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Unstable laboratory *Wolbachia* strain *w-Anga* is negatively correlated with *Plasmodium falciparum* in wild malaria vectors

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Spread of insecticides resistance threatens the control of malaria. In this context, biological control using an endosymbiotic bacterium *Wolbachia* is being explored as a complementary method for its control. However, for optimal use of this bacterium in biocontrol strategies, it is imperative to characterize it. So, *Anopheles gambiae* complex mosquitoes were collected, morphologically identified, then blood fed and gravid female mosquitoes oviposited individually. After oviposition, the species of parent was molecularly determined, along with their *w-Anga* infection status. Additionally, we performed *16SrRNA* gene sequencing of *w-Anga*-positive mosquitoes to determine their phylogeny. Finally, we amplified gene encoding the *circumsporozoite protein* to determinate their *Plasmodium falciparum* infection status and assessed the stability of *w-Anga* transmission of positive females and their offspring. From the results obtained, our *w-Anga* strains cluster with other *Wolbachia* Supergroup B strains. However, the prevalence of *Plasmodium falciparum* infection was lower in *Wolbachia*-infected females (4.59%) than in those uninfected (22.02%). Furthermore, the transmission frequency of this bacterium in infected *Anopheles coluzzii* females of the F0 generation to F1 offspring was 10.64% and 16.67% from infected females of the F1 generation to F2 offspring. This study results will serve as preliminary data for the possible use of *Wolbachia* in malaria control.

Keywords Malaria, *Anopheles gambiae* complex, *w-Anga*, Phylogeny, Stability, *Plasmodium falciparum*

Abbreviations

An. arabiensis	Anopheles arabiensis
An. coluzzii	Anopheles coluzzii
An. gambiae	Anopheles gambiae
CSP	Circumsporozoite protein
CTAB	Cetyl Trimethyl Ammonium Bromide
DNA	DeoxyRibonucleic Acid
PCR	Polymerase Chain Reaction
P. falciparum	Plasmodium falciparum
rRNA	ribosomal Ribonucleic Acid
SIT	Sterile Insect Technique
w-Anga	*Wolbachia* strain originated from *Anopheles gambiae* mosquitoes.
wAlbB	Strain B of *Wolbachia* originated from *Aedes albopictus* mosquitoes.
wAu	*Wolbachia* from Australia.
wMelPop	*Wolbachia* from *Drosophila melanogaster*.

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Wolbachia is an alphaproteobacterium, non-spore forming and Gram-negative common to several arthropod species including 70% of insects, as well as some nematodes[1]. It is an endosymbiotic bacterium currently being tested in biological vector control approaches[1].

These control approaches, based on mosquito population replacement or suppression strategies, possess two essential properties: cytoplasmic incompatibility (CI) and the ability to inhibit pathogens, particularly blocking viral replication.

Cytoplasmic incompatibility is a property of *Wolbachia* that enables its spread within uninfected mosquito populations through a sterility syndrome. This occurs when a *Wolbachia*-infected male mates with an uninfected female (unidirectional CI) or when both individuals are infected with different *Wolbachia* strains (bidirectional CI), initially reducing wild mosquito populations (a suppression strategy employed through the Sterile Insect Technique (SIT)). This reduction provides a selective advantage to *Wolbachia*-infected females, which produce numerous infected offspring, ultimately replacing the wild mosquito population.

Indeed, previous studies involving field releases of *Aedes aegypti* mosquitoes trans-infected with different strains of *Wolbachia* (*wMelPop*, *wAlbB*, *wAu*) carried out in Australia, Malaysia and Brazil have shown stability and high prevalence of the bacterium in *Aedes sp*. populations. This led to a reduction in the incidence of Dengue fever cases in these localities, suggesting the use of this strategy in the eradication of this arbovirosis[2–4]. The success in exploiting the potential of *Wolbachia* against *Aedes aegypti* mosquito led some researchers to consider the use of this bacterium against *Anopheles* mosquitoes, the malaria vector. Unfortunately, the results of these studies were not fruitful and led the authors to consider that *Anopheles* mosquitoes cannot carry *Wolbachia* infection[5,6]. However, this view was reversed in 2014 when the first evidence of natural *Wolbachia* infections was found in *Anopheles gambiae* and *Anopheles coluzzii* collected in Burkina Faso: this bacterial strain was named "*w-Anga*" and belongs to a new potential phylogenetic supergroup specific to *Anopheles*, which is related but distinct from supergroups A and B associated with arthropods and evolutionarily linked[7]. Subsequently, similar evidence of natural infections of *Anopheles* with *Wolbachia* was observed across several countries in Africa[8,9]. In 2017 a natural *Wolbachia* strain different from that in Burkina Faso was identified in *An. gambiae* complex mosquitoes of Mali[8]. Furthermore, in 2019, a study carried out in Gabon confirmed the presence of natural *Wolbachia* infections in sixteen mosquito species, including all the main malaria vectors in Central Africa (*An. gambiae*, *An. coluzzii*, *An. funestus*, *An. nili* and *An. moucheti*)[10] .

In order to use these natural *Wolbachia* strains in vector control strategies against *Anopheles* mosquitoes, it would be necessary to have knowledge on the phylogeny of *w-Anga* strain used and the stability of its transmission over the generations of mosquitoes. It would also be necessary to assess the potential impact of the bacterium on the prevalence of *Plasmodium* in *Anopheles* mosquitoes.

Results

Frequency of natural *w-Anga* infections according to localities, collection periods and mosquito species

The current study showed that the overall frequency of *w-Anga* infection among the collected mosquitoes was 13.84%. This frequency varied according to localities, collection periods and mosquito species. Also, the frequency of natural infection with *w-Anga* was 3.88% (8/206) for Soumousso, 13.93% (51/366) for VK5 "Vallée du Kou Sector 5" and 24.23% (47/194) for VK7 "Vallée du Kou Sector 7". However, this frequency was statistically higher for mosquitoes originating from VK7 compared to those originating from VK5 ($P = 0.00676$) and Soumousso ($P < 0.0001$). Furthermore, the prevalence of *w-Anga* infection varied from 2.4% (3/125) for June, 4.41% (19/431) for July, 1.52% (1/66) for August and 57.64% (83/144) for September over all study sites. However, this frequency of infection was higher for September compared to June, July and August with a statistical value of $P < 0.001$. Among the mosquitoes collected during our study, *An. coluzzii* was the most infected species with *w-Anga* at 12.79% (98 /766) frequency, followed by *An. gambiae* with a frequency of 1.04% (8/766). However, no infection with the bacterium was recorded in *An. arabiensis*. Due to the high frequency of *w-Anga* within wild *An. coluzzii*, we performed the rest of the experiment on this species.

Phylogenetic analysis of *w-Anga* strains

Phylogenetic analysis of *16SrRNA* sequences used in our study was based on 33 sequences from NCBI, 4 from our studies and a positive control of the *Wolbachia* strain *wAlbB*. This resulted in a midpoint-rooted phylogenetic tree (Fig. 1). This tree showed that the 16 S sequences of our *Wolbachia* strains cluster with other Supergroup B strains such as *wAnga VK5 3.1b* for our sample *wAnga03*, *wAns* for *wAnga02* and *wAnCa* for *wAnga02* & *wAnga04* (97–99% nucleotide identity).

Impact of natural *w-Anga* infections on *Plasmodium falciparum* parasite presence within wild caught *Anopheles coluzzii* mosquitoes

From our analysis, it appears that the prevalence of *P. falciparum* infection was lower in *w-Anga* positive *An. coluzzii* females compared to *w-Anga* negative females. Indeed, only 5/109 (4.59%) *w-Anga* positive females were infected with *P. falciparum* compared to 24/109 (22.02%) *w-Anga* negative females that were infected with *P. falciparum*. Furthermore, the correlation test showed a significant negative correlation ($P = 0.0003309$) between natural *w-Anga* infection and *P. falciparum* infection (Fig. 2).

Impact of natural *w-Anga* infections on fecundity and fertility of wild caught *Anopheles coluzzii* mosquitoes

The impact of natural *w-Anga* infections on the fecundity and fertility of 107 *An. coluzzii* females (divided into 34 *w-Anga*-infected females and 73 uninfected females) was investigated by comparing the mean number of eggs and larvae of *w-Anga*-infected females with those of uninfected females. Thus, the mean number of eggs

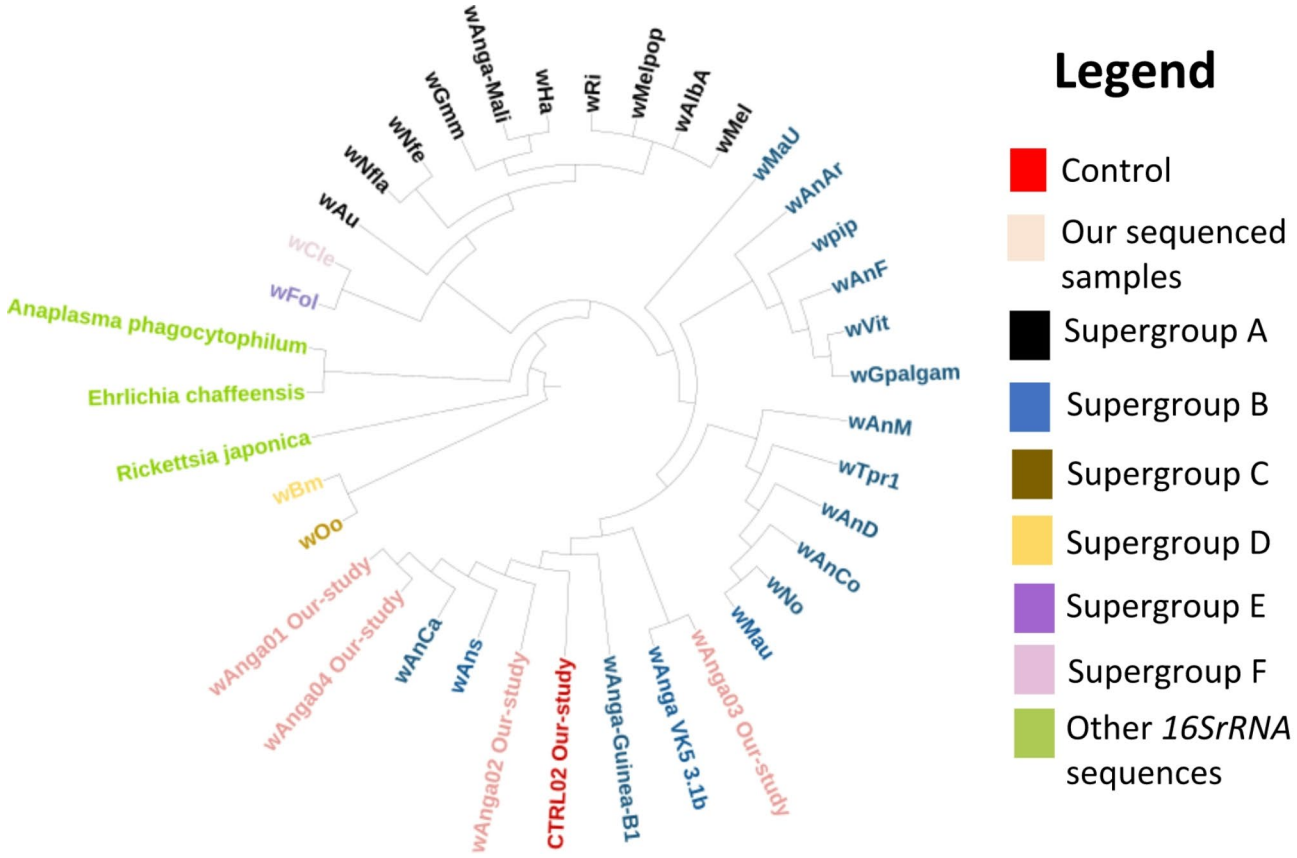

Fig. 1. Mosquito samples collected have been to *16SrRNA* Nested PCR to obtain amplicons positive for *Wolbachia* strain *w-Anga*. These amplicons underwent *16SrRNA* gene sequencing by Sanger technology using GENEWIZ/AZENTA's internal formulation of BigDye V3 chemistry on an ABI3730xl sequencer. The sequences obtained were subjected to phylogenetic analysis, which involved aligning them with data available in the GenBank database using BLAST. Following the BLAST alignment, we established a phylogenomic relationship between our samples (five strains, including four derived from our *16SrRNA* amplicons and a positive control of *wAlbB* strain) and 33 *Wolbachia* strains from NCBI by constructing a phylogenetic tree using the "Fasttree" program in the "Jupyter Notebook" application, version 4.2.4. This phylogenetic tree was visualized and annotated using "ITOL v6.9.1." The assembly names on the phylogenetic tree were color-coded based on the identity of the supergroups (A-F) and other *16SrRNA* sequences.

laid by *w-Anga* infected females was statistically higher ($P = 0.019$) than that of the uninfected. It was 56.63 ± 4.82 eggs for the infected and 42.42 ± 2.55 eggs for the uninfected. The mean number of larvae was statistically higher ($P = 0.0022$) in *w-Anga* infected females than in uninfected females. The average number of larvae was 39.81 ± 4.90 for infected females and 23.66 ± 1.91 for uninfected females (Fig. 3).

Stability of *w-Anga* over generations in *Anopheles coluzzii* mosquito lines

Out of the 223 females that laid eggs individually, only 32 (14.35%) were infected with *w-Anga* (F0 generation). From the F1 offspring of infected females, we obtained 329 mosquitoes, including 169 males and 160 females. For this F1 generation, *w-Anga* was detected in 35 mosquitoes, including 9 males and 26 females. The frequency of transmission of *w-Anga* from infected females of the F0 generation to F1 offspring was therefore relatively low (10.64%; Table 1). The F2 offspring of infected F1 females produced 18 mosquitoes, divided into 12 males and 6 females. For this generation, *w-Anga* was detected in 3 mosquitoes, including 1 male and 2 females, with a transmission frequency of 16.67% (Table 1). However, there was no statistically significant difference between the frequency of *w-Anga* infection in the F1 generation and that in the F2 generation ($P = 0.696$).

Discussion

The detection of *16SrRNA* sequences specific to *w-Anga* bacterial strain in two of the three *An. gambiae sensus lato* species, on different ecological sites and during the rainy in Western Burkina Faso suggests that wild *Anopheles* mosquitoes naturally harbor *Wolbachia*. This suggests that more efforts should be made to exploit this infection in malaria control. However, the frequencies of *w-Anga* infection recorded in the two collection sites were relatively low compared to frequencies found previously in the same localities[11]. Thus, these low infection frequencies could be due to the presence of extremely low densities of *Wolbachia* in *An. gambiae complex* mosquitoes in nature. Furthermore, the variation of *w-Anga* infection frequencies within *An. gambiae*

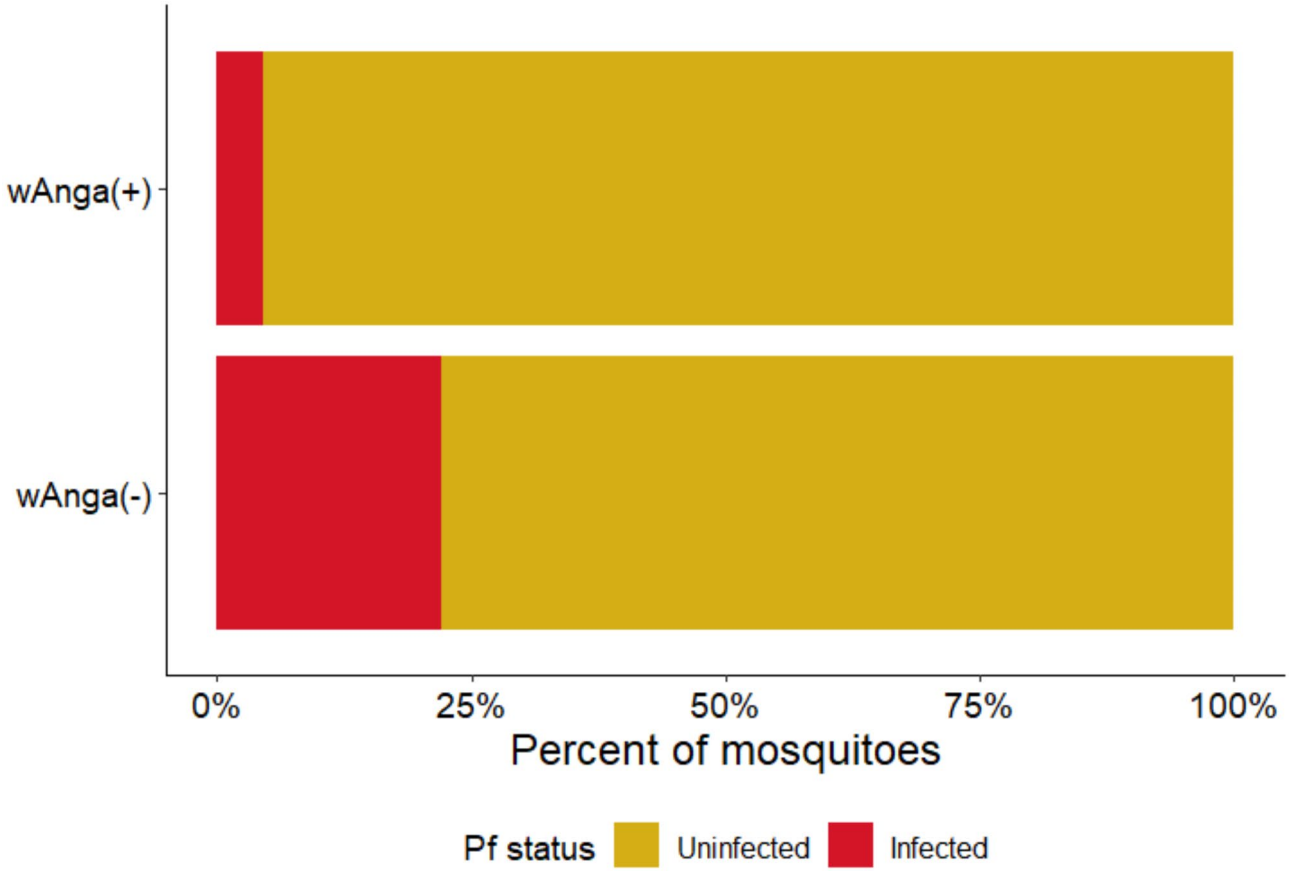

Fig. 2. *Plasmodium falciparum* infection status based on *w-Anga* infection within wild caught *Anopheles coluzzii*. The proportions of *Anopheles coluzzii* mosquito females infected with *Plasmodium* among *w-Anga*-positive females (5/109) and among *w-Anga*-negative females (24/109) are shown in red (with a statistically significant difference between the two proportions, $P = 0.0003309$).

sensus lato in our study over the collection period may be caused by ecological factors including temperature variations. Indeed, previous studies have reported a reduction in *Wolbachia* density in *Aedes albopictus* and *Aedes aegypti* following a temperature increase[12,13]. Referring to the initially isolated strains from one of our sites and considering their potential evolution and bacterial diversity, a deeper analysis of the genetic identity of our *Wolbachia* strains is crucial to determining their phylogenetic relationships with previously identified *Wolbachia* strains.

The different *Wolbachia* strains isolated in our study from *An. gambiae* complex mosquitoes, particularly from *An. coluzzii*, one of the species within this complex, belong to supergroup B.

Furthermore, referring to *Wolbachia* strain "*wAnga VK5 3.1b*" which was previously isolated in 2014 from *Anopheles* mosquitoes at one of our collection sites "VK5"[7], we report identity percentages of 99.20% for our sample *wAnga01*, 99.74% for *wAnga 02*, 99.73% for *wAnga03* and 99.19% for *wAnga 04*. These facts allow us to assert that the *wAnga* strains isolated from our collected mosquitoes are similar to those isolated in 2014 in the same locality with the difference of a few nucleotides. This slight nucleotide variation between *wAnga* strains could be explained by the occurrence of mutations over time. Our *w-Anga* strain is similar to "*wAnga VK5 3.1b*" strain, what about its transmission within this species under laboratory conditions?

The stable transmission of *w-Anga* infection is theoretically expected to reach a frequency of 100% across generations[14]. However, in our study, we observed low frequencies, indicating that the transmission of *w-Anga* infection was not stable between successive generations (F0–F1 and F1–F2) of *An. coluzzii* mosquitoes under laboratory conditions. Several hypotheses may explain this instability. The first hypothesis is based on the presence of residual genomic DNA from dead bacteria in certain females classified as positive, which would limit the transmission of bacterial infection to their offspring[15,16]. A second hypothesis could be contamination of the midgut lumen of *An. coluzzii* females by the *Wolbachia* bacterium or traces of its DNA[17]. Such contamination might originate either from plants previously fed on by insects carrying *Wolbachia*[17] or from the cohabitation of *Anopheles* and *Aedes* mosquitoes infected with *Wolbachia* at collection sites, for example, in water storage containers where the simultaneous presence of larvae from both species is associated with the detection of *Wolbachia*[18]. In such cases of contamination, *Wolbachia* would not be present in the germ cells of mosquitoes, preventing transovarian transmission within *Anopheles* lineages[19]. Finally, another hypothesis could involve mutual exclusion between *Wolbachia* and certain bacterial genera, such as *Asaia spp.*, in the reproductive organs

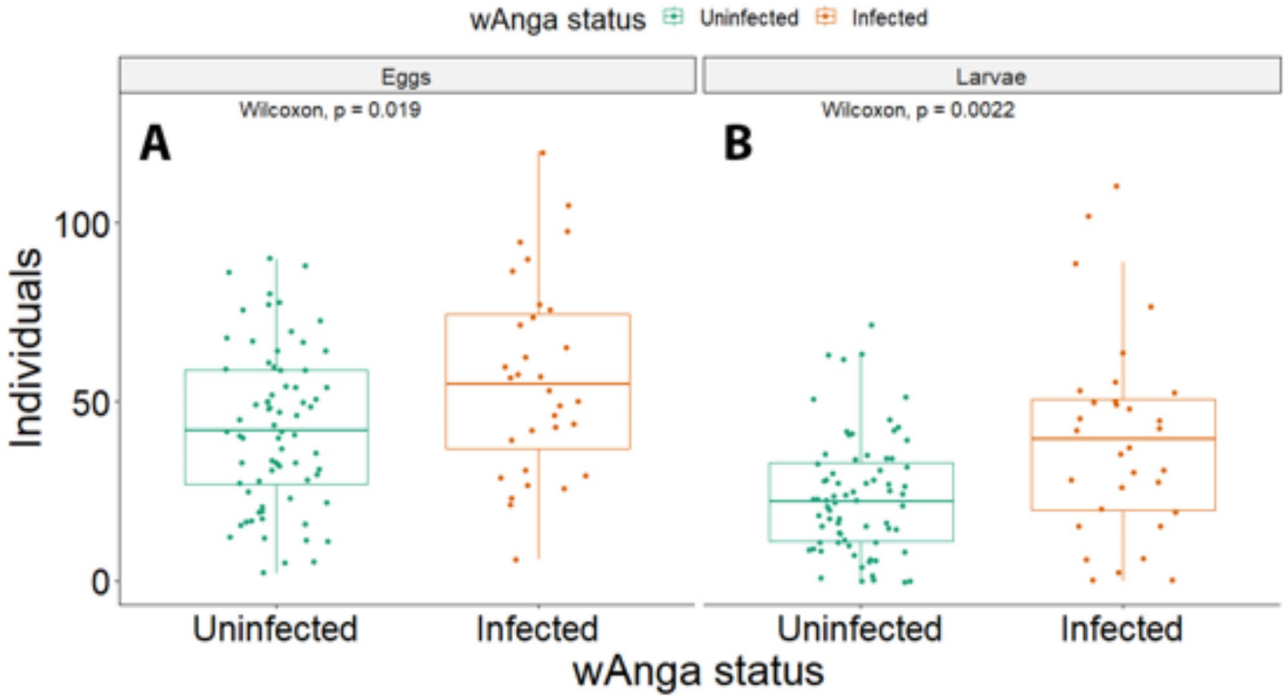

Fig. 3. Impact of natural *w-Anga* infections on fecundity and fertility of wild caught *Anopheles coluzzii* mosquitoes. (**A**) Average number of eggs uninfected and infected to *w-Anga*. (**B**) Average number of larvae uninfected and infected to *w-Anga*.

	Generation		
***w-Anga* status**	**F0**	**F1**	**F2**
Uninfected	191/223 (85.65%)	294/329 (89.36%)	15/18 (83.33%)
Infected	32/223 (14.35%)	35/329 (10.64%)	3/18 (16.67%)

Table 1. Stability of *w-Anga* over F0-F1 and F1-F2 generations adults wild caught *Anopheles coluzzii*.

of vector mosquitoes, a phenomenon recently demonstrated in a study[20]. Moreover, given the instability of *w-Anga* infection transmission in our *An. coluzzii* populations, an essential question arises: what is the impact of this bacterium on the fecundity and fertility of these mosquito populations? From the study of the impact of *w-Anga* on the fecundity of *An. coluzzii* in the laboratory, we found that the average number of eggs was statistically higher in infected females than in uninfected ones. A similar fact was observed in *Drosophila melanogaster* by Eva Fast et al.[21]. They found that *Wolbachia* infected flies laid four times more eggs than uninfected flies. They explained this phenomenon by the stimulation of germline stem cell division in infected females by *Wolbachia*, but also by the reduction of "programmed" death by *Wolbachia* in the organ where the eggs develop[21]. Furthermore, our study on *An. gambiae* complex mosquitoes as well as that of Dobson et al., on *Aedes albopictus* have shown that the presence of *Wolbachia* affects the fertility of their host[22]. This is because the average number of larvae hatched from the eggs of *Wolbachia* infected females was statistically higher than that of uninfected females.

Beyond its impact on fecundity and fertility of *An. coluzzii*, *w-Anga* may impact the development of *Plasmodium* in the mosquito as we found that the prevalence of *P. falciparum* infection was lower in *Wolbachia*-infected females than in those uninfected. Thus, this impact could be the inhibition of *Plasmodium* by *w-Anga*. Based on this hypothesis, we wonder about the mechanisms by which this inhibition could be achieved. One of these mechanisms would be the induction of potent anti-pathogenic effects following the activation of the immune system by the *w-Anga* bacterium. This activation of the immune system could then lead to an inhibitory effect on *Plasmodium* infection in the mosquito[23,24]. Another mechanism of inhibition would be the establishment of competition between *w-Anga* bacterium and *Plasmodium* parasite for some cellular components, such as cholesterol and other fatty acids of the host that are essential for them[25]. Also, competition would occur between *w-Anga* and *Plasmodium* for nutrient resources contained in the blood meal of the mosquito. Indeed, following a blood meal, large quantities of resources such as lipoproteins are circulated. These same lipoproteins are necessary for *Plasmodium* to escape the mosquito's immune system[26]. However, their potential diversion by *Wolbachia* could lead to increased rates of *Plasmodium* destruction by the immune system. In addition, bacteria from the mosquito microbiome could compete with *Wolbachia* following a blood meal[27] and divert the

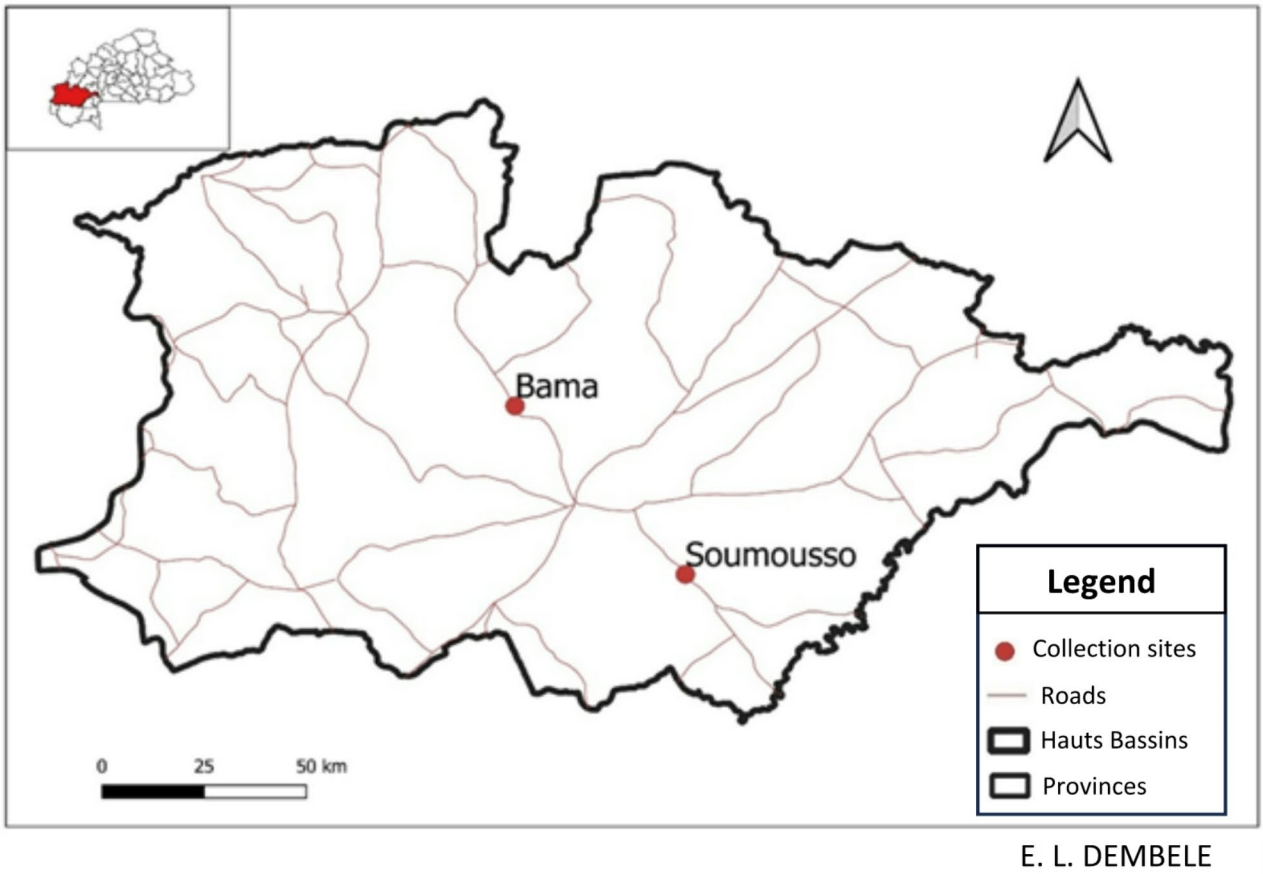

Fig. 4. Hauts Bassins region in Burkina Faso representation, in which the two collection sites Bama and Soumousso are located in red dots.

development resources of the *Plasmodium*. It is not clear whether the negative correlation we observed between *Wolbachia* and *Plasmodium* infection is driven by *Wolbachia* or *Plasmodium* prevalence. However, *Wolbachia* is typically received vertically, and so would be present before female mosquitoes are challenged with *Plasmodium* following maturation and blood-feeding.

Methods

Field mosquito collection, morphological identification and molecular differentiation of *Anopheles gambiae* complex species

Mosquito collections were carried out monthly during the rainy season from June to September 2021. They consisted of capturing male *Anopheles* as well as blood fed and gravid female using the Residual Fauna Capture method in Bama "Vallée du Kou" (11°23'N, 4°24'W) and in Soumousso (11°04'N, 4°03'W); two localities located in Western Burkina Faso (Fig. 4)[28]. "Vallée du Kou" is a rice-growing area with an annual rainfall of about 1200 mm/year. It is characterized by highly productive and almost permanent mosquito breeding grounds due to the presence of the Kou River. Malaria transmission in this locality is essentially ensured by *An. gambiae sensus lato* and secondarily by *An. funestus*[28].

As for Soumousso, it is a Savannah area with annual rainfall between 1000 and 1200 mm. It is characterized by the presence of a semi-permanent stream that feeds mosquito breeding grounds. In this locality, the main species ensuring the transmission of malaria are *An. coluzzii*, *An. gambiae*, *An. arabiensis*, *An. funestus* and *An. nili*, with *An. gambiae* majority year round[28].

A total of 766 mosquitoes were collected from dwellings and enclosures at the study sites. Wild caught mosquitoes were brought to the IRSS/Centre Muraz insectarium and identified morphologically using the Gillies & Coetzee dichotomous key. Females of the *An. gambiae* complex were selected and oviposited individually. The genomic DNA of those that laid eggs was extracted with Cetyl Trimethyl Ammonium Bromide (CTAB) on whole mosquitoes following the protocol of (Myriam & Céline (2003)). Subsequently, the DNA extracts were amplified by a SINE 200X PCR approach following the protocol of (Santolamazza *and al.*, (2008)), using a pair of primers (S200×6.1 F: TCGCCTTAGACCTTGCGTTA; S200×6.1R: CGCTTCAAGAATTCGAGATAC) in order to identify their species. This SINE 200X PCR was performed under the following conditions: 10 min at 94 °C for denaturation followed by 35 cycles of 94 °C during 30 s, 54 °C during 30 s, and 72 °C during 1 min, with a final extension step at 72 °C during 10 min. Following amplification, electrophoresis was performed on

a 2% agarose gel and bands were observed at 479 bp for *An. coluzzii*, 249 bp for *An. gambiae sensus stricto* and 223 bp for *An. arabiensis*.

Molecular detection of the *w-Anga* strain in wild caught *An. gambiae* complex mosquitoes

The detection of *w-Anga* was performed by a Nested PCR technique targeting a variable region of the conserved *Wolbachia 16 S rRNA* gene. For this amplification, two primer pairs were used (W-Spec F: CATACCTATTC GAAGGGATAG; W-Spec R: AGCTTCGAGTGAAACCAATTC) for the first phase of the amplification and (16SNF: GAAGGGATAGGGTCGGTTCG; 16SNR: CAATTCCCATGGCGTGACG) for the second primer set. Nested PCR was performed under the following conditions: 5 min at 95 °C for denaturation followed by 2 cycles of 2 min at 95 °C (a), 1 min at 60 °C (b), 1 min at 72 °C (c), followed by 30 s at 95 °C, 1 min at 60 °C, 45 s at 72 °C and repeat a, b and c in 40 cycles, with a final extension step at 72 °C for 5 min for the first primer set and 15 min at 95 °C for denaturation, followed by 35 cycles of 15 s at 95 °C, 15 s at 60 °C and 25 s at 72 °C, with a final extension step at 72 °C for 5 min for the second primer set. Electrophoresis of the second primer set amplicons was performed on a 1% agarose gel and bands were obtained at 412 bp for bacterial positive samples.

16SrRNA gene sequencing and phylogenetic tree realization

The phylogenetic tree realization of *w-Anga* positives samples was performed on conserved *16SrRNA* sequences using Sanger technology. To do this, ten *w-Anga* PCR products were purified using Wizard® SV Gel and PCR Clean-Up purification kit, (Promega, USA). After purification, the samples have been sent to GENEWIZ from Azenta to perform *16SrRNA* Sanger sequencing using an GENEWIZ-internal formulation of BigDye V3 chemistry on a ABI3730xl sequencer. At the end of sequencing, the data obtained were compared with the data available in the GenBank database (www.ncbi.nlm.nih.gov) using the BLAST (Basic Local Alignment Search Tool) search under default parameters and multiple sequence comparison. This alignment was used to confirm that the reads produced were the *Wolbachia 16SrRNA* gene. As a result of the BLAST alignment, we selected 30 *Wolbachia* sequences belonging to supergroups A to H and 03 other *16SrRNA* sequences with which we created the phylogenetic tree using the web application Jupyter Note Book version 4.2.4. On Jupyter Note Book, all of these sequences were used as imput into the program Mafft version v7.526 and aligned using the default parameters. After alignment, the phylogenetic tree was generated using Fasttree, visualized and annotated by ITOL v6.9.1.

Determination of the spatio-temporal frequency of the bacterial strain *w-Anga* in *Anopheles gambiae* complex mosquitoes

The spatio-temporal frequency of *w-Anga* was determined by performing a molecular screening of the bacterium from June to September 2021 at different study sites. This screening consisted of determining the proportion of *w-Anga* using the Nested PCR technique for the detection of the said bacterium in *An. gambiae* complex mosquitoes collected at VK5, VK7 (Two localities of Vallée du Kou) and Soumousso. Subsequently, the species of these mosquitoes was determined by SINE 200X PCR technique.

Molecular detection of *Plasmodium falciparum* between wild caught of *Anopheles coluzzii* mosquitoes infected and uninfected to *w-Anga*

The impact of *w-Anga* on the presence of wild *P. falciparum* was assessed by determining the status of *P. falciparum* infection between wild caught *An. coluzzii* females carrying *w-Anga* and those not carrying the bacterium. The detection of *P. falciparum* consisted of the search for sporozoites by a classical PCR technique using a pair of primers (Pf1: GGAATGTTATTGCTAACAC; Pf2: AATGAAGAGCTGTGTATC) targeting the gene coding for the parasite-specific *circumsporozoite protein* (*CSP*). This PCR was performed under the following conditions: 3 min at 94 °C for denaturation, followed by 35 cycles of 30 s at 94 °C, 1 min 15 s at 56 °C and 1 min at 68 °C, followed by 10 min at 68 °C. Electrophoresis of the amplicons was performed on a 2% agarose gel and bands were observed at 501 bp for sporozoite positive samples.

Determination of the impact of natural *w-Anga* infections on fecundity and fertility of female mosquitoes *Anopheles coluzzii*

The impact of natural infection with *w-Anga* on fecundity and fertility of *An. coluzzii* females was assessed by comparing fecundity and fertility parameters in *w-Anga* positive F0 females versus females without *w-Anga*. For the assessment of fecundity, egg counts of *w-Anga* positive and *w-Anga* negative females were carried out using a hand-held counter by observation with a hand magnifying glass. For fertility, a count of larvae hatched from eggs (previously counted) of *w-Anga* infected females as well as uninfected females was carried out. This count was carried out using a transfer pipette.

Laboratory monitoring of the transmission stability of natural *w-Anga* infection in *Anopheles coluzzii* mosquito populations

The transmission of *w-Anga* in *An. coluzzii* mosquitoes was monitored in the laboratory (in a controlled enclosure under conditions of 26 ± 2 ℃ temperature and 80 ± 2% relative humidity) over two generations to verify the stability of the bacterium's transmission in wild-caught mosquitoes. This activity, based on the determination of the generational infection rate (F0 females to F1 offspring and F1 to F2) to the bacterium, involved selecting cups containing the eggs of *w-Anga*-positive *An. coluzzii* females and hatching them. On emergence, a total of 329 offspring were obtained for the F1 generation and 18 mosquitoes for the F2 generation. All offspring from these two generations were tested for *w-Anga* using Nested PCR to verify the stability of this bacterial transmission.

Data analysis

In this study, data analysis and graphs realization were conducted using R (R Core Team, 2021), RStudio (Rstudio Team, 2021), and the packages reshape2 (Wickham, 2007), multcomp (Hothorn, 2008) tidyverse (Wickham, 2019), ggplot2 (Wickham, 2016), scales (Wickham, 2020), broom (Robinson, 2022), and ggpubr (Kassambara, 2020). The tables were made with Microsoft Word 2016. The Chi-squared Pearson and Wilcoxon tests were used for comparisons. p-values < 0.05 were considered statistically significant.

Conclusion

The development of new approaches to malaria vector control is imperative. For this purpose, *Wolbachia* bacteria could be a promising alternative for vector control. This study shows that *Anopheles* mosquitoes from "Vallée du Kou" and Soumousso harbor *w-Anga* infection at a fairly high frequency, and that bacterial strains isolated from "Vallée du Kou" belong to supergroup B. However, under laboratory conditions, the transmission of this infection was unstable in *An. coluzzii* mosquitoes. Moreover, a negative correlation was observed between *w-Anga* infection and *P. falciparum* infection in these mosquitoes. This correlation suggests that *Wolbachia* could potentially serve as a biological control tool.

Nevertheless, to better understand the various properties of this bacterium and guide future research, it is important to consider the limitations of the present study. These include the lack of seasonal variability in sample collection, the restriction of collections to a single ecological niche (the Southern Sudanian Zone), and the use of a single mosquito species (*An. coluzzii*) for experiments. These factors limit the extrapolation of results to other ecosystems and other species within the *An. gambiae* complex. This underscores the need to expand the study by including data from different ecological niches and seasons while assessing the effects of *w-Anga* on malaria transmission by other significant vectors such as *An. gambiae* and *An. arabiensis*.

Despite these limitations, the study highlights two main key findings: the instability of *w-Anga* infection transmission over *An. coluzzii* mosquitoes' generations, and the negative correlation between *w-Anga* infection and *Plasmodium* presence within these mosquitoes.

Data availability

The R code and data for all analyses in this article are available as supplementary files.

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Author contributions

EB, AD, ELD designed the experiments; ELD, IS, EJG, BL and EB performed the experiments and analyzed the data. EB, ELD, MVM, BL, SS and AD wrote the manuscript. EB and AD are the guarantors of the study. All authors read and approved of the final manuscript.

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Declarations

Competing interests

The authors declare no competing financial interests.

Ethical approval

Experiments with animals were carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. In addition, Experiments followed the IRSS Animal Welfare Assurance A5926-01. Trained personnel and veterinarians cared for animals involved in this study and all efforts were made to minimize suffering. All work with *w-Anga* was performed under biosafety containment level II requirements.

Consent for publication

All authors have approved the final manuscript and consent for the publication.

Additional information

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