metabolize pyrethroids as well as DDT are consistently over-expressed in resistant *An. gambiae* and *An. coluzzii* [30], [31], [32][33], [34], as are the *Cyp6aa/p* and *Cyp9k1* clusters together with the glutathione-S-transferases *(Gste-2*) and esterases[33], [34]. Carboxylesterases further degrade organophosphates, whereas copy-number variation amplifies P450 clusters, *Gste-2* and *Ace-1*, elevating detoxification beyond transcriptional control [35], [36], [37]. Because a single locus frequently harbours distinct alleles in different siblings, *An. gambiae* and *An. coluzzii* meet identical selective pressure by largely independent mutational routes thus leading to a multi-genic, multi-allelic and geographically heterogeneous architecture in which neighbouring populations carry different causal variants, greatly complicating surveillance and resistance

management across the complex[38]. How these complex allelic interactions enable adaptation in local vectors will support custom anti vector interventions.

For nearly three decades, insecticide-resistance surveillance in malaria vectors has relied predominantly on single-locus PCR genotyping of *kdr* and *Ace-1*[19], [23], which is comparatively inexpensive and rapid, yet blind to the wider mutational landscape. By assessing only a few pre-defined loci, such assays overlook novel alleles, copy-number variation and the evolutionary processes that propagate resistance, thereby systematically underestimating its true burden. Whole-genome sequencing (WGS) transcends these limitations and thus is resolving species and cryptic sub-populations[39], [40], full allelic spectrum associated with resistance across insecticide classes, copy-number variation, together with gene flow between populations and taxa[41], [42], [43]. Genome-wide H12 scans [44] across Africa *An. gambiae* complex WGS within the MalariaGen Ag1000G database [36], [45] have localized selective sweeps, haplotype and diplotype clustering providing some evidence about the origins of SNPs, those acquired as an introgression between species and hence providing some clue to trace how resistance alleles spread [41]. Crucially, WGS, phenotypic bioassay and PCR are complementary rather than interchangeable, a distinction that should reshape how surveillance assays are designed for various endemic communities based on resources and data needs [38] In Cameroon, local vector density and malaria transmission intensity are shaped not only by ecological niche and urbanization, but also by rainfall, larval-habitat availability and micro-environmental conditions[7], [12]. The outcome of this has been seasonal transmission in the Sudano-Sahelian zone, contrasting with stable perennial transmission across the forest ecological zone[13]. Vector control has been countered by consistent reports of pyrethroid, carbamate and organochlorine resistancea cross Cameroon. Meanwhile, the genomic architecture determining these adaptive traits have not been determined, even for regions like the South-West region(a recognized malaria hotspot) where agro-industrial vector interventions may be impacting evolution of resistance in *An. gambiae* complex. Preliminary WGS analysis of *An. gambiae* complex around Mount Cameroon resolved *An. coluzzii* into two genetically distinct sub-populations, A and B, classified as single specie based on conventional PCR[104]. That study did not, however, compare their resistance associated genetic variation, leaving unresolved the distribution of selective sweeps, metabolic mechanisms and GABA-linked resistance across these siblings, together with its relationship to the resistance phenotype. Here, we address this gap by pairing the genome-wide discovery with field-based confirmation across two altitudinal zones in the same Mount Cameroon communities: whole-genome sequencing for screening for resistance, alongside phenotypic analysis of a parallel cohort from the same locality by WHO bioassay. In addition, we validated the variants observed in the population by PCR-RFLP for *Kdr-West* and *Ace-1*. Together, these multi-targets methods analyzed, taxon-specific resistance architecture of *An. gambiae* and *An. coluzzii* (A and B) across three insecticide classes generated converging genomic and phenotypic evidence of modes of insecticide resistance to guide taxon-aware vector control and malaria elimination

Methods

Mosquito Sampling

Sample collection was done around the slopes of Mount Cameroon, which sits directly on the coast of the Southwest Region of Cameroon, rising steeply and extending inland right from the edge of the Bight of Bonny. Adult *An. gambiae* complex mosquitoes were collected by human landing catch while eggs and larval stages of *An. gambiae* complex mosquitoes were collected by dipping methods. The collections were done in six localities grouped as lower (18–197 mas) and intermediate (371–584 mas) altitude (Fig. 1) in Buea, Limbe and Tiko Health Districts. These towns have a mild climate with an average temperature ranging from 23–25°C annually. The region is characterized by an average annual rainfall of 2625 mm making it lush with tropical vegetation and rainforest, high relative humidity (75–80%) and precipitation (2000–10,000 mm) [53] and two major seasons; a long rainy season (March–November) and a short dry season (November–February).

Adult anopheline mosquitoes were collected both indoors and outdoors in different houses across the communities between 6:00 PM and 6:00 AM using a locally design mouth aspirator and placed in plastic cups. The samples were then transported to an insectary at the Molecular Epidemiology and Drug Resistance Unit (MEDRU) at the University of Buea. Adult anophelines were morphologically identified with key. Insecticide bioassay analysis was conducted on 4–5 days old female *An. gambiae* complex adults obtained after rearing the larva from different collection sites. The adult mosquitoes that were collected via HLC were immersed in 80% ethanol and stored at − 20°C based prior to sequencing and analysis.

Whole genome sequencing of An. gambiae complex species

From a total of 1730 *An. gambiae* complex mosquitoes that were collected by human landing, 480 identified as *An. gambiae* complex mosquitoes (240 each from low and intermediate altitude) were sent for WGS at the Wellcome Sanger Institute as part of the MalariaGEN Vector Observatory project (https://www.malariagen.net/vobs/). The samples were sequenced using the protocols outlined in the MalariaGEN user guide (https://malariagen.github.io/vector-data/ag3/methods.html).

An. gambiae complex insecticide resistance analyses

To estimate the levels of insecticide target-site resistance from the WGS data, the frequency of single nucleotide polymorphisms (SNPs) was computed at known insecticide resistance genes (i.e., VGSC/para (AGAP004707; 2L: 2358158–2431617) for pyrethroids, *ACE1* (AGAP001356; 2R: 3438301–3497400) for carbamates and organophosphates, GSTE2 (AGAP009198; 3R: 28597652–28598640) for pyrethroids and organophosphates, and GABA/Rdl (AGAP006028; 2L: 25363652–25434556) for dieldrin. In addition, copy number variations for genes or clusters associated with metabolic insecticide resistance (i.e., several Cyp450 clusters (Cyp6aa/p cluster; 2R: 28 460 000–28 580 000 and Cyp9k1 (AGAP00818; X: 15240572–15242864)) for pyrethroids, ACE1 and GSTE2 for carbamates and organophosphates) were assessed. Similarly to the case for SNPs, the frequencies of amplification of these genes for the previously defined cohorts was computed. Garud's H12 statistic[44] was additionally

computed genome-wide to provide a complementary, haplotype-frequency-based assessment of selective sweeps, enabling detection of both hard and soft sweep signatures. H12, which quantifies the combined frequency of the two most common haplotypes at a given locus, provides a sensitive measure of both hard and soft sweep signatures, thereby complementing extended haplotype homozygosity-based statistics (XP-EHH and iHS) and strengthening the evidence for recent positive selection at insecticide resistance loci. To further characterize the selection sweep signals detected across the *An. coluzzii* and *An. gambiae* genomes, the H12 statistic [44] was additionally computed within identified sweep regions. For these analyses, the samples were separated according to taxon and windows of 1000 bp were used. Finally, haplotype clusters were computed in the regions of the target-site genes to observe if the signals were shared between populations to determine the extent of insecticide resistance spread.

Larva/pupa collection, breeding and Insecticide resistance Bioassay

To characterize the phenotypic insecticide resistance profiles of *Anopheles* populations across altitudinal gradients on the slopes of Mount Cameroon, larvae and pupae were sampled from two distinct communities with contrasting altitudes: Missellele at lower altitude and Dibanda (Mile 14) at intermediate altitude. Following controlled field colonization, 625 physiologically vibrant female F_o *An. gambiae* complex mosquitoes, selected from 1,250 which were 4–5 days old were considered suitable for insecticide bioassay. Phenotypic resistance was assessed using WHO tube bioassays (WHO, 2022). Twenty-five mosquitoes were introduced into 4 different test tubes, each containing an insecticide-impregnated paper, with a parallel control group of 25 mosquitoes simultaneously exposed to non-impregnated sterile Whatman filter paper. Mosquitoes were exposed to bendiocarb (0.1%) and deltamethrin (0.05%, 0.05% + PBO, 0.1%, and 0.1% + PBO) for one hour. In addition, synergist assays were performed by pre-exposing mosquitoes to piperonyl butoxide (PBO) prior to deltamethrin exposure to assess the contribution of metabolic resistance mechanisms. Knockdown rates were recorded immediately following the one-hour exposure period, and mortality was assessed for 24 hours post-exposure. Resistance status was classified according to WHO threshold criteria.

All post-exposure mosquitoes, both dead (phenotypically susceptible) and surviving (phenotypically resistant) were individually preserved in Eppendorf tubes containing silica gel and stored at − 20°C for downstream molecular analyses

DNA Extraction and Molecular speciation of An. gambiae complex following assessment of phenotypic resistance.

From a total of 625 *An. gambiae* complex obtained following WHO insecticide bioassays, 265 phenotypically resistant mosquitoes previously exposed to different concentrations of pyrethroid (deltamethrin and deltamethrin + PBO) and carbamate (bendiocarb) insecticides were randomly selected sampled communities across low and intermediate altitudes (135 from low altitude and 130 from intermediate altitude) for molecular characterization. Genomic DNA was extracted from whole

mosquitoes using the QIAGEN kit according to the manufacturer's instructions. Members of the *An. gambiae* complex were identified by multiplex PCR following the protocol of Scott et al. (1993). PCR amplicons were separated on 2% agarose gels containing ethidium bromide (10 mg/mL) and visualized under a UV illuminator (TOYOBO Trans Model TM-20) against a 1 kb ladder. Amplicons from samples identified as *An. gambiae* s.s. were subsequently digested with the restriction enzyme (*Hha*I) in a water bath at 38°C for 3 hours and the products resolved by electrophoresis to determine the molecular forms (M and S) of *An. gambiae* s.s following the [1] protocol.

Detection of mutation in the Vgsc and Ace-1 genes

Detection of the Kdr-west (1014F or L995F) mutation

The presence of the resistance allele (L1014F) in the *Vgsc* gene was detected by PCR following the described protocol [19] with slight modifications. The reaction conditions were as follows: an initial denaturation at 94°C for 2 min; 35 cycles of denaturation at 94°C for 30 s, primer annealing at 94°C for 30 s, and extension at 72°C for 30 s; a final extension at 72°C for 5 min; and a hold at 4°C. Each reaction was set up in a total volume of 15 µL, consisting of 7 µL GoTaq Green Master Mix; 1 µL each of primers Agd1 (5'-ATAGATTCCCCGACCATG-3'), Agd2 (5'-AGACAAGGATGATGAACC-3'), Agd3 (5'-AATTTGCATTACTTACGACA-3'), and Agd4 (5'-CTGTAGTGATAGGAAATTTA-3'); 3 µL nuclease-free water; and 1 µL of a 1:5 dilution of the mosquito DNA sample. Amplicons were separated by electrophoresis on a 2% agarose gel stained with ethidium bromide and visualised using a Gel Doc system (version 2.0). Members of the *An. gambiae* complex were classified as resistant or susceptible to deltamethrin based on fragment lengths of 293 bp and 195 bp or 137 bp, respectively.

Detection of the Ace-1 gene (G119S) mutation

The G119S mutation in the *Ace-1* gene was identified by RFLP following PCR [23]. A 541-bp DNA fragment was amplified using the PCR GoTaq® Flexi DNA polymerase kit (Promega, USA) in a total volume of 25 µl containing 10 µM of each primer Ex3AGdir(5'-GATCGTGGACACCGTGTTCG-3') and Ex3AGrev (5'-AGGATGGCCCGCTGGAACAG-3'). The DNA fragment was amplified using the following PCR reaction condition; Initial denaturation 94℃ for 3 min, 35 cycles of denaturation, annealing of primers and extension at 94℃ for 30 sec, 60℃ for 30 sec, 72℃ for 30 sec respectively, final extension at 72℃ 5 minutes. For digestion reaction, 7.5 µl of PCR product were used with 5U of *Alu*I enzyme (New England Biolab) in a final volume of 25 µl and incubated at 37°C for three hours. Digestion products were analyzed by electrophoresis on a 2% agarose gel. Susceptible homozygous (SS) individuals displayed 403 bp and 153 bp long fragments, and two fragments of 253 bp and 150 bp for resistant homozygous (RR) while heterozygous (RS) reveal a combination of susceptible and homozygous resistant bands[55].

Data analysis for phenotypic resistance bioassay and PCR genotyping

The *An. gambiae* populations were classified as susceptible, suspected resistant or resistant if mortality rate recorded after 24 h following exposure were ≥ 98%, 90 and 97% or < 90% respectively.

Data were analyzed using the statistical software, SPSS (SPSS Inc. Released 2009). Association between allele frequencies in *An. gambiae* complex populations between cohorts were compared using Pearson's χ2-test. Where expected frequencies fell below 5 (as in the *Kdr* SS), pairwise Fisher's exact tests were additionally performed, since Fisher's exact test does not rely on large-sample approximations. For each *P*-value < 0.05, the difference was considered significant.

Results

An. gambiae sibling diversity and markers of resistance to insecticides.

An. gambiae sibling species.

A total of 276 *An. gambiae* complex samples out of the 480 sent for sequencing passed quality assessment. Out of these 276 mosquitoes, 178 were identified as *An. gambiae s.s*, 93 as *An. coluzzii (A and B)* and 4 as *An. melas*. Additionally, 1 sample could not be classified and is excluded from further analysis.

Target-site resistance to insecticides

1) Pyrethroids: Vgsc/Para

Several SNPs in the *Vgsc* gene of *Anopheles* mosquitoes have been reported to confer resistance to pyrethroid insecticide. The most prevalent, L995F (*Kdr-West*), was present at near-fixation levels across all cohorts except *An. coluzzii* B, where it reached 60% (Fig. 2A). A second key variant, L995S (*Kdr-East*), was absent from all *An. coluzzii* cohorts at both altitudes but detected at low frequency (< 20%) in all *An. gambiae* s.s. cohorts. On the contrary, the *Vgsc* double mutation V402L+I1527T was absent from all *An. gambiae* s.s. cohorts yet present in all *An. coluzzii* cohorts, reaching intermediate frequency in *An. coluzzii* B and low frequency in *An. coluzzii* A. Similarly, R254K, previously associated with pyrethroid resistance, was observed at high frequency exclusively in *An. gambiae* s.s cohorts. Finally, L1667M, still not linked to insecticide resistance, was detected at high frequency in *An. coluzzii* A, low frequency in *An. coluzzii* B, and was absent from all *An. gambiae* s.s. cohorts. The new variants N1345I and K1603T were found specifically in the *An. coluzzii* B, at a moderate/low frequency.

The frequency distributions of all SNPs are summarized in Fig. 2A.

2) Carbamates and organophosphates: **Ace-1**

The *Ace-1* resistance-associated mutation G280S was completely absent from all *An. coluzzii* cohorts, including *An. coluzzii* B. In contrast, it was detected at intermediate frequencies across all *An. gambiae*

s.s. cohorts, suggesting a species-specific distribution of organophosphate and carbamate resistance at the target site (Fig. 2B).

3)Pyrethroids and organochlorines: **Gste-2**

The *Gste-2* mutation I114T, associated with pyrethroid and organochlorine resistance, showed a markedly contrasting distribution between species. It was absent in some communities and present at very low frequency in all *An. gambiae* s.s. cohorts, whereas *An. coluzzii* cohorts harboured this mutation at intermediate to high frequency. Notably, the mutation I114T displayed by *An. coluzzii* B is comparatively lower in frequency than the other *An. coluzzii* cohorts (Fig. 2C). While the I114T was almost absent in the *An. gambiae*, the frequency of Gste2-L119V mutation appears moderate and more specific to this taxon compared to *An. coluzzii* where it is only found in *An. coluzzii* B. Additionally, a new variants Gste-2-T154S implicated in deltamethrin and permethrin resistance was also detected in all species, while occurring at a higher frequency in *An. gambiae* than *An. coluzzii*(A and B). Also, the F120L was also specific to *An. gambiae s.s* and across all cohorts.

4)Dieldrin: GABA/Rdl

The γ-aminobutyric acid receptor gene(*Rdl*), which mediates resistance to dieldrin, harbours two functionally relevant SNPs with divergent species distributions (Fig. 2D). The A296S/T345S mutation was present at near-fixation levels in all *An. coluzzii* A cohorts but absent or at very low frequency across all *An. gambiae* s.s. cohorts and *An. coluzzii* B. Conversely, A296G/T345M was detected at intermediate frequency in all *An. gambiae* s.s. cohorts and in *An. coluzzii* B, yet absent from the remaining *An. coluzzii* A cohorts. The opposing distributions of A296S and A296G between *An. coluzzii* A and *An. gambiae* s.s. suggest that dieldrin resistance at this locus is maintained through distinct mutational routes in each taxon.

Metabolic resistance to insecticides

Several genes, including some cytochrome P450, carboxylesterase and glutathione S-transferase genes, are known to affect metabolic resistance in members of the *An. gambiae* complex through amplification. We examined some well documented targets to estimate the risk of possible metabolic resistance present in the Southwest cohorts.

1)Pyrethroids: C*YP*6aa/p cluster and C*YP*9k1

Many cytochrome P450 genes in the *CYP6aa/p* cluster have been shown to be linked to metabolic resistance. None of the cohorts that we analyzed (Fig. 3) shows a higher frequency of the gene exceeding 10% of amplification across all *An. gambiae* complex samples and cohorts. Amplification in another cytochrome P450 gene (*CYP9K1*) has also been reported to be highly involved in the metabolic resistance of pyrethroids insecticides. For all cohorts, there were intermediate to high frequency of amplification (46–100%) (Fig. 3) in all samples, while amplification of this gene was particular fixed

(100% amplification) in the *An. coluzzii* B. Additionally, though CNV frequency for the amplification of *CYP6aa1* and *CYP6aa2* gene were absent in all anopheline cohorts, it was particularly present at a low frequency (10%) at *An. coluzzii* B.

2)Carbamates and organophosphates: *Ace-1*

The acetylcholinesterase 1 (*Ace-1*) gene is not only involved in target-site resistance but also in metabolic resistance with copy number variants (CNVs) of this gene playing this important role (Fig. 3). There was no amplification of this gene observed in any *An. coluzzii* cohort (*An. coluzzii A* and *An. coluzzii B* cohorts), but the gene amplification was present at intermediate frequency (41–51%) in all *gambiae s.s* cohorts.

3) Pyrethroids and organochlorines: Gste-2

The glutathione S-transferase epsilon 2 gene (Gste2) is also known to cause metabolic resistance when amplified. These amplifications were only found at low frequencies (1–9%) in all cohorts for both *An. gambiae* and *An. coluzzii* A, while in the *An. coluzzii* B cohort appears at intermediate frequency with AGAP009198(GSTE2)(20–30%) (Fig. 3).

Spatial and introgressive gene flow

After the observed differences in term of insecticide resistance profiles between the various cohorts analyze, the evaluation for the risk of resistance spreading from one population to another based on the patterns of haplotype sharing between these cohorts was necessary. The focus was on the same loci for insecticide resistance previously explored. Pairwise genetic distances (number of SNPs) across the *Vgsc* gene for *An. gambiae* s.s, *An. coluzzii* and *An. melas* were strongly right-skewed, with most sample pairs sharing near-zero distances (supplementary Figure: 1.a). Also, a small subset predominantly involving *An. gambiae* s.s and *An. coluzzii* were substantially less diverse (supplementary Figure: 1.a). There were also haplotypes shared between *An. coluzzii* A and *An. coluzzii* B cohorts and between locations at this insecticide resistance locus (supplementary Figures: 1.b).

On the VGSC/para, one cluster of haplotypes was observed to be specific to *An. gambiae s.s.* while others were composed exclusively of *An. melas* and *An. coluzzii* samples respectively. However,there were variant clustering between both taxons (*An. gambiae* s.s and *An. coluzzii*) suggesting shared ancestry or some degree of introgression (supplementary Figure: 1.a). As for *An. coluzzii* samples only (supplementary Fig. 1b), the haplotypes were observed to be shared between *An. coluzzii* A and *An. coluzzii* B cohorts and between samples from different locations. Similarly, while the Gste2 haplotype cluster displayed low genetic distances, with extensive intermixing among *An. gambiae, coluzzii A*, and *coluzzii B* haplotypes across the tree (supplementary Figure: 1.c), the Rdl haplotype cluster showed pronounced genetic distances, with two major branching largely separated by population identity: *An. coluzzii* A haplotypes form a distinct, tightly grouped branch, while *An. gambiae* and *An. coluzzii* B show more internal structure (supplementary Figure: 1.d).

Additionally, diplotype cluster analysis was performed to get a complete picture on the haplotypes under selection. The main difference between the two *An. gambiae* complex siblings was the presence of the *Vgsc*-R254K mutation in the *An. gambiae s.s* cohorts which was absent in the *An. coluzzii* cohorts. Also, the *An. coluzzii* A cluster carried the *Vgsc*-L1667M mutation specific to that haplotype and may be driving the haplotype sweep selection in *Vgsc*-L995F background (supplementary Fig. 2.a). For the GSTE2 loci, pairwise genetic distances between *An. gambiae* s.s., *An. coluzzii*, and *An. melas* were minimal compared to the *Vgsc* gene, with two distinct haplotypes shared in *An. gambiae s.s* (supplementary Fig. 1.a), characterize with respectively the L119V and F120L variants (supplementary Fig. 2.b). Furthermore, *An. coluzzii* is characterised by its own distinct haplotype, driven by the I114T mutation (supplementary Fig. 2.b & c), which is predominantly observed in *An. coluzzii* A and a subset of *An. coluzzii* B. Finally, pairwise genetic distances at the *Rdl* locus reveal higher divergence compared to other loci, with pairwise differences reaching up to 400 SNPs (supplementary Fig. 1c). *An. gambiae* s.s. and *An. coluzzii* display distinct distance distributions, consistent with their divergent carriage of the A296S and A296G/T345M resistance alleles, driving their distinct recent haplotypes. The broad spread of values indicates substantial haplotypic diversity at this dieldrin resistance locus across the *An. gambiae* complex.

Selection sweeps

Strong, well characterised selection signals were observed at *GABA/Rdl, Vgsc/para, Cyp6aa/p, Cyp9k1, Ace1, Gstes* regions after computing H12 for every chromosome, using cohorts defined according to taxon (*An. gambiae s.s.*, *An. coluzzii* A and *An. coluzzii* B). Beyond these established targets, the genome scan also revealed a set of signals in far less characterized regions, and the distribution of these signals across cohorts followed a clear pattern of taxon specificity (Fig. 4).

On 2RL, the *An. gambiae* s.s cohort carried a peak at *Cyp6aa/p*, coinciding with the carboxylesterase gene *COEAE6O*, together with a distinct nearby signal at *CYP4AA1/SP8905*. Immediately downstream of *Rdl*, around 2L:28.4 to 28.7 Mb, a further signal was shared by *An. gambiae* s.s and *An. coluzzii* A but was not detected in *An. coluzzii* B. This region contains the carboxylesterase gene *COEAE2F* and the alpha esterase AGAP006227, both implicated in insecticide resistance, alongside several glucosyl/glucuronosyl transferases genes(AGAP006222, AGAP006223), aldehyde oxidases (AGAP006220, AGAP006221, AGAP006224 to AGAP006226), the ubiquitin conjugating enzyme E2C AGAP006238, involved in antiviral immune defense, and a cluster of cysteine rich venom proteins (AGAP006252, AGAP006253), a family of salivary and venom proteins known in *Aedes aegypti* to facilitate flavivirus replication in the midgut through interaction with host prohibitin. The *An. coluzzii* A cohort showed a broad, cohort specific signal around 2R:47.6 to 48.0 Mb, spanning SG9, the serine protease genes *SP11838, SP11706* and *SP21408*, the sensory ionotropic receptor *IR40a*, implicated in host seeking and environmental adaptation, and the Methuselah like GPCRs *GPRMTH5* and *GPRMTH6*, associated with stress response and neuroendocrine signaling. Further *An. coluzzii* A additional specific peaks were detected toward the end of 2R, around 55.0 to 55.5 Mb, encompassing the odorant receptors

Or26, Or27, Or56 and *Or57*, and a larger region between 58.0 and 61.0 Mb containing the putative GABA-B receptor *GPRGBB2*, the ABC transporters *ABCC4, ABCC6* and *ABCG4*, the dopamine receptor *GPRDOP1*, glutamate dehydrogenase (AGAP004622), several sodium independent sulfate anion transporters (AGAP004635, AGAP013218, AGAP004636) and the cytochrome P450 *CYP306A1* which may be implicated in insecticide resistance. The *An. coluzzii* B cohort instead displayed its own private unique signal, not recorded in the other two cohorts, around 2R:40.6 to 41.4 Mb. This region contains the ubiquitin carboxyl terminal hydrolase 34 (AGAP003643) and the E3 ubiquitin protein ligase RNF139 (AGAP003666), both implicated in antiviral immune responses, together with serine proteases (*SP8905*, SP8907 and SP8898) and the aldehyde dehydrogenase AGAP003652, involved in insecticide detoxification.

On 3RL, the shared *Gste2* signal and a peak at *CYP9M1/CYP9M2* (3R:31.7 to 31.9 Mb) was shared by *An. gambiae s.s.* and *An. coluzzii* A. A closely positioned but distinct signal around 3R:30.2 to 30.3 Mb, corresponding to a beat protein encoding gene (AGAP009267) and a SAM domain containing protein (AGAP029470) implicated in host-parasite interaction, was instead shared between *An. coluzzii* A and *An. coluzzii* B. A further peak, specific to *An. coluzzii* A, was found around 3R:38.5 to 38.7 Mb at AGAP009688, encoding a leucine rich repeat and immunoglobulin like domain protein involved in innate immunity. On 3L, two additional regions were shared by *An. gambiae* s.s. and *An. coluzzii* A only, one around 3L:27.5 to 28.0 Mb at AGAP011564, a glucosyl/glucuronosyl transferase and key Phase II detoxification enzyme, and one around 3L:38.2 to 38.3 Mb at *GPRNPR4*, a predicted neuropeptide receptor implicated in mosquito behaviour and physiology.

On the X chromosome, the *An. gambiae* s.s cohort showed the expected peak at Cyp9k1/CPR15, which was more pronounce in *An. coluzzii* A and B, while the *An. coluzzii* A cohort carried it two own distinct signal at NADPH cytochrome P450 reductase *Cpr* gene and an elevated region of H12 spanning *GSTU1, CTL7* and *Osi23*. Together, these results show that beyond the shared insecticide resistance loci, each cohort carries a distinct repertoire of additional selection signals. Several of these involve detoxification gene families which are carboxylesterases, GSTs, CYPs, UGTs and aldehyde dehydrogenases.

Insecticide bioassay, mortality rates and molecular speciation of An. gambiae complex

Overall, of the 1250 (635 samples from each community across the two altitudes) F_0 female adult mosquitoes exposed, their mortality rate was highly dependent on the insecticide used (pyrethroids or carbamates). The mortality rates were 68% (68/100),77% (77/100), 37% (37/100),73% (73/100) in Missellele (low altitude) and 72% (72/100), 75% (75/100), 29% (29/100), 78% (78/100) in Mile 14 (intermediate altitude) after being exposed to 0.05% deltamethrin, 0.05 deltamethrin + PBO, 0.1% deltamethrin and 0.1% deltamethrin + PBO respectively. Furthermore, the mortality rate for 66% (66/100) was recorded for the bendiocarb insecticide at Missellele, 57% (57/100) at Mile 14 while no mortality was recorded in the control tubes (Fig. 5.A). However, none of the samples that were knocked down during exposure recovered 24 hours after.

All 265 randomly selected mosquitoes from a pool of 1250 *An. gambiae* complex for molecularly speciation and *Kdr/Ace-1* genotyping were all deltamethrin resistant. Out of this 265 F_0 *An. gambiae* complex, 135 were from Missellele (low altitude) and 130 from Mile 14 (intermediate altitude). They were all 100% (265/265) identified as *An. gambiae s.s* following PCR. The restriction of the PCR products produced two distinct band sizes (Table 1). For banding patterns after RFLP: a)257bp + 110bp bands corresponded to the S-form now known as *An. gambiae* while b) 367 bp corresponded to M-form now known as *An. coluzzii*.

Table 1
Communities and species distribution post PCR

Community	Altitude (m.a.s.l)	PCR_1(speciation) Amplicon size	PCR_2(speciation) Amplicon size(s)	Species count(n)	
		Pre-restriction	Post-restriction (Hhal)	*An. gambiae*	*An.coluzzii*
Missellele	45–70	390 bp	257bp and 110bp	40	95
Mile 14	360–390		367bp	58	72

Distribution and frequency of the Kdr-West (L1014F also known as L995F) Allele

One hundred and forty-two (142) randomly selected *An. gambiae s.s* previously exposed to different concentrations of deltamethrin: 62 from Mile 14 Buea and 80 from Missellele were genotyped for the *Kdr-West* SNP.

Table 2
Proportions of *Kdr-West* & *Ace-1* SNPs in *An. gambiae* complex along the slopes of mount Cameroon

Gene	**Genotype**	**Variant proportions[%(n)]**			
		Total (n)	**Mile 14 [100(62)]**		**Missellele [100(80)]**
			An. gambiae	*An. coluzzii*	*An. gambiae*	*An. coluzzii*
Kdr-West	RR	125	35.5(22)	48.4(30)	43.8(35)	47.5(38)
	SS	15	14.5(9)	1.6(1)	3.8(3)	2.5(2)
Ace-1	RS	96	40.3(25)	46.8(29)	16.3(13)	36.3(29)
	SS	27	8.1(5)	1.6(1)	21.3(17)	5.0(4)

Kdr-West gene was successfully genotyped in 140 samples: 62 from Mile 14 and 78 in Misselle. The genotyping revealed near-fixation of the *Kdr-West* resistant allele across both taxa and sites, with homozygous-resistant (RR) genotypes predominating (125/140) and susceptible (SS) genotypes rare. At Mile 14, RR frequency was significantly higher in *An. coluzzii* than *An. gambiae* ($p = 0.013$), a species

difference that disappeared at Missellele (p = 0.67). For *Ace-1*, heterozygotes genotype (RS) dominated, with homozygous-susceptible genotypes being uncommon overall amongst species. The *Ace-1* genotype distribution differed markedly between sites within *An. gambiae*: susceptible (SS) genotypes rose from 8.1% at Mile 14 to 21.3% at Misselele (p = 0.003) while there was no significant different (p = 0.36) for the SS genotype in *An. coluzzii* samples between these two communities. Consequently, at Missellele, the two species differed significantly in *Ace-1* genotype with *An. gambiae* mostly susceptible (p = 0.0004), whereas no such difference was detected at Mile 14 (p = 0.20) (Fig. 5.B).

Distribution of the ACE-1 (G119S/G280S) resistant allele

The *Ace-1* gene (541 bp) was amplified in 123 *An. gambiae* s.s. mosquitoes: 60 from Mile 14, Buea (intermediate altitude) and 63 from Missellele (lower altitude).Digestion of the amplicon with 5 U of *Alu*I on a 2% agarose gel yielded 403 bp and 153 bp fragments for the homozygous-susceptible (SS) genotype, and 403 bp, 253 bp and 153 bp fragments for the heterozygous-resistant (RS) genotype. Heterozygous genotypes predominated across all cohorts. This signal was driven mainly by Missellele *An. gambiae*, which showed a markedly higher SS frequency than Mile 14 *An. gambiae* (p = 0.003), Mile 14 *An. coluzzii* (p < 0.0001) and Missellele *An. coluzzii* (p = 0.0004) (Table 2). Overall, most mosquitoes were heterozygous-resistant (RS; 78.1%, 96/123), and the remainder were homozygous-susceptible (SS; 21.9%, 27/123). No homozygous-resistant (RR) genotypes were detected at either altitude. The RS and SS frequencies differed significantly between sampling sites (p < 0.001), Fig. 5C).

Discussion

Policies to sustaining the long-term efficacy of insecticide-based vector interventions need to be guided by data tracking how insecticide-resistance mechanisms emerge and spread across the heterogeneous malaria transmission and diverse vector landscapes of sub-Saharan Africa. Insecticide resistance development is impacted by vector diversity, behaviour, ecological niche, and the uncontrolled use of insecticides and pesticides [5], [56]. Here we assessed populations of malaria vectors across the altitude gradient along Mount Cameroon, where malaria is endemic, and the surrounding landscape is dominated industrial farming that heavily depend on pesticides. We used whole-genome sequencing to discovery variation, characterize the genomic architecture and assess operational-scale allele frequencies of insecticide resistance along the slopes of Mount Cameroon, accompanied by phenotypic resistance bioassays and PCR-RFLP genotyping. Integrating these three complementary approaches enhances future surveillance and elimination planning. Although nearly 50 *Anopheles* species transmit *Plasmodium* across Africa [57], [58] *An. Funestus*[52],[59],, *An. gambiae* and *An. coluzzii*[1], [2], [3],[46], [60] and, to a lesser extent, *An. melas* [13] and *An. ziemanni* [61], are the principal vectors in Cameroon and most of sub-Saharan Africa. The recent identification of a potentially novel taxon (*An. coluzzii* B) within the *An. gambiae* complex[104] demonstrates the need for genome-based approaches in revealing the complexity of vectors that may be missed by conventional PCR-based identification targeting the SINE or a single X-chromosome centromeric locus[1], [2], [3].

Genetic markers of resistance in *Anopheles* arise mainly through point mutations in genes encoding key insecticide targets such as acetylcholinesterase and the voltage-gated sodium channel [21], [24], [43], [62]. All Anophelines across localities and altitudes carried pyrethroid-resistance markers, and phenotypic bioassays confirmed the reduced susceptibility predicted by WGS. Three independent *Vgsc/para* target-site mutations were detected: L995F (*Kdr-West*), near fixation across all cohorts; L995S (*Kdr-East*), at low frequency in *An. gambiae* s.s. [63], [64]; and the double mutation V402L + I1527T, exclusive to *An. coluzzii*, consistent with reports across Africa [26], [62], though some studies found these SNPs absent [47], [51]. The taxon-specific segregation of alleles at the same loci indicates that *An. gambiae* and *An. coluzzii* are responding to the same selective pressure through largely alternative mutational routes, reflecting parallel but non-identical *Kdr* evolution across African vectors [26], [33], [41]. These findings corroborate earlier Cameroonian reports[10], [50], [65] and align with West and Central African resistance patterns [66], [67], [68], [69].This confirms that pyrethroid resistance is a continent-wide crisis rather than a localized phenomenon. The data presented here is one of the earliest reports of *Kdr-East* in the Southwest region of Cameroon, although this variant has been previously recorded in central Cameroon [63], [64] while reported absent elsewhere [47], [70]. This may point to potential gene flow sharing the resistance locus between ecological settings. The co-occurrence of all three mutations at varying frequencies signals an escalating pattern that warrants immediate intervention.

The *Ace-1* G280S mutation, conferring carbamate and organophosphate resistance[23], was absent in all *An. coluzzii* cohorts but present at intermediate frequency in *An. gambiae* s.s. This species-specific pattern shows some weakness in operational value i.e organophosphate- and carbamate-based interventions may remain effective against *An. coluzzii* even where pyrethroid resistance is widespread, whereas *An. gambiae* would show reduced susceptibility [48], [71], [72], [73]. This reinforces the case for high resolution community-level genotyping to guide insecticide choice rather than uniform implementation of interventions [74],[75]. The *Gste-2* I114T mutation, conferring pyrethroid and organochlorine resistance [33], showed the opposite pattern: intermediate-to-high frequency in *An. coluzzii* but largely absent in *An. gambiae* s.s., with *An. coluzzii* B again diverging from *An. coluzzii* A. As organochlorines are not WHO-prequalified here, this signature most plausibly reflects residual pyrethroid exposure or agricultural pesticide use[76]. In addition, the absence of I114T and V402L in *An. gambiae* suggests that carbamate/organophosphate IRS would could control this species as previously reported [56], [66], [67], but unfortunately, its present in *An. coluzzii*, would only maintain the continuous escalation of malaria incident in communities “blessed” with *An. coluzzii* from across Africa [40], [77], [78]

The GABA/*Rdl* locus showed the most taxon-resolved pattern, with A296S/G, T345S and N530K differentially distributed across cohorts have also been reported in *An. gambiae* complex[40], [79], *An. sinensis* [80] *a*nd *An. stephensi* [81] across different geographical landscape, making a vital mutation for intervention.

The A296S and A296G mutations confer dieldrin resistance [28], [82]. A296S approached fixation in *An. coluzzii* A but was essentially absent in other siblings, while A296G occurred at intermediate frequency in *An. gambiae* s.s. and *An. coluzzii* B but not *An. coluzzii* A. These discrepancies; present in *An. coluzzii* yet

absent in *An. gambiae* s.s., particularly A296S is unexpected given its proposed origin through introgression from *An. arabiensis* and *An. gambiae* into *An. coluzzii* [83]. T345S was confined to *An. coluzzii* A and N530K to *An. gambiae* cohorts. As dieldrin is no longer used operationally, persistence of these mutations may reflect residual cross-resistance selection from current insecticide classes or a low fitness cost of carriage [28], meriting longitudinal monitoring. Although A296S/G occurs in *An. sinensis* in Asia[80] and mainly in *An. funestus* in Cameroon[49], [84] and across Africa[76], [85], [86], this is its first report in *An. gambiae* in Cameroon's Southwest region. T345S and N530K are also reported here for the first time in Cameroon, signaling a risk of multi-class resistance that requires wider monitoring.

Metabolic markers reinforced this taxon- and locus-specific patterns. *Cyp6aa/p* amplification remained low (< 10%) across all cohorts, whereas *Cyp9k1* amplification was widespread and reached fixation in *An. coluzzii* B. This was consistent with its role as a major pyrethroid-resistance driver in West and Central Africa [87], previously reported in West [39], [88] and East Africa[31]. *Ace-1* copy-number amplification occurred exclusively in *An. gambiae* s.s, mirroring its target-site pattern and indicating possible convergent, taxon-restricted selection through two independent mechanisms, while *Gste-2* amplification was low except in *An. coluzzii* B. Haplotype sharing at *VGSC/para* revealed substantial gene flow between *An. coluzzii* A and B, paralleling long-range *Kdr-West* haplotype sharing across Africa [41] and risking accelerated spread of resistance alleles independently of local selection. Selection-sweep analysis (H12) confirmed strong signals at established loci (*VGSC/para*, GABA/*Rdl*, *Cyp6aa/p*, *Cyp9k1*, *Ace-1*, *Gste-2*) but additionally provided novel signals atcarboxylesterase loci (*COE15O, COE2G/3G/3H*) and the *Keap1* region, showing that targeted genotyping panels alone cannot capture the full genomic landscape under selection from insecticide and pesticide use.

Beyond this well-characterized resistance associated with loci, whole genome-wide scan revealed several less characterized adaptive signatures. Alongside the classical sweeps (*VGSC/para*, GABA/*Rdl*, *Cyp6aa/p*, *Cyp9k1*, *Ace-1*, *Gste-2*), additional taxon-specific peaks emerged in poorly characterized regions [89], [90], suggesting that different members of the *An. gambiae* complex face distinct ecological pressures or have evolved different solutions to similar challenges. The strongest non-classical signals involved detoxification pathways: *An. gambiae* s.s. and *An. coluzzii* A shared a large selective region on chromosome 2RL containing carboxylesterases, aldehyde oxidases and Phase II enzymes including UDP-glucuronosyltransferases (UGTs), pointing to selection on coordinated detoxification modules rather than single genes. *COEAE60*, *CYP4AA1* and UGTs (AGAP011564, AGAP006222) genes have been previously reported as overexpressed in resistant mosquitoes, and copy-number expansion of *COEAE2F* is linked to enhanced esterase-mediated resistance [89][90][91].

Selection also targeted cuticular resistance genes, notably *CPR15* and the NADPH cytochrome P450 reductase (*Cpr*). Evidence of the role of cuticular modification in metabolism-independent barrier to insecticide penetration is growing [92], [93]. *Cpr* supplies the electron donor for P450-mediated detoxification, and its knockdown raises susceptibility in several insects [94]. Its selection exclusively in *An. coluzzii* A suggests an additional layer of metabolic adaptation, and the co-selection of detoxification enzymes, cuticular proteins and *Cpr* implies complementary mechanisms not fully captured by

conventional synergist bioassays. This study also revealed genetic signatures of selection around loci associated with behavioural and ecological adaptation. *An. coluzzii* A was uniquely enriched for loci in chemosensory perception, neuronal signaling and neuroendocrine regulation - *IR40a*, odorant receptors (*Or26*, *Or27*, *Or56*, *Or57*), Methuselah-like GPCRs, GABA-B receptors and the putative dopamine receptor *GPRDOP1* [95], [96]. Because D1-like dopamine receptors govern locomotion, arousal and olfactory learning [97], [98], these signals suggest variations in sensory and neuronal pathways that could shift host-seeking, biting or resting behaviour and reduce contact with indoor interventions- a form of behavioural resistance. *An. coluzzii* A also uniquely showed selection of ABC transporter genes, implicated in resistance through xenobiotic efflux and overexpressed in pyrethroid-resistant *An. gambiae* and *An. sinensis* [95], [99], suggesting several complementary mechanisms evolving simultaneously in this taxon.

The scan further revealed immune-related genes better known for antiviral defense than anti-*Plasmodium* immunity- RNF139, ubiquitin carboxyl-terminal hydrolases, the ubiquitin-conjugating enzyme AGAP006238, and CTL7, a C-type lectin facilitating flavivirus entry in *Aedes* [100]. Their functional role in *Anopheles* is unknown, but they hint that pressures beyond malaria parasites shape immune evolution, with possible implications for arbovirus susceptibility and vector competence that merit study.

To corroborate WGS results, WHO phenotypic bioassays and PCR-RFLP genotyping for *An. gambiae* complex siblings were performed. The phenotypic assays corroborated the resistance predicted by whole-genome sequencing (WGS). Susceptibility to Deltamethrin- bendiocarb both fell below the WHO susceptibility threshold for all cohorts even at higher intensity dose, indicating concurrent pyrethroid and carbamate resistance, furthering evidence of multi-class resistance that may now be widespread in African *An. gambiae* complex [101]. Also, the partial restoration of deltamethrin susceptibility after PBO pre-exposure implicates cytochrome P450–mediated detoxification alongside target-site mechanisms, consistent with the *Cyp9k1* and *Cyp6aa/p* amplifications recovered here and with P450-driven metabolic resistance reported elsewhere in West Africa [97].

The single-loci species speciation PCR clearly resolved *An. gambiae* complex into *An. gambiae* s.s and *An. coluzzii*, occurring in sympatry as previously reported [12], [102], [103]. Resistance genotyping also showed the L995F(*kdr-west*) allele predominated among phenotypically resistant *An. gambiae* s.s., and its strong genotype–phenotype association validated the near-fixation *VGSC/para* signal. Although PCR-RFLP determined prevalence of *kdr-west* was consistently lower than the WGS estimate; its detection reflects the confirmatory versus discovery roles of the two, approaches., Since single-locus PCR resolves one canonical mutation whereas WGS captures the full allelic spectrum, including V402L and metabolic variants[43]. The *Ace-1* locus showed a marked excess of heterozygotes and no resistant homozygotes, consistent with the established fitness cost of the G280S mutation and its resolution through gene duplication and balancing selection [25]. Because both major vectors are present at every elevation sampled, any single-insecticide strategy will simultaneously confront the divergent, taxon-specific resistance architectures resolved by WGS, reinforcing the operational case for taxon-aware deployment rather than uniform application. All the samples across different communities along the slope of Mount

Cameroon had a mutation that confers some level of resistance to at least 3 out of the 4 main insecticide classes commonly used for vector control.

In a nutshell, the cross-platform concordance validates a tiered surveillance model coupling genomic discovery with field-deployable confirmation. Together, these findings show that contemporary adaptation in the *An. gambiae* complex is shaped by multiple interacting pressures, not insecticide exposure alone. Beyond the classical loci, signatures spanning detoxification, cuticular permeability, chemo-sensation, neuronal signaling and immunity reveal taxon-specific evolutionary trajectories within Cameroon. Surveillance relying solely on classical resistance markers will therefore miss emerging mechanisms; integrating genome-wide selection scans with transcriptomics, phenotypic assays and functional validation will offers a deeper understanding of vector evolution, and a stronger basis for anticipating both malaria and arboviral control challenges.

Conclusion

This study reveals that insecticide resistance in *Anopheles gambiae* and *An. coluzzii* along Mount Cameroon's slopes is multi-mechanistic and taxon-specific, spanning four insecticide classes and including a previously undescribed *An. coluzzii* sub-population with a distinct resistance architecture. Introgressive gene flow at resistance loci across taxa and localities, shaped by the region's altitudinal ecological gradients, indicates resistance alleles can spread beyond their taxon of origin. These findings highlight a genomically complex, evolving resistance landscape that current single-locus surveillance likely underrepresents, with direct implications for insecticide selection and intervention timing across Cameroon

Abbreviations

FST
Fixation Index
PCA
Principal Component Analysis
SNPs
Single Nucleotide Polymorphisms
WGS
Whole Genome Sequencing
PCR
Polymerase chain reaction
Vgsc
Voltage gated sodium channel
HLC
Human landing catch

Declarations

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Data availability

The datasets generated and analysed during the current study are available as part of the Malaria Vector Genome Observatory repository, included in the 1270-VO-MULTI-PAMGEN (https://www.malariagen.net/vobs/) study part of the Ag3.7 release. Raw data has also been deposited to ENA, details, including accession number: PRJEB42254.

Declarations Ethics approval and consent to participate Ethical clearance was obtained from the Cameroon National Ethics Committee (Ref. N0 2019/09/1188/CE/CNERSH/SP and 2020/12/1315/CE/CNERSH/SP). Administrative authorisations were sought from the Regional Delegation of Public Health for the Southwest region, and the District Medical Service of Buea and Tiko Health districts. At community level, authorization was obtained from Chiefs or Quarter heads where appropriate. Only prospective participants who volunteered to participate by signing a written informed consent, after sensitization were enrolled as mosquito trappers. All experimental procedures involving human subjects were conducted in accordance with the 1964 Declaration of Helsinki guidelines and regulations.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Author Contribution

PNK: coordinated the study, collected samples, performed experiments, and drafted the manuscript TOA: conceptualized, designed, coordinated the study, and drafted the manuscript. JB and AS: performed data

analysis. BEA,NWK,SBE,AEN and LNT: collected samples and performed experiments. SBG,JBD and CC: provided critical review of the manuscript. AAN:conceptualized, designed, provided logistics, coordinated and substantially improved on the manuscript. All authors were involved in the review of the manuscript.

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Data Availability

The datasets generated and analysed during the current study are available as part of the Malaria Vector Genome Observatory repository, included in the 1270-VO-MULTI-PAMGEN (https://www.malariagen.net/vobs/) study part of the Ag3.7 release. I attach a manifest containing 2 columns: the first one is the sample ID that is used in the metadata to identify the sample, the second is the Biosample accessions. These can be used in the ENA browser (e.g., https://www.ebi.ac.uk/ena/browser/view/SAMEA13204185 for the first sample), the Biosamples browser (e.g., https://www.ebi.ac.uk/biosamples/samples/SAMEA13204185), or the NCBI browser (e.g., https://www.ncbi.nlm.nih.gov/biosample/SAMEA13204185/).

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105. Supplementary. Figures.

Figures

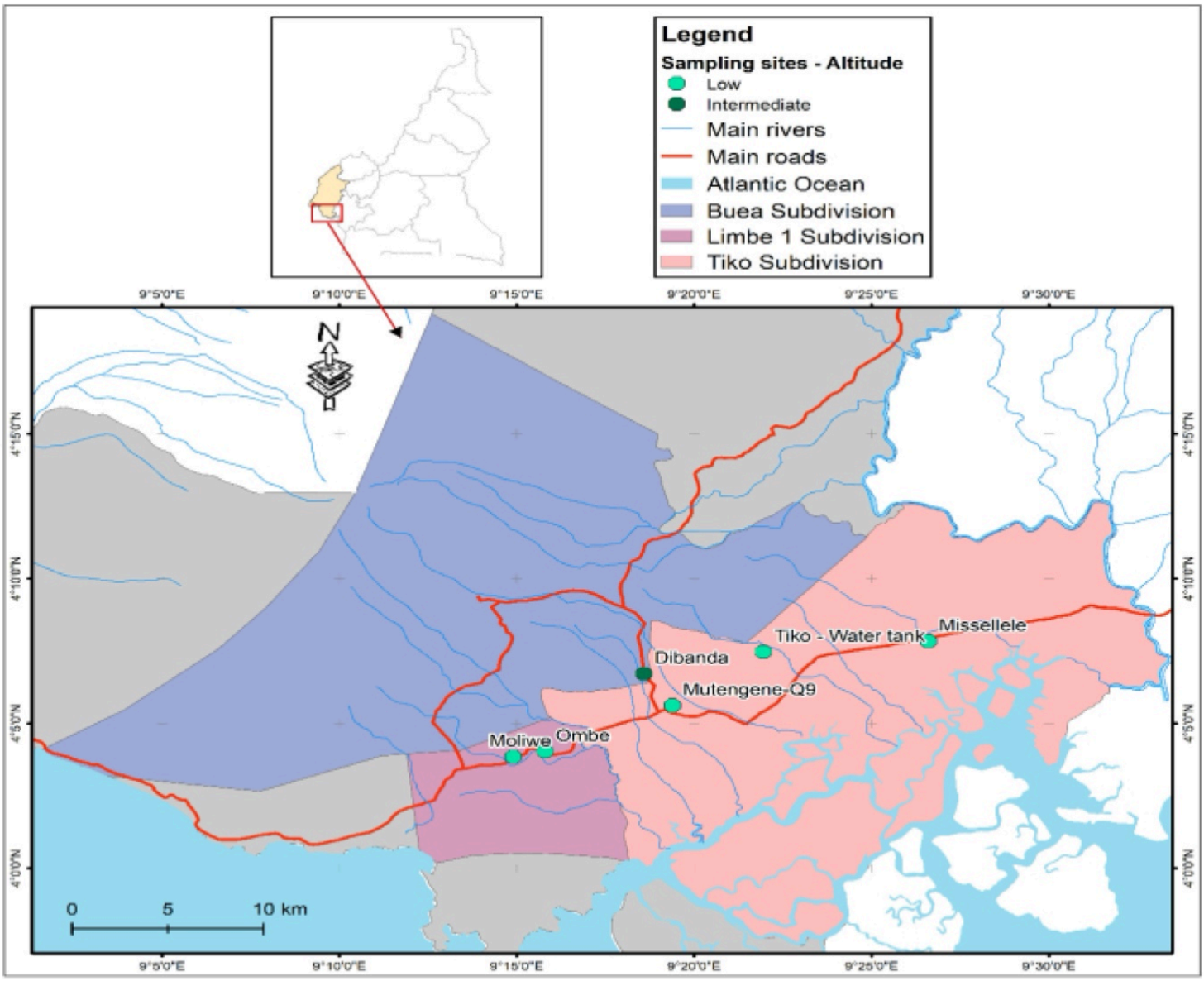

Figure 1

Map of sample collection sites (dots colour-coded by altitude) along the slope of Mount Cameroon.

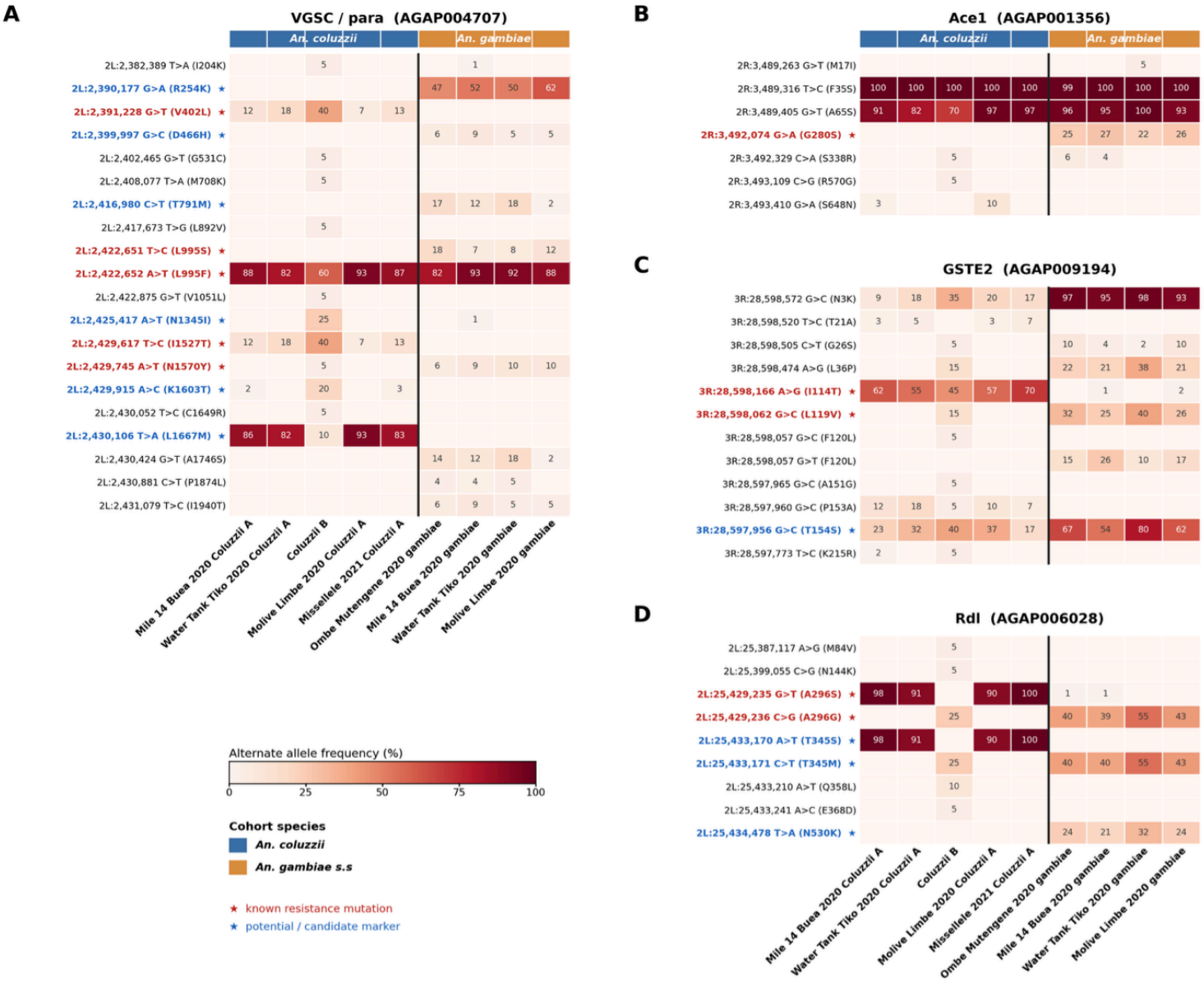

Figure 2

A. Frequencies of single nucleotide polymorphisms of the Vgsc/para gene. B. Frequencies of single nucleotide polymorphisms of the *Ace1* gene. C. Frequencies of single nucleotide polymorphisms of the *GSTE-2* gene. D. Frequencies of single nucleotide polymorphisms of the GABA/Rdl gene

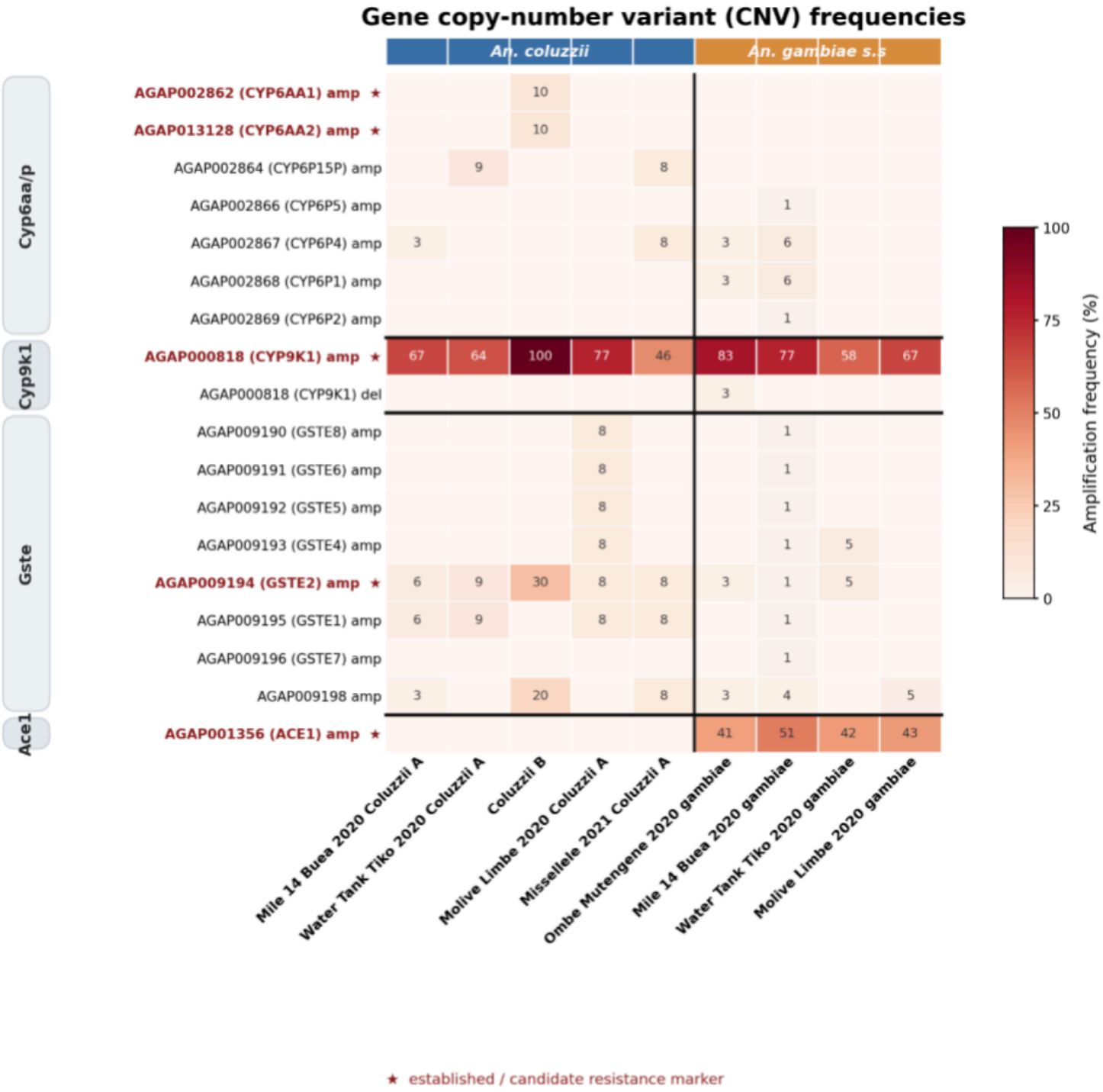

Figure 3

Frequencies of copy number variations across Ace-1, Gste-2 and cytochrome P450 luci. Frequencies of variants amplifications and deletions on the *Cyp6aa/p*gene cluster, *CYP9k1* gene cluster, *Ace-1* gene and in the *Gste* gene.

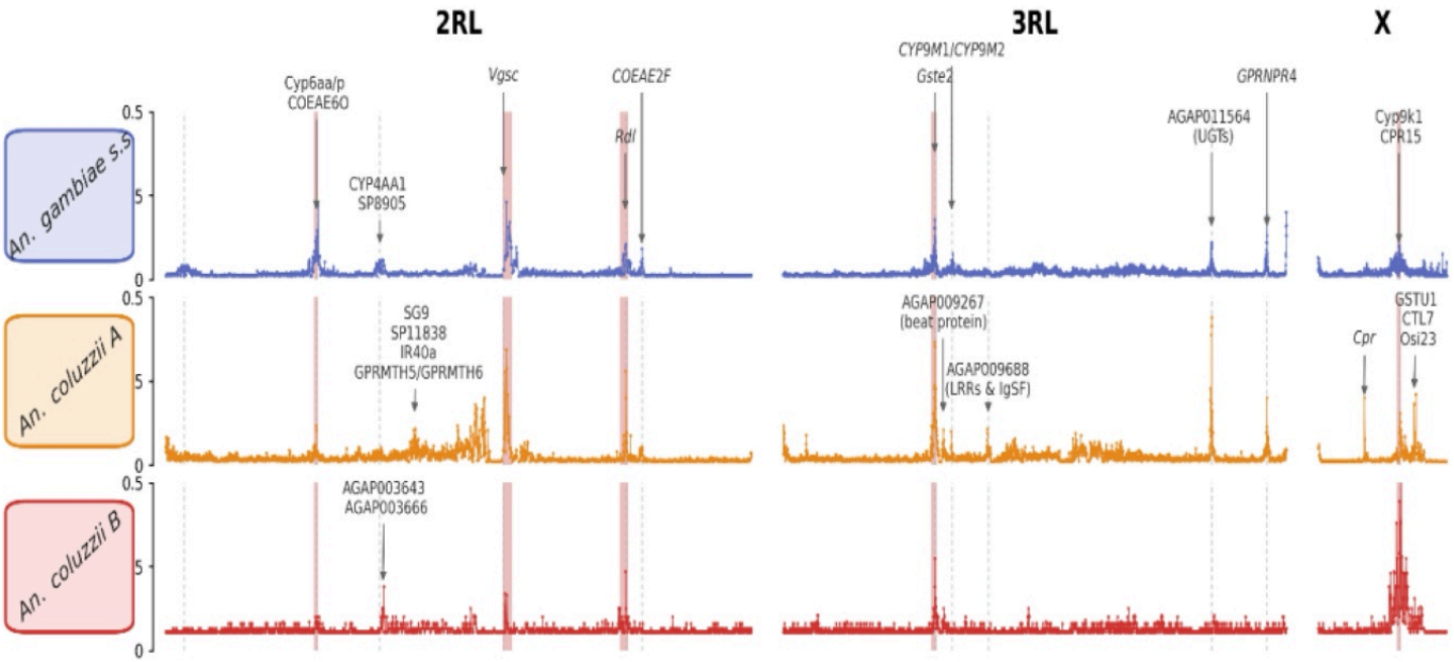

Figure 4

A. H12 analysis on chromosome arm 3L using windows of 1000 bp on all *An. coluzzii* A cohorts. B. H12 analysis on chromosome arm 2L using windows of 1000 bp on all *An. coluzzii* A cohorts. C. H12 analysis on chromosome arm 2R using windows of 1000 bp on the *An. coluzzii* B cohort

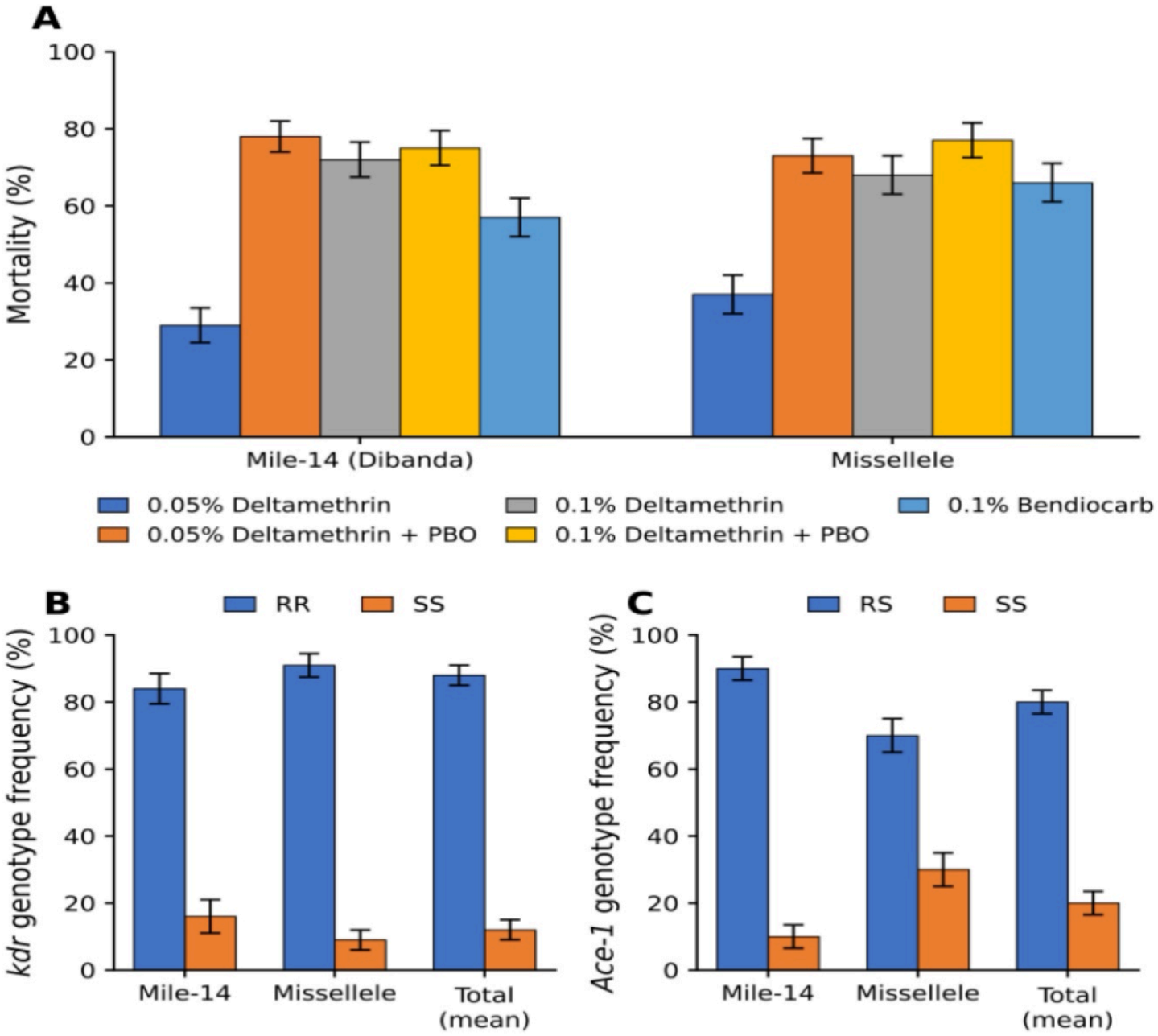

Figure 5

A. Mortality status of *An. gambiae* complex from low and intermediate altitudes around the slopes of mount Cameroon. B. *An. gambiae s.s Kdr-West* genotypic frequency at low (Missellele) and intermediate altitude (Mile 14 Buea). **C.** *An. gambiae Ace-1*genotype frequencies in Missellele (low) and Mile 14 (intermediate altitude) respectively.

Supplementary Files

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