

SUPPLEMENTARY INFORMATION S1

Structure of *APL1* alleles in Ngousso

An insertion at the 5' end of the *APLIA*² variant encodes multiple repeats or variations of the peptide PANGGL, an amino acid repeat region normally only seen in *APLIC*. In addition, *APLIA*² has a 144 bp in-frame deletion that reduces the LRR domain by 48 aa as compared to the LRR domains found in the other two *APLIA* alleles.

At the 3' end, two small insertions, 8 and 9 bp in length, respectively, are diagnostic for *APLIA* alleles in Ngousso and neither is found in the PEST reference [1]. The 8bp insertion point is situated at position 2L:41271509-41271510 in the *APLIA* reference sequence and results in a frame-shift that suppresses the stop codon (2L:41271509-41271511). The insertion of 9 bp is situated at the very 3' end of the gene at position 2L:41270995-41270996. The *APLIA*¹ allele has only the 8 bp insertion, which extends the ORF to a stop codon at position 2L:41270938-41270940, leading to a protein with a C-terminal coiled-coil domain. The *APLIA*² allele, in addition to the N-terminal PANGGL repeat, has both, the 8 and 9 bp insertion. Here the 8 bp insertion is followed 69 bp further downstream (position 2L:41271439-41271441) by a premature stop codon due to a point mutation, resulting in an *APLIA*² protein lacking the C-terminal coiled-coil region. The *APLIA*³ allele only displays the 9 bp insertion at the 3' end. This allele is similar to the *APLIA* allele of PEST, encoding a protein structured as the *APLIA*¹ form at its N-terminus but lacking the coiled-coil domain (Supplementary Figure 2A).

*APLIB*² and *APLIB*³ variants have a 3 bp insertion (2L:41267132-41267133). The corresponding *APLIB* alleles form a haplotype with the *APLIA* variants that do not express the coiled-coil domain, *APLIA*² and *APLIA*³, respectively.

In addition to the observed variations in the PANGGL encoding regions, the *APLIC* alleles of the *APL1* haplotypes 2 and 3 (variants *APLIC*² and *APLIC*³, respectively) harbor a 7 bp deletion (at position 2L:41257813..41257819) compared to *APLIC*¹ and to the PEST reference genome sequence. This deletion, located 57 bp after the stop codon of *APLIC* (2L:41257877..41257879), does not change the gene product.

Homology of *APL1* proteins

The three *APLIA* proteins display a high level of homology. The LRR regions, the common part of all three *APLIA* alleles, show 87% amino acid identity. Furthermore, the *APLIA*³ protein is 94% identical to the *APLIA*¹ variant.

Concerning the *APLIB* products, if the premature stop codon in the *APLIB*³ allele is ignored and its coding sequence is virtually extended to the same length as the other *APLIB* variants, the three products share 93 to 97% amino acid identity in pair-wise comparisons. Similar results are found for the *APL1C* proteins. In addition, as illustrated by polydot graphs [2] the *APLIA*, *B* and *C* genes share extensive amino acid identity to each other (Figure S5).

Frequencies of *APLIA* alleles and genotypes in the Ngousso colony

Using the *APLIA*-RFLP typing assay, we determined allele and genotype frequencies in Ngousso mosquitoes from three independent infection series (with n=300 mosquitoes per replicate). The Ngousso colony displays different frequencies for the six *APLIA* genotypes, but despite variations in the genotype distributions, the frequencies of the three different *APLIA* alleles varied only slightly between the experiments (Figure S4). The three types of *APLIA* homozygotes are present in different frequencies in the Ngousso population with *APLIA*¹/*APLIA*¹ homozygotes showing the lowest prevalence (Figure S4B). In all three experiments the genotypes were found to be in Hardy Weinberg equilibrium.

REFERENCES

1. Vectorbase <http://www.vectorbase.org/>.
2. EMBOSS <http://emboss.sourceforge.net/>.