DEET Efficacy Increases With Age in the Vector Mosquitoes Anopheles gambiae s.s. and Aedes albopictus (Diptera: Culicidae)

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Abstract

Old mosquitoes are the most likely to transmit pathogens due to the higher probability that they will be exposed to pathogens, and the time required before a mosquito becomes infectious, the extrinsic incubation period (EIP). However, old mosquitoes are rarely considered in the evaluation of control tools. This study evaluated the effect of mosquito aging on the repellent efficacy of N,N-diethyl-3-methylbenzamide (DEET) in two vector mosquitoes, Anopheles gambiae s.s. (Giles) (Diptera: Culicidae) and Aedes albopictus (Skuse) (Diptera: Culicidae), respective vectors of malaria parasites and arboviruses such as dengue, chikungunya, and Zika viruses. For both mosquito species, DEET-induced inhibition of blood-feeding was assessed in three age classes. Life-history traits related to mosquito fecundity and survival following DEET exposure were also measured. Results showed that, in both species, bloodmeal inhibition induced by DEET was significantly higher in old females (>18 d old) than in younger ones (<13 d old). Life history traits recording showed no combined effects of DEET and aging on mosquito blood engorgement, oviposition rate, number of eggs laid nor survival; however, age effects are reported for all these traits. These results highlight the need for taking into account mosquito age in the evaluation of integrated mosquito management tools. They also suggest that the control of vector-borne pathogens with a long EIP could be improved by targeting old mosquitoes and supports the efficacy of repellents in the fight against mosquito-borne diseases.

Key words: vector, mosquito, aging, DEET, repellency

In mosquito vector populations, age is a critical parameter when considering the ability of individuals to transmit pathogens. To maintain pathogen circulation, a vector must live long enough to survive all the steps before becoming infectious, which involve infection, pathogen multiplication, and migration into the salivary glands. The time from exposure to the pathogen and infectiousness, i.e., the extrinsic incubation period (EIP) varies with pathogens, vectors and environmental conditions (Ohm et al. 2018). Human malaria parasites are transmitted by Anopheles mosquitoes and require an EIP of about 10 d before the transmissible stages reach the salivary glands (Vanderberg and Yoeli 2016, Vaughan et al. 2016a, Vaughan et al. 2016b). For mosquito-borne viruses such as dengue or Zika, the EIP in Aedes vector mosquitoes lasts approximately 6–15 d for dengue viruses (WHO 2009a, Chan and Johansson 2012) and 10 d for Zika virus (Roundy et al. 2017). Also, the life-expectancy of mosquitoes range from 14 to 21 d for most of the Anopheles vectors of Plasmodium in Sub-Saharan Africa (Carnevale and Robert 2009) and from 10 to 39 d for Aedes albopictus (Skuse) (Diptera: Culicidae) and Ae. aegypti (L.) (Diptera: Culicidae) (Delatte et al. 2009, Goidin et al. 2015). This implies that young mosquitoes cannot transmit these pathogens and highlights that, in a vector population, only a small proportion is actually infectious to the vertebrate hosts, which may have a substantial impact on the efficacy of integrated mosquito management programs (Bellan 2010). In this context, old mosquitoes should be considered as a priority in the development and evaluation of control tools. However, the process of aging has been largely neglected until recent years and practical limitations still restrain the recommendations for insecticide and repellent testing to 5- to 7-d-old mosquitoes (WHO 2009b).

Deleterious metabolic modifications have been shown to be related to mosquito aging (Ryan et al. 2015), such as changes in flight performances (Rowley 1968), immune functions (Hillyer et al. 2005), salivary gland structure (Beckett 1990) and fertility (McCann et al. 2010). The decline in mosquito performances with age could influence
mosquito response to control tools such as repellents and insecticides, as well as its ability to detoxify these chemicals. Consistently, various studies have documented the effect of mosquito aging on the efficacy of insecticides, which remain the most common tool against vector mosquitoes. Literature highlights increases in insecticide susceptibility alongside increased aging (Hodjati and Curtis 1999, Glunt et al. 2011, Chouboua et al. 2012, Aizoun et al. 2014, Aldridge et al. 2017). Interestingly, this effect was also observed in mosquitoes carrying insecticide resistance alleles (Rowland and Hemingway 1987, Lines and Nassor 1991, Hodjati and Curtis 1999, Jones et al. 2012, Kulma et al. 2013). Contrasting with insecticides, the effect of mosquito aging on repellents efficacy has received very little attention. However, due to the spreading of insecticide resistance, blocking vector and human contact with the use of repellents remains a valuable tool to control mosquito-borne disease transmission (Fradin and Day 2002, Licciardi et al. 2006, Nguesan et al. 2006, Prideon et al. 2009, Pennetier et al. 2010). In *Ae. albopictus*, DEET was more efficient to inhibit bloodmeal in 15-d-old females than in younger and older females in arm-in-cage assays (Lao et al. 1995). However, contrasting results were observed, as other authors did not find any correlation between mosquito aging and DEET efficacy, using the same experimental design (Barnard 1998). These discrepancies may be attributed to the lack of reproducibility in experiments when using host odors due to inter-host variability. To this regard, studies with standardized protocols must be conducted in order to better understand the potential effect of mosquito aging on repellents efficacy. In this study, we tested the effect of aging on DEET efficacy against two main vectors of human pathogens, *Anopheles gambiae* s.s. (Diptera: Culicidae) and *Ae. albopictus* (Diptera: Culicidae), when exposed to a bloodmeal through a DEET-impregnated net. We also tested the effect of DEET exposure on life history traits related to fecundity and survival, in order to show the impact of repellent exposure along a mosquito lifespan.

**Materials and Methods**

**Biological Material**

Experiments were performed on adult females of two vector mosquitoes, *An. gambiae* and *Ae. albopictus*. Because the neurotoxicity of DEET may interplay with insecticide resistance (Swale et al. 2014), we used an *An. gambiae* colony that is fixed for the L1014F homozygote mutation (*kdr-west* allele) in the gene coding for the voltage-gated sodium channel. This mutation confers resistance to pyrethroids and DDT and is widespread among malaria mosquito vector populations (WHO 2016). The colony was obtained by introgression into the Kismu genome the *kdr-west* allele obtained from pyrethroid resistant mosquitoes in Kou Valley, Burkina Faso (Alout et al. 2013). For *Ae. albopictus*, an insecticide-susceptible strain was used, as insecticide resistance is emerging but remain less documented (Moyes et al. 2017). This strain was collected in 2011 in Montpellier, France. Mosquitoes were reared at 27°C and 80% RH with a photoperiod of 14:10 h (L:D). Larvae were fed TetraMin (Tetra, Germany) and adults a 10% honey solution. Mosquitoes were reared in the technical/ research platform dedicated on vectors at Institut de Recherche pour le Développement (IRD) Montpellier Center, France.

**Selection of Different Age Classes**

To measure the effect of mosquito age on DEET efficacy, three age classes each separated by 7 d were selected for each mosquito species. Experiments were performed on females that had the opportunity to mate. They were 4, 11 and 18 d post-emergence for *An. gambiae* and 6, 13, and 20 for *Ae. albopictus*. Oldest females (18 and 20 d, respectively) were given the opportunity to blood feed at day 8 and day 15 post-emergence. Females of intermediate age (11 and 13 d, respectively) were given access to one bloodmeal at day 8 post-emergence. After the bloodmeal, females were given the opportunity to oviposit. Youngest females (4 and 6 d, respectively) were never given access to blood feeding before the assay. This protocol design allows all age classes to be blood-starved for the same period the day of the experiment, which represents 4 d for *An. gambiae* females and 6 d for *Ae. albopictus* females.

**Exposure to DEET: Blood Feeding Assays**

Females were sugar-starved for 6 h before the assay. For both mosquito species, the three age classes were tested simultaneously. DEET efficacy was tested by blood feeding assays through DEET-impregnated nets. Preliminary assays were performed to select a DEET concentration that corresponds to a mean inhibition between 25 and 75% compared to the control group without repellent. This dose induces repellency but also allows some of the females to blood feed and thus to observe either an increase or a reduction in DEET efficacy between age classes. These assays indicated that a net impregnated with a dose of 25 µg/cm² for *An. gambiae* and 7.5 µg/cm² for *Ae. albopictus* entails such inhibition range in our experimental setup. To perform the assays, batches of 25 females were placed in paper cups (L: 10 cm, ø: 7 cm). Each cup containing mosquitoes was covered by a 15 x 17 cm piece of polyester net that has been previously impregnated either with DEET in ethanol at the selected concentration or with ethanol as a negative control, and left to dry for 1 h at room temperature. Cups were placed under glass feeders, so mosquitoes would contact the impregnated net when blood feeding. Feeders were linked to a water bath circulation (Julabo Labotechnik, Seelbach, Germany) that each maintained 500 µl of rabbit blood at 37°C. They were sealed on one end with Parafilm membrane allowing mosquitoes to feed through it. This device allows for the simultaneous exposure of 800 females for each replicate. After 1 h of exposure, the proportion of blood-fed females was counted for each treatment and each age class, and blood-fed females were maintained in the same conditions as during the rearing for subsequent recording of life history traits.

**Recording of Life History Traits**

**Bloodmeal Size**

The quantity of excreted haematin during blood digestion was used to assess the quantity of ingested hemoglobin during the bloodmeal (Briegel 1980). After exposure, all fed females were individually maintained in plastic vials and supplemented with 10% honey. They were removed after 48 h and excreta was eluted in 1 ml of 1% lithium carbonate solution. Absorbance of the eluate was measured at 405 nm by a TECAN Sunrise absorbance microplate reader (Tecan, Männedorf, Switzerland). The quantity of excreted haematin by individual females was estimated by using the standard curve obtained from known concentrations of porcine haematin (1–100 µg/ml) (Sigma Aldrich, Lyon, France) and used as a proxy of the bloodmeal size.

**Fecundity and Survival**

After ingestion of the bloodmeal and digestion, females were individually transferred into paper cups and supplemented with 10% honey. The bottom of the cup was covered with moist cotton and filter paper to allow oviposition. The proportion of females that laid eggs (oviposition
rate) was assessed, and eggs were counted by using the software Egg Counter (Mollahosseini et al. 2012). The number and identity of dead females were recorded every day to determine their survival.

Statistical Analysis
All statistical analyses were performed using the R software (R Development Core Team 2008).

Blood feeding. Logistic regression by generalized linear mixed-effects model (glmer, binomial distribution, logit link, lme4 package; Bates et al. 2015) was used to compare the proportion of blood-fed females between age classes for both mosquito species, considering replicates and batches as random effects. Treatment, age, and their interaction were included in the model as fixed effects. Post hoc comparisons between the three age classes were performed using multiple comparisons (Tukey’s tests, multcomp package; Hothorn et al. 2008).

Life-history traits. For each age class and species, bloodmeal size (measured as the quantity of excreted haematin) was analyzed using a linear mixed-effects model with a normal error distribution (lmer, lme4 package). Oviposition rate was compared using glmer with a binomial error distribution. The number of eggs laid per female was analyzed using a hurdle model with negative binomial distribution (pscl package; Zeileis et al. 2008). The probability of the presence versus absence of eggs was modeled using a binomial distribution. The effects of treatment and age on survival were assessed using a Cox proportional hazards regression model (packages survival, coxme; Therneau and Grambsch 2000, Therneau 2015). For each analysis, age, treatment, and their interaction were included as fixed effects, and replicate was added as random effect. For comparison of the number of eggs laid, bloodmeal size was also included as a fixed factor. The contribution of each explanatory variable and the interaction between age and treatment were assessed sequentially using analysis of variance (ANOVA) function, with nonsignificant terms removed from the model. Model selection was performed using the Akaike Information Criterion (AIC) and analysis of the residuals (plotresid, package RVAideMemoire; Hervé 2017).

Results
Interplay Between Mosquito Age and DEET on Blood-Feeding Success
Blood-feeding success on a DEET or ethanol-treated net was tested among 4,057 females for An. gambiae and 3,492 for Ae. albopictus, both across five replicates. For the two species, the concentrations of DEET (25 µg/cm² of net for An. gambiae and 7.5 µg/cm² of net for Ae. albopictus) significantly affected blood feeding, with a mean inhibition across age classes of 75% for An. gambiae ($X^2 = 138.55$, df = 1, $P = 2.2e^{-16}$) and 55% for Ae. albopictus ($X^2 = 64.50$, df = 1, $P = 9.66e^{-16}$) compared to control groups.

In both species, aging significantly affected blood feeding in presence of DEET ($X^2 = 13.17$, df = 2, $P = 0.0014$ for An. gambiae and $X^2 = 17.55$, df = 2, $P = 0.00015$ for Ae. albopictus) (Fig. 1). In An. gambiae, the proportion of blood-fed females was significantly higher in young females than in old ones ($P < 0.001$). No differences were observed between young females and intermediate-aged females ($P = 0.20$) and between intermediate-aged females and old females ($P = 0.43$) (Fig. 1A). In Ae. albopictus, young females were significantly less inhibited by DEET than females of intermediate age ($P = 0.0074$) and old females ($P < 0.001$). No differences in the blood feeding were observed between females of intermediate age and old females ($P = 0.34$) (Fig. 1B). In both mosquito species, blood feeding was not affected by mosquito aging in the control group ($X^2 = 1.49$, df = 2, $P = 0.47$ for An. gambiae and $X^2 = 1.27$, df = 2, $P = 0.53$ for Ae. albopictus) (Fig. 1A and B).

Effects of DEET Exposure and Age on Mosquito Life-History Traits
Life-history traits were measured in 600 females of each mosquito species across five replicates.

Blood Meal Size
For both species, bloodmeal size was not affected by DEET treatment compared to control groups ($X^2 = 0.0083$, df = 1, $P = 0.93$ for An. gambiae and $X^2 = 1.71$, df = 1, $P = 0.19$ for Ae. albopictus).

No significant interaction was found between age and treatment for this trait ($X^2 = 3.84$, df = 2, $P = 0.15$ for An. gambiae and $X^2 = 2.21$, df = 2, $P = 0.33$ for Ae. albopictus). Blood meal size was only influenced by mosquito age ($X^2 = 24.12$, df = 2, $P = 5.80e^{-6}$ for An. gambiae and $X^2 = 21.16$, df = 2, $P = 2.54e^{-6}$ for Ae. albopictus). Observations were highly similar between species, with young females taking larger bloodmeal than intermediate-aged females ($P = 0.022$ for An. gambiae and $P = 0.019$ for Ae. albopictus) and old females ($P < 0.001$ for An. gambiae and $P < 0.001$ for Ae. albopictus) (Fig. 2).

Oviposition Rate
Oviposition rate was not affected by DEET treatment compared to control groups neither in An. gambiae ($X^2 = 2.22$, df = 1, $P = 0.14$) nor in Ae. albopictus ($X^2 = 0.68$, df = 1, $P = 0.41$)
nor in *Ae. albopictus* (*X^2^ = 3.40, df = 1, P = 0.065*). No significant interaction was found between age and treatment for this trait in the two species (*X^2^ = 5.79, df = 2, P = 0.055 for *An. gambiae* and *X^2^ = 0.52, df = 2, P = 0.77 for *Ae. albopictus*). In *An. gambiae*, mosquito aging significantly influenced oviposition rate (*X^2^ = 21.70, df = 2, P = 1.94e−4*), with young females laying significantly less than intermediate-aged females (*P < 1e−4*) and old females (*P = 0.0026*). Although a similar trend was observed in *Ae. albopictus*, mosquito age did not significantly influence oviposition rate (*X^2^ = 1.28, df = 2, *P = 0.53*) (Fig. 3).

**Number of Eggs Laid**

For both species, number of eggs laid was not affected by DEET treatment compared to control groups (*X^2^ = 1.22, df = 1, *P = 0.27* for *An. gambiae* and *X^2^ = 2.00, df = 1, *P = 0.16* for *Ae. albopictus*). There was no significant interaction between age and treatment for this trait (*X^2^ = 4.75, df = 2, *P = 0.31* for *An. gambiae* and *X^2^ = 1.78, df = 2, *P = 0.41* for *Ae. albopictus*). In both mosquito species, number of eggs laid seem to be influenced by mosquito age with intermediate-aged females laying more eggs than young and old females, although the difference was not statistically different in *An. gambiae* (*X^2^ = 2.23, df = 2, *P = 0.33*). In *Ae. albopictus*, mosquito age did show a significant effect on number of eggs laid (*X^2^ = 6.90, df = 2, *P = 0.03*), with old females laying significantly less eggs than intermediate-aged females (*P = 0.012*) (Fig. 4).

**Survival**

DEET treatment did not affect survival in either *An. gambiae* (*X^2^ = 0.33, df = 1, *P = 0.56*) or in *Ae. albopictus* (*X^2^ = 0.61, df = 1, *P = 0.43*) compared to control groups. Age and treatment showed no significant interaction for this trait (*X^2^ = 3.16, df = 2, *P = 0.21* for *An. gambiae* and *X^2^ = 3.90, df = 2, *P = 0.14* for *Ae. albopictus*). Survival was only influenced by mosquito age at the time of exposure to bloodmeal and/or the number of blood meals with or without repellent (*X^2^ = 7.35, df = 2, *P = 0.025* for *An. gambiae* and *X^2^ = 7.77, df = 2, *P = 0.02* for *Ae. albopictus*). A similar trend was observed in the two species, with females exposed to bloodmeal when old surviving longer than other classes. Yet, in *An. gambiae*, survival was significantly longer in females that were exposed when old than in intermediate-aged females (*P = 0.04*). In *Ae. albopictus*, survival was significantly longer in females that were exposed when old to the bloodmeal than in females that were exposed when young (*P = 0.015*) (Fig. 5).

**Discussion**

This study evidenced increases in DEET efficacy alongside increasing aging in two vector mosquitoes. In both *An. gambiae* and *Ae. albopictus*, DEET induced a significantly higher inhibition of bloodfeeding in old females (18-d-old for *An. gambiae* and 20-d-old for *Ae. albopictus*) than in young females (4- and 6 d-old). In *Ae. albopictus*, intermediate-aged females (13-d-old) were also more inhibited by DEET than young females (6-d-old). These results support a previous study showing that DEET repellency lasted longer for 15-d-old than 5-d-old mosquitoes (*Xue et al. 1995*). Taken together, these data highlight that mosquito aging both reduces blood feeding in the presence of DEET and increases the duration of DEET efficacy. It is worth noting that, under our experimental set-up, age and number of blood meals are combined, which do not allow to assess the effects of each independently. This design is, however, the most biologically relevant considering that these two factors are also associated in nature, as gonotrophic cycles and blood feeding take place throughout the whole female lifespan (*Carnevale and Robert 2009*). Interestingly, the observed increase of DEET efficacy with mosquito age appears to be conserved across mosquito species and insecticide resistance status, as our results were highly consistent in pyrethroid-resistant *Anopheles* and in insecticide-susceptible *Aedes*. The correlation between mosquito aging and increased susceptibility to chemicals irrespectively of insecticide resistance status is reminiscent with previous observations on insecticides (*Rowland and Hemingway 1987*, *Lines and Nassor 1991*, *Hodjati and Curtis 1999*, *Glunt et al. 2011*, *Chouaibou et al. 2012*, *Jones et al. 2012*, *Kulma et al. 2013*, *Aizoun et al. 2014*, *Aldridge et al. 2017*). DEET is the most used mosquito repellent; however, its mode of action remains under debate. It is thought to exert deterrent as well as insecticidal effects in insects (*Corbel et al. 2009*, *Legeay et al. 2018*). In this context, the increased susceptibility to DEET observed in old females may be attributed to decreases in the efficacy of detoxification mechanisms. Consistently, mosquito aging has been shown to be associated with decreases in the concentration of enzymes involved in DEET detoxification (*Mourya et al. 1993*, *Hellestad et al. 2011*). These decreases have been observed in both insecticide-resistant and susceptible old mosquitoes and are also responsible for increased susceptibility to insecticides (*Hazelton and Lang 1983*, *Mourya et al. 1993*). Alternative and non-exclusive hypotheses to explain the observed changes in susceptibility to DEET along mosquito lifespan could also be increased in the rate of cuticle permeability, decreases

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*Fig. 2.* Mean quantity of excreted haematin after blood feeding for the three age classes in *An. gambiae* (A) and *Ae. albopictus* (B). DEET and control treatments are grouped together, as variability was not explained by DEET treatment but only by mosquito age. Results are presented as mean ± SE. Different letters indicate significant differences (post hoc chi-squared tests with a Tukey correction, *P* < 0.05, **P** < 0.01, ***P*** < 0.001). *N* = 600 females of each mosquito species across five replicates.
in the rate of xenobiotic excretion or changes in the DEET detection by gustatory or olfactory receptors. For instance, changes in antennal olfactory sensitivity with aging have already been documented in other insect species (Seabrook et al. 1979, Crnjar et al. 1990, Den Otter et al. 1991). However, further studies will need to be implemented, as the evaluation of DEET efficacy regarding age-related changes of these parameters has, to our knowledge, never been investigated. Our results did not show any combined effect of DEET and aging on the quantity of blood intake, oviposition rate and number of eggs laid, nor survival after exposure. This is inconsistent with a reduced ability for chemicals detoxification in old females (Hazelton and Lang 1983, Mourya et al. 1993) and suggests that, if DEET induces fitness costs in exposed mosquitoes, they are not expressed in this study. DEET has been shown to present insecticidal effects by inhibiting insect cholinesterases, but its action is reversible (Corbel et al. 2009). One possible explanation could be that mosquitoes are impacted when contacting DEET during blood feeding, but that the impact does not persist beyond exposure due to the reversible nature of DEET action. Under our experimental setup, the reported life-history traits are only influenced by mosquito age and/or the number of blood meals taken, with observations being highly consistent between the two mosquito species tested. Yet, young females displayed a higher quantity of blood intake associated with a lower oviposition rate. For this age class, the assay corresponds to the first bloodmeal and then to the first gonotrophic cycle. Observations are in accordance with literature showing that, in the first gonotrophic cycle, the bloodmeal is used to replenish metabolic reserves to the detriment of eggs development (Charlwood et al. 2003, Carnevale and Robert 2009). The increased longevity observed in females exposed when old may rely on the fact that, throughout gonotrophic cycles, females progressively increase the use of blood lipids for metabolism to the detriment of fecundity, and then accumulate more energetic reserves (Briegel et al. 2002). The decreases in the quantity of blood intake and in the number of eggs laid observed in old females are also consistent with the deleterious modifications associated with mosquito senescence previously documented (Styer et al. 2007, McCann et al. 2010).

Altogether, these observations explain the natural variability in fecundity that occurs throughout female gonotrophic cycles as well as on the relationship between the number of gonotrophic cycles and mosquito longevity. They also reveal the need for considering mosquito age and parity in predictive models of disease transmission as keystone factors that could impact life history traits and, then, pathogen circulation (Ryan et al. 2015).

Our observations highlight that, in two main vectors of pathogens, DEET efficacy increases with mosquito aging. The expected risk of being bitten despite the presence of DEET is then reduced in old mosquitoes, which are the more susceptible to harbor infectious stages of pathogens. This result corroborates previous observations performed on insecticides (Rowland and Hemingway 1987, Lines and Nassor 1991, Hodjati and Curtis 1999, Glunt et al. 2011, Chouaibou et al. 2012, Jones et al. 2012, Kulma et al. 2013,

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**Fig. 3.** Mosquito oviposition rate for the three age classes in *An. gambiae* (A) and *Ae. albopictus* (B). DEET and control treatments are grouped together, as variability was not explained by DEET treatment but only by mosquito age. Results are presented as mean ± SE. Different letters indicate significant differences (post hoc chi-squared tests with a Tukey correction, \( *P < 0.05 \), \( **P < 0.01 \), \( ***P < 0.001 \)). \( N = 600 \) females of each mosquito species across five replicates.

**Fig. 4.** Mean number of eggs laid per female for the three age classes in *An. gambiae* (A) and *Ae. albopictus* (B). DEET and control treatments are grouped together, as variability was not explained by DEET treatment but only by mosquito age. Results are presented as mean ± SE. Different letters indicate significant differences (post hoc chi-squared tests with a Tukey correction, \( *P < 0.05 \), \( **P < 0.01 \), \( ***P < 0.001 \)). \( N = 600 \) females of each mosquito species across five replicates.
Aïzoun et al. (2014), Aldridge et al. (2017), and suggests that old mosquitoes may be more susceptible to chemical-based control tools. This may have significant epidemiological consequences for integrated mosquito management. First, it suggests that the control of vector-borne pathogens with a long EIP could be achieved by selectively eliminating old mosquitoes. This age-structure alteration, referred to as late-life acting intervention (LLA), has been proposed a few years ago, and involves specifically targeting the most dangerous vectors (Koella et al. 2009, Huijben and Pajjmans 2017). The idea behind this evolutionarily sustainable strategy is to target old mosquitoes, before the pathogen reaches salivary glands, in order to reduce the infectious reservoir. The LLA could then enable the containment of transmission without intense selection for resistance, as it reaches mosquitoes after most of the reproduction has been accomplished (Read et al. 2009). Yet, the increased susceptibility of old mosquitoes to a repellent observed in our study supports the feasibility of such LLA strategies. Besides, this study highlights the need for taking into account mosquito age in the development and evaluation of integrated mosquito management tools. Indeed, bioassays that include only newly-emerged mosquitoes do not reflect the overall population susceptibility level. Here, the increased susceptibility observed in old mosquitoes suggests that the quantity of chemicals necessary to fight infectious vector mosquitoes could be adjusted, which could allow to delay the spread of resistance mechanisms, as well as reduce the damages in the environment and the public health challenges caused by the use of chemicals.

This study, together with previous observations, shows that mosquito aging reduces blood feeding success and increases behavioral repellency to DEET (Xue et al. 1995). Moreover, this increased susceptibility alongside aging seems to be a common trait to mosquito species, and affects both insecticide-susceptible and resistant mosquitoes. Altogether, these data encourage the integration of repellents in LLA strategies. However, it is worth noting that, as fecundity and longevity of old females were not shown to be affected by DEET, repellents may not be used alone but rather as part of integrated control strategies that both deter and kill old mosquitoes. Also, mosquito age is not the only parameter of epidemiological relevance and susceptible to influence the efficacy of control tools. Thus, further studies are warranted to evaluate the combined effects of mosquito infection and aging on DEET efficacy. In the long-term, this would help public health policies in designing personal protection strategies that target the most dangerous mosquitoes and reduce the infectious reservoir.

**Fig. 5.** Mean survival for the three age classes in *An. gambiae* (A) and *Ae. albopictus* (B). DEET and control treatments are grouped together, as variability was not explained by DEET treatment but only by mosquito age. Results are presented as mean ± SE. Different letters indicate significant differences (post hoc chi-squared tests with a Tukey correction, *P* < 0.05, **P** < 0.01, ***P*** < 0.001). N = 600 females of each mosquito species across five replicates.

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