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Aedes albopictus

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Abstract

Background: The success of the Sterile Insect Technique for area-wide vector control relies crucially on the ability of sterile males to outcompete their wild counterparts for mating with wild females. Competitiveness tests are commonly performed for assessing quality sterile males intended for field release programs, but there are few reports on the optimal age and ratio for sterile *Aedes albopictus* male release in a SIT program.

Objective: Here, a series of mating competitiveness experiments with different age (1, 3, and 5 d post-emergence) and ratios (1:1, 5:1, and 10:1) of sterile to wild males were carried out in a walk-in field cage under semi-field conditions to examine variations in the mating competitiveness of irradiated adult *Ae. albopictus* males.

Methods: Pupae from laboratory-reared *Ae. albopictus* were irradiated at 35 Gy and introduced in field cages at different ages post emergence and at different ratios with a cohort of wild males and females. The mating competitiveness of sterile versus fertile males, and sterility induction into wild females measured through the egg hatch were observed.

Results: The age of sterile males *Ae. albopictus* significantly affected their competitiveness when confronted with wild males of the same age. Competitiveness of sterile males was significantly lower after emergence (1-day old) than when they were older (3-5 days old). Presumably, 3-days old sterile males may be considered as the optimal age for sterile male releases. At this optimal age, induced sterility rates increased as the proportion of irradiated males relative to the wild male increased.

Conclusion: Our study points to the fact that sugar feeding prior to field release, could be administered for 3 days post-emergence in order to enhance the performance of sterile males. Consistent with early investigations under competitive situations differing in sterile male-to-wild male ratio, the level of induced sterility in the wild females increased as the proportion of irradiated males relative to the wild male increased.

Keywords

Chikungunya; Dengue; Sterile insect technique; SIT; Irradiation

Introduction

Aedes albopictus (Skuse) (Diptera: Culicidae), a proven competent vector for several arboviruses including Dengue and Chikungunya viruses, has expanded its distribution area worldwide and now exceeds the limits of its natural tropical range. This *Aedes* mosquito (*Stegomyia*) species is considered as the most abundant mosquito species in the Islands of the Southwest Indian Ocean. It is an introduced invasive species [1] that has almost replaced the previously established *Ae. aegypti* L. in natural or urban habitats in Reunion Island [2]. *Aedes albopictus* has been incriminated as the main vector in the recent Chikungunya epidemic outbreaks that occurred in the islands of the Southwest Indian Ocean from 2005 to 2007 [3-5].

Beside vector surveillance, current strategy for controlling *Ae. albopictus* in Reunion Island involves the classical approach, which strictly enforces breeding site elimination through community participation, and the use of insecticide treatments in targeted areas by vector control authorities. During the dengue (in 2004) and chikungunya epidemics (in 2005/2006), both larvicidal (including temephos and fenithrothion), and adulticidal treatment (e.g. permethrin), were used from 2004 until the beginning of 2006 and then replaced respectively by Bti (*Bacillus thuringiensis var. israelensis* toxins) and deltamethrin until 2007 [6]. But as in other countries, there are concerns over the use of broad-spectrum insecticides, including the potential selection for insecticide resistance that could affect their efficacy [7-9], in addition to the risk of environmental contamination with possible consequences on non-target organisms [10-12]. Due to the limitations of the current control strategies, there is a renewed interest in alternative or complementary solutions for an efficient and eco-friendly integrated control of *Ae. albopictus.* Following the Chikungunya outbreak in Reunion Island, considerable attention has been given to the possibility of its prevention by the Sterile Insect Technique (SIT). The SIT is based on inundative and repeated releases of sterile males that induce sterility in the wild population, and consequently suppress the target pest species [13,14], while reducing the use of insecticides. Suitable applications of SIT rely on the use of competitive sterile males that are able to locate and compete for mates in the wild and to transfer their sterile sperm. The fitness of sterile males is thus a key target for SIT programs [15,16] but this is a complex process reflecting the diverse physiological demands of the mass reared males.

To reach the high numbers of sterile males needed for release, technical and material conditions for mass rearing are required [17-21]. Every step of mass production, from colonization to release through the irradiation, has the potential to modify the biology of the sterile insects as well as their ability to survive in the field, and compete with wild males for mating with wild females [22-25]. In *Ae. albopictus*, colonization increases inbreeding depression [26] and sterilization by irradiation may strongly impact male's fitness traits such as longevity, flight performance or mating behaviour proportionate to irradiation dose [27]. To prevent such damages while maintaining the competitive mating ability of irradiated versus wild-type males, an established dose of 35 Gy has been chosen to sterilize *Ae. albopictus* males [28,29].

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Various tests have been developed to estimate the life history traits of produced sterile males that influence their efficiency in the field [24]. One of the most classically used is the competitiveness tests. For *Ae. albopictus*, such tests have been performed in small lab cages or in semi field cages [29-31]. Bellini et al. [31] and Madakacherry et al. [30] showed that irradiated *Ae. albopictus* males were as competitive as wild males, while Oliva et al. [29] showed significant decrease of competitiveness in the presence of wild counterparts. One of the major differences between those experiments was the age of males at the time of release with females. In studies reported by Bellini et al. [31] and Madakacherry et al. [30] the males were 3 days old as opposed to 1 day and 5 days old for males used in another related study by Oliva et al. [29]. In insects, male mating ability, quantity and quality of sperm produced vary with age [32-35]. The first purpose of the study reported herein was to determine the optimal age of sterile males intended for release in an SIT program in Reunion Island. Variation in mating competitiveness of sterile *Ae. albopictus* males aged 1 day, 3 days and 5 days old post-emergence, when confronted with wild males of the same age was examined.

Classically, releases of sterile males should usually be performed with "over flooding" ratios in order to compensate the reduced competitiveness of irradiated males or to increase the efficiency of the SIT technique by increasing the chances of insemination of wild females by sterile males [36-38]. Previous results from laboratory studies on irradiated male *Ae. albopictus* released at a ratio of 1:1:5 (wild females: wild males : sterile males), have shown a reduction of 50% of the fertility in wild females [29], while Madakacherry et al. [30] observed 80% of reduction. Following the first set of experiments which was designed to determine the optimal age for release, another study aimed to separately evaluate the effect of three treatments differing in sterile male-to-wild male ratio (i.e. 1:1, 5:1 and 10:1) on the female fertility under semi field conditions. This experiment was done for confirmatory purpose and to provide direct information that can serve as a guide in the determination of optimum release strategy in future field testing in Reunion Island where the SIT is currently being developed as a component of area-wide campaigns to suppress *Ae. albopictus*.

Material and Method

Production and irradiation of *Ae. albopictus* males

The *Ae. albopictus* strain used in the present study was collected as eggs in January 2009 at Saint Benoit (La Réunion). The colony is routinely maintained in a climate-controlled insectary (T: 27 ± 2°C, RH: 75 ± 2%, light: 12L: 12D). The females are routinely fed with 2.5 ml of sheep blood through a Parafilm® membrane on the Hemotek® system [39] (Discovery Workshops, Accrington, Lancashire, United Kingdom). Eggs from the two generations F25 and F26 were used for the tests. Egg hatching took place in water with dehydrated rabbit food (hay pellet, Compagnie des Grains du Capricorne, Le Port, Reunion Island) left overnight in the rearing water during 24h. Larvae were collected and reared at a density of approximately 500 larvae (L1) per tray (30×40 cm) containing 1 litre of water. They were fed with dry pellets composed of 50% of rabbit-food and 50% of fish-food (Sera Koi Food, Sera, Heinsberg, Germany). When pupae appeared, they were collected for the experiment purpose. Virgin mosquitoes were obtained by separating females and males as pupae based the genitalia morphology as observed under optical microscope (Leica MZ6). After sex separation, female pupae and a portion of the male pupae were allowed to emerge into separate adult cages without further treatment (fertile group). To produce sterile males, separate batches of pupae (aged between 24 and 30 h) from the same larval cohort were randomly retrieved and sterilized with 35 Gy of ionizing radiation (2.35 Gy min-1 for 15 min) from a Cs-137 source (Gammacell IBL 437, Cis Bio International, Germany) at the Blood bank located at the Bellepierre hospital, St Denis de La Réunion. While the dose required to induce 100% sterility in *Ae. albopictus* males is about 40-60 Gy [28], other evaluations of the dose-sterility response in *Ae. albopictus* males indicated that the 35 Gy sterilizing dose used in our study represents a better trade-off between high sterility (approx. 95-97%), and fitness [30,31,40]. At emergence, random batches of 100 sterile males each were maintained in separate cages with continuous access to a 10% (w/v) sucrose solution for 1, 3, and 5 days following emergence to allow ageing, the day of emergence being considered as day 0 of an experimental trial. During the ageing process neither sugar solution nor water was limiting in any treatment.

Production of wild males and females

In addition to the sterile males produced from the laboratory stock, wild (non-sterile) males and females used in the competitiveness test were derived from eggs collected from different field sites (Sainte-Suzanne, Bras-Panon, Saint-Benoît, Sainte-Clotilde CIRAD campus) with oviposition traps (ovitraps). Ovitrap consisted of a 1 liter black plastic jar filled with 650 ml of water collected from a river were placed at ground level in a shaded location. The oviposition substrate was made of a brown germination paper (ovistrip) clipped on a 15 × 10 cm stiff PVC sheet, which was placed inside the ovitrap pot. After 2-3 days, the ovistrips were collected and brought to the laboratory, where each was rinsed with tap water and placed in the dark inside plastic tray covered by a Plexiglas for two days allowing gentle drying and egg maturation. From the third day, the Plexiglas cover was removed to allow faster drying and on day 5, the dried egg paper was placed for 72h in 250 ml of tap water (taken one day earlier) with powdered Rabbit food. Larvae from the hatched eggs were then reared as described above and pupae were collected, sexed by their genitalia and the males and females were placed in separate cages awaiting the experiments. Unless otherwise specified, wild males and females that had emerged from the same field cohorts were used in each semi-field experiment. They were maintained in separate laboratory cages (30×30×30 cm^3) with continuous access to a 10 % (w/v) sucrose solution for 1, 3 and 5 days as described below.

Effect of sterile male age on competitiveness in semi-field cages

Mating experiments were conducted under semi-field conditions set at the CIRAD (Centre International de Recherche Agronomique pour le Development) campus in Sainte-Clotilde (20°90'55"S, 55°49'78" E), La Réunion. The experiment took place 2 months in March and April 2013. Five identical walk-in field cages (180×150×150 cm^3) were used at the same time so all treatments could be done simultaneous for each replicate. In the competitiveness tests, the release ratio was, in each case, 100:100:100 (numbers of sterile males: wild males: wild females). In addition, control cages have been set up in following ratios: 0:100:100 (fertile control) and 100:0:100 (sterile control). Three age categories have been tested: 1 day-old, 3 days-old and 5 days-old post-emergence. For each selected age at release, three replicates were performed with different batches of virgin mosquitoes. Sterile and wild males were kept with females in the cages for 6 consecutive days from the time of release. Resting places for mosquitoes were provided in the form of a wooden table

on top of which a 10% (w/v) sucrose solution was placed for the duration of the experiment. This experimental setup was exposed to ambient environmental conditions at 28°C ± 5°C, 80-90% RH. Caged rabbit was provided for female blood-feeding during the first 3 days, after which, the rabbit was removed and three oviposition cups were placed for 3 days consecutively. Subsequently, eggs laid on paper were collected and counted, while adult mosquitoes were collected with a vacuum aspirator and killed by etherisation. Egg counting was performed manually when less than 600 eggs were present on the paper, and for greater number of eggs, counts were done automatically using scanning and counting image J [41]. All eggs were allowed to mature for 2 days under laboratory conditions and then placed in a tray containing tap water with small amount of food (rabbit food) to allow hatching and L1 were counted the 3 days following the egg paper immersion.

Effect of different ratios of sterile to wild males on population sterilization process under semi-field conditions

The effect of three different ratios of wild to sterile males on the female fertility was evaluated under the same semi-field conditions as described above. At the start of each experiment, a fixed "optimal" age of males was chosen according to the results obtained in the experiment described above. Different ratios of sterile and wild males of the same age were released into 5 semi-field cages where they were allowed to compete for mating with virgin females of the same age for six consecutive days. Three ratios (sterile males number: wild males number: wild females number) have been tested simultaneously in different field-cages: 100:100:100, 500:100:100 and 1000:100:100. In addition, two other ratios of 0:100:100 (fertile control) and 100:0:100 (sterile control) were included in each experimental trial. Three replicate trials were performed for each release ratio, usually at weekly intervals, using random batched of newly emerged mosquitoes. The five field cages used were randomly assigned to different ratios and the position of each of the sterile/wild male ratio was changed after each replicate trial.

Parameters recorded and statistical analysis

For each treatment and control group in each experiment, the average number of eggs laid per female was established. Subsequently, the observed egg hatching rate was calculated by dividing the number of 1st instars larvae (corresponding to number of hatched eggs) by the total number of eggs laid. The Fried competitiveness index, 'C' [42] was calculated using hatch rates from the fertile control (Hn), sterile control (Hs) and the treatment groups (Ho) as follows: C= N/S * (Hn -Ho)/Ho-Hs), where N is the number of 'normal' males (wild), S is the number of sterile males.

The analysis of variance (ANOVA) with Tukey's honestly significant difference post hoc tests and Student's t-test were used to test differences in hatching rates and competitiveness indices between experimental groups and among replicates. Prior to statistical analyses observed values of egg hatchability were arcsin transformed to increase their fit to the normal distribution. All statistical analyses were carried out using Microsoft Excel 2007 for Windows and Minitab version 2000.

Ethical considerations

The permission for the use of rabbits under semi-field conditions was obtained from the institutional ethical committee after demonstrating that all measures were taken to avoid unnecessary suffering and severe damage to the animals. Animal care was in accordance with the principles and guidelines of the French Ministry of Environment. The corresponding protocol was approved by the Institutional Ethical committed for Animal Care and Experimental Use, GIP CYROI, Sainte Clotilde, Reunion – France.

Results

Effect of sterile male age on competiveness in semi-field cages

The average numbers of eggs produced by females after mating are presented in Figure 1. Regardless of the age at release, there was no evidence of significantly statistical difference in the average numbers of egg produced by females between the treatments and the controls ($F_{4,10}=2.94$, $P=0.07$, Figure 1). The mean egg hatching rates significantly differed between treatments and controls ($F_{4,10}=63.5$, $P<0.001$). However, due to a relatively high variability of the observed hatch rates in the 5 days old treatment, there was no statistical evidence of significantly variation between eggs hatching rates according to the age of males. Overall, there was no significant effect of age of male on the competitiveness index (CI=0.24 ± 0.08, 0.51 ± 0.11 and 0.56 ± 0.39 for sterile males aged 1, 3 and 5 days old, respectively). The lack of age dependent regulation of sterile mating competitiveness probably occurred as a result of the high variability in the results obtained in the 5 days old treatment. If analyzed without the 5 days old results, the competitiveness index of 1 day old sterile males and 3 days old are significantly different (T test, $t=11.56$, $df=5$, $P=0.027$).

Effect of different ratios of sterile to wild males on population sterilisation process under semi-field conditions

Although there was an apparent variability in the number of eggs produced among treatment groups and controls, the differences in the average number of eggs recorded were unrelated to variations in the sterile: wild male ratio ($F_{4,10}=2.03$, $P=0.16$, Figure 2). The mean egg hatching rates were significantly different according to the treatment ($F_{4,10}=160.17$, $P<0.001$) and compared to controls (Figure 2). As expected, there was generally a marked decline in the observed egg hatching rates between the 1:1:1 and 5:1:1 and 10:1:1

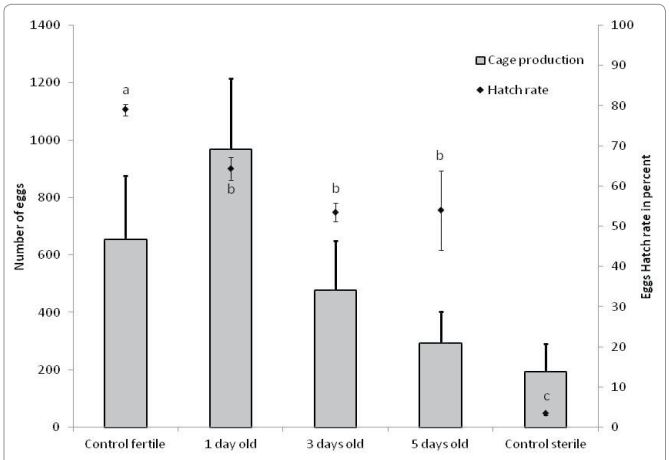

Figure 1: Variation in the mean of egg numbers and hatch rates (± SE) for egg batches laid by females in field cages containing either irradiated *Ae. albopictus* males only (Control sterile), wild males only (Control fertile), and an even ratio of sterile to wild males of three different ages classes (1, 3 and 5 days old). The number of mosquitoes used for each experiment (sterile males: wild males: wild females) was 100:100:100 in the treatment, 0:100:100 in the control fertile group, and 100:0:100 in the control sterile one × 3 replicates. Different letters indicate significant difference in Tukey's posthoc tests following ANOVA test for eggs hatch rate.

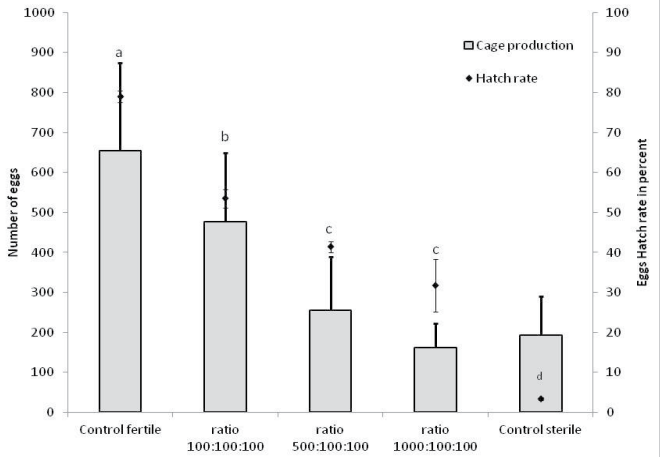

Figure 2: Variation in the mean of egg numbers and hatch rates (± SE) for egg batches laid by females in field cages containing either three different ratio of sterile to wild *Ae. albopictus* males (sterile males number: wild males number: wild females number) 100:100:100, 500:100:100 and 1000:100:100 , irradiated males only (ratio 0:100:100, Control sterile) and wild males only (ratio 100:0:100, Control fertile). Different letters indicate significant difference in Tukey's posthoc tests following ANOVA test for eggs hatch rate.

ratios', confirming that the level of sterility induced among females is influenced by the ratio of sterile males. Surprisingly, the treatment ratio of 1000:100:100 showed high variability in the egg hatching rate, precluding significant differences with the ratio of 500:100:100, but results obtained in these treatment were significantly different from the two controls (fertile and sterile). In parallel, full induced sterility was not attained during the experiments, even with the highest sterile to wild-type ratio 10:1, though the latter ratio yielded significantly higher level of sterility compared to the sterile control group. The induced sterility rates were 33.6 ± 5.2, 49.7 ± 1.6 and 61.7 ± 11.0% for the ratio of 100:100:100, ratio 500:100:100, ratio 1000:100:100 respectively. Competitiveness index significantly varied according to the release ratio ($F_{2,6}=15.15$, $P<0.005$), with 0.51 ± 0.11, 0.20 ± 0.02 and 0.18 ± 0.09 recorded for the ratio of 100:100:100, 500:100:100, 1000:100:100, respectively.

Discussion

Under semi-field experimental conditions in Reunion Island, gamma irradiated *Ae. albopictus* males exhibited a CI value that varies according to the age, ranging from 0.24 for 1 day old males to 0.51 and 0.56 for 3 days old and 5 days old, respectively (where a value of 1.0 suggests equal competitiveness). Mating competitiveness was significantly lower when sterile males were confronted with wild males soon after emergence (1-day old) than when they were 3 days old, though holding sterile males under laboratory conditions for 5 days prior to confrontation with wild males did not allow improving the CI as compared with other age classes. This suggests that 3 days old sterile males could be the most competitive compared to the other younger and older ones. These results were consistent with the increase of competitiveness observed by Oliva et al. [40] between one day old and five days old males released in semi field cages with wild males. Presumably, such an increase in CI with age could be linked to the variation of mating activity intensity during adult life in most mosquitoes. The peak of mating activity of *Anopheles stephensi* Liston [43] and *An. culicifacies* [44] takes place between the third and the seventh days. In *Anopheles gambiae* and *Anopheles arabiensis*, mating ability of seven-day-old males is higher than the one of males younger than three days old [45]. However, the observed change in competitiveness value with age may not be due to an increase of intrinsic male mating ability, but instead associated with the sugar meals supplied for 2-3 days following emergence, leading to an increase in fitness of sterile males [40]. Indeed, the provision of energetic substances to adults prior to release could offset the reduction of competitiveness caused by radiation and mass rearing process, thereby improving the competitiveness of sterile males [18,46,47]. Bellini et al. [48] thus created a sugar feeding device (associated with the releasing device) on which newly emerged sterile males (released as pupae) could acquire sugar meal prior to disperse into the wild. Such sugar fed released males showed an increase of about 25% of the value of competitiveness index compared to unfed males.

Even if no significant difference in competitiveness has been observed between irradiated males aged 3 days and 5 days old, high variability in the competitiveness of 5 days old sterile males suggests that ageing may also affect the mating competitive ability of sterile males. A loss of sperm cell present in male storage organ with time has already been observed in irradiated *An. arabiensis* males [49]. Examination of the testes of irradiated virgin males kept for 6 days showed four times less sperm than non-irradiated males of same age. Such phenomena could be suspected in *Ae. albopictus*. Indeed, Oliva et al. [50] have already shown that the sterile males that copulate with 4-5 successive females until the third day post-emergence were unable to replenish their sperm supply, suggesting that the immature sperm cells may have been impaired by irradiation exposure. Collectively, these findings suggest that despite a significant decline in male fitness following radiation sterilization, holding sterile males under optimal conditions with access to sugar meal ideally for 3 days post-emergence may be influential enough to consider the pre-release period.

Regardless of the age, the sterile *Ae. albopictus* males used in our conditions were at their best (in ratio 100:100:100) approximately half as competitive as wild-type males, suggesting a significant impact of irradiation and/or mass rearing. Moreover, the Competitiveness Index found in our experiment was comparable to that found by Oliva et al. [29] 0.14 for 1 day old and 0.55 for 5 day old males. In contrast, our results are very different from those found in previous experiments in Italy [31] and in Vienna [30], which showed that irradiated *Ae. albopictus* males were as competitive as wild males when they were 3 days old. The results concordance with Oliva et al. [29] could be due to the fact that similar methodologies regarding cage sizes, release ratios and adult densities have been used, while experimental conditions were strongly different from Bellini et al. [31] and Madakacherry et al. [30] set up. However, the same range of Competitiveness Index was found in the latter investigations, although the experimental setups were relatively different. Beside the difference in experimental conditions, another reason that may explain the discrepancy between the previous results and our findings is probably the difference in strains of *Ae. albopictus* used (Reunion strain for the current study and Oliva et al. [29] and Rimini strain for Bellini et al. [31] and Madakacherry et al. [30]). To the best of our knowledge, very few information exist in the literature regarding potential difference in mating competitiveness between different strains in *Ae. albopictus*. Intraspecific variations in life history traits such as rates of larval growth [51], diapause [52], female longevity and reproduction [53], fitness and hybrid production [54] or competitive interactions with *Ae. aegypti* [55], has been already documented in *Ae. albopictus* from distinct geographic origins. Thus, different

Ae. albopictus strains from distinct geographic regions likely exhibit different male quality after mass rearing or irradiation treatment. This may have direct implications in the efficiency of the sterile male insect technique as a control strategy across borders.

The efficiency of the sterile male insect technique as a control strategy relies on releases of sterile males in numbers adequate to obtain high over-flooding ratios in the field. In the experiment reported herein, despite a positive relationship between induced sterility and ratio of the wild to sterile males, meaningful difference in induced sterility was visible when sterile males were confined with wild males and females in the ratio of 500:100:100 (5:1:1) compared to the 1000:100:100 (10:1:1). The level of induced sterility at the ratio of 5:1 (49.7%) as observed in the present study is lower than the 81% observed in Rimini (Italy) strain *Ae. albopictus* [30], but relatively closed to the 53% previously recorded with 1 day old La Réunion strain *Ae. albopictus* [29]. This again might be related to strain differences in the competitiveness of sterile *Ae. albopictus* males when confronted with wild rivals at different ratios. Gaining a fuller understanding of strain-specific control of mating competitiveness in irradiated males will be important in the future before establishing any long term sustainable strategy for the suppression of *Ae. albopictus* by sterile male releases in different field situations.

This situation in *Ae. albopictus* competitiveness suggests that even a higher sterile-to-wild male ratio would be required to achieve meaningful population suppression in the field situations. Proof-of-principle demonstrations of this idea that a ratio much higher than 10:1 may be sometimes needed to achieve a meaningful suppression have been reported in previous SIT programs against other insects. For example, a significant decline in fertility of a wild population of Mediterranean fruit flies (*Ceratitis capitata*) in Guatemala was obtained when sterile males were released in the field at a ratio of 100:1 sterile male: wild male [37], while ratios of 40:1 were required for the suppression of codling moths (*Cydia pomonella*) populations in British Colombia, Canada [36]. Taking into consideration the effect of radiation on the life history parameters of *Ae. albopictus* [31,29] some uncertainty will remain on whether the ratio superior to 10:1 will be sufficient to effectively suppress the population of the naturally occurring *Ae. albopictus* mosquitoes in diverse field situations. Results from the current and related studies at least motivate further evaluation in small-scale open release trials with increasing ratios of sterile to wild males. However, mathematical models and numerical simulations may help gaining an appreciation on how experimental trials such as that reported here, and real-world baseline ecological data can be brought together to establish the appropriate release strategies for long-term suppression of wild *Ae. albopictus* populations.

Under our studied conditions, sterile *Ae. albopictus* males show a decrease of competitiveness with the increase of sterile male ratio and that could explain why high levels of sterility were not obtain when increasing the release ratio from 5:1 to 10:1. Such reduction of CI with the increasing ratio is difficult to interpret. Few papers deal with this ratio effect on male competitiveness, Maiga et al. [56] observed such decrease for *Anopheles coluzzii* while Yamada et al. [57] does not observe that for *An. arabiensis*. No real explanation has been proposed. One hypothesis could be that the higher density of male lead to higher disturbance between males during copulations due to the known aggressive behaviour of *Ae. albopictus* males under laboratory or semi-controlled conditions. In 100:100 ratios, each male probably could mate "quietly" while in 500:100 and 1000:100 ratios, disturbances and fights probability increase in which normal male could be the winner against the irradadiated male. Indeed, as observed in other some insect species, somatic damages link to the irradiation could have affect locomotor activity [58], flight ability [59] or male vigour [60] and could be detrimental during the fights.

Conclusion

As observed in numerous studies that have been set to develop or assess the efficacy of Sterile Insect Technique (SIT), irradiation inevitably impacts on the competitiveness of sterile males. Consistent with previous studies, we found that the age of the sterile *Ae. albopictus* males at release interacted with other physiological factors (such as access to sugar meal after sterilization) to affect their mating competitiveness. In our study, 3 days post-emergence was found optimum and thus may be considered in the future application of SIT against *Ae. albopictus*. Oliva et al. [29] suggested the release of 5 days old male in their paper but high variability of mating ability observed in the current study suggests an age-dependant increasing effect of irradiation process. Considering this requirement, our results further indicate that effective field release strategies against *Ae. albopictus* may require sterile-to-wild male ratios greater than 10:1. Keeping this in view, an inundative sterile male release may be very effective in reducing the density of the target vector population if used as a component of area-wide integrated vector management, depending on technical constraints and specifics field situations. Indeed some other eco-friendly techniques could be used to enhance the efficiency of the SIT as the association with Wolbachia-induced phenotypes, such as cytoplasmic incompatibility and pathogen interference [61,62], or behavioural-based control programs as "boosted SIT" (Auto-dissemination of a juvenile hormone analogue the pyriproxyfen by sterile males [63]) and mosquitocidal botanicals [64]. Such integrated vector management could lead to a limitation of the high numbers of sterile males to release to a threshold economically viable and need to be tested in further experiments.

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Authors' Contributions

Conceived and designed the experiments: LCG, DD. Performed the experiments: OD, GLG and CL. Analyzed the data: DD and LCG. Wrote the paper: DD and LCG. All authors read and edited the manuscript and approved the final version.

Competing Interest

The authors declare no competing interests.

References

1. Juliano SA, Lounibos LP (2005) Ecology of invasive mosquitoes: effects on resident species and on human health. Ecol Lett 8: 558-574.
2. Bagny L, Delatte H, Quilici S, Fontenille D (2009) Progressive decrease in Aedes aegypti distribution in Reunion Island since the 1900s. J Med Entomol 46: 1541-1545.
3. Higgs S (2006) The 2005-2006 Chikungunya epidemic in the Indian Ocean. Vector Borne Zoonotic Dis 6: 115-116.

4. Schuffenecker I, Iteman I, Michault A, Murri S, Frangeul L, et al. (2006) Genome microevolution of chikungunya viruses causing the Indian Ocean outbreak. PLoS Med 3: e263.

5. Delatte H, Dehecq JS, Thiria J, Domerg C, Paupy C, et al. (2008) Geographic distribution and developmental sites of Aedes albopictus (Diptera: Culicidae) during a Chikungunya epidemic event. Vector Borne Zoonotic Dis 8: 25-34.

6. Delatte H, Paupy C, Dehecq JS, Thiria J, Failloux AB, et al. (2008) Aedes albopictus, vector of chikungunya and dengue viruses in Reunion Island: biology and control. Parasite 15:3-13.

7. Hemingway J, Ranson H (2000) Insecticide resistance in insect vectors of human disease. Annu Rev Entomol 45: 371-391.

8. Tantely ML, Tortosa P, Alout H, Berticat C, Berthomieu A, et al. (2010) Insecticide resistance in Culex pipiens quinquefasciatus and Aedes albopictus mosquitoes from La Réunion Island. Insect Biochem. Insect Biochem Mol Biol 40: 317-324.

9. Vontas J, Kioulos E, Pavlidi N , Morou E , della Torre A, et al. (2012) Insecticide resistance in the major dengue vectors Aedes albopictus and Aedes aegypti. Pesticide Biochem Physiol 104:126–131.

10. Naqvi SM, Vaishnavi C (1993) Bioaccumulative potential and toxicity of endosulfan insecticide to non-target animals. Comp Biochem Physiol C 105: 347-361.

11. Wiktelius S, Chiverton PA, Meguenni H, Bennaceur M, Ghezal F, et al. (1999) Effects of insecticides on non-target organisms in African agroecosystems: a case for establishing regional testing programmes. Agric Ecosyst Environ 75: 121-131.

12. Biondi A, Mommaerts V, Smagghe G, Viñuela E, Zappalà L, et al. (2012) The non-target impact of spinosyns on beneficial arthropods. Pest Manag Sci 68: 1523-1536.

13. Knipling EF (1955) Possibilities of insect control or eradication through the use of sexually sterile males. J Econ Entomol 48:459-469

14. Knipling EF, Laven H, Craig GB, Pal R, Kitzmiller JB, et al. (1968) Genetic control of insects of public health importance. Bull World Health Organ 38: 421-438.

15. Oliva CF, Damiens D Benedict MQ (2014) Male reproductive biology of Aedes mosquitoes. Acta Trop 132 Suppl: S12-19.

16. Lees RS, Knols B, Bellini R, Benedict MQ, Bheecarry A, et al. (2014) Review: Improving our knowledge of male mosquito biology in relation to genetic control programmes. Acta Trop 132 Suppl: S2-11.

17. Balestrino F, Benedict MQ, Gilles JR (2012) A new larval tray and rack system for improved mosquito mass rearing. J Med Entomol 49: 595-605.

18. Puggioli A1, Balestrino F, Damiens D, Lees RS, Soliban SM, et al. (2013) Efficiency of three diets for larval development in mass rearing Aedes albopictus (Diptera: Culicidae). J Med Entomol 50: 819-825.

19. Balestrino F1, Puggioli IA, Bellini R, Petric D, Gilles JR (2014) Mass production cage for Aedes albopictus (Diptera:Culicidae). J Med Entomol 51: 155-163.

20. Zheng ML, Zhang DJ, Damiens DD, Yamada H, Gilles JR (2015) Standard operating procedures for standardized mass rearing of the dengue and chikungunya vectors Aedes aegypti and Aedes albopictus (Diptera: Culicidae)-I-egg quantification. Parasit Vectors 8: 42.

21. Zheng ML, Zhang DJ, Damiens DD, Lees RS, Gilles JR (2015) Standard operating procedures for standardized mass rearing of the dengue and chikungunya vectors Aedes aegypti and Aedes albopictus (Diptera: Culicidae)- II - Egg storage and hatching. Parasit Vectors 8: 348.

22. Bartlett AC (1984) Genetic changes during insect domestication: Advances and Challenges in Insect Rearing. Agricultural Research Service, New Orleans, USA

23. Clarke GM, McKenzie LJ (1992) Fluctuating asymmetry as a quality control indicator for insect mass rearing processes. J Econ Entomol 85: 2045-2050.

24. Calkins CO, Parker AG (2005) Sterile insect quality: In Sterile Insect Technique. (1stedn), Springer, Netherlands

25. Benedict MQ, Knols BG, Bossin HC, Howell PI, Mialhe E, et al. (2009) Colonisation and mass rearing: learning from others. Malar J 8 Suppl 2: S4.

26. O'Donnell D, Armbruster P (2010) Inbreeding depression affects life-history traits but not susceptibility to infection by Plasmodium gallinaceum in the Asian tiger mosquito, Aedes albopictus. Infect Genet Evol 10: 669-677.

27. Proverbs MD (1969) Induced sterilization and control of insects. Annu Rev Entomol 14: 81-102.

28. Balestrino F, Medici A, Candini G, Carrieri M, Maccagnani B, et al. (2010) Gamma ray dosimetry and mating capacity studies in the laboratory on Aedes albopictus males. J Med Entomol 47: 581-591.

29. Oliva CF, Jacquet M, Gilles J, Lemperiere G, Maquart PO, et al. (2012) The sterile insect technique for controlling populations of Aedes albopictus (Diptera: Culicidae) on Reunion Island: mating vigour of sterilized males. PLoS One 7: e49414.

30. Madakacherry O, Lees RS, Gilles JR (2014) Aedes albopictus (Skuse) males in laboratory and semi-field cages: release ratios and mating competitiveness. Acta Trop 132 :124 –129

31. Bellini R, Balestrino F, Medici A, Gentile G, Veronesi R, et al. (2013) Mating competitiveness of Aedes albopictus radio-sterilized males in large enclosures exposed to natural conditions. J Med Entomol 50: 94-102.

32. Jones TM, Elgar MA (2004) The role of male age, sperm age and mating history on fecundity and fertilization success in the hide beetle. Proc Biol Sci 271: 1311-1318.

33. Radwan J, Michalczyk L, Prokop Z (2005) Age dependence of male mating ability and sperm competition success in the bulb mite. Anim Behav 69: 1101-1105.

34. Dean R, Bonsall MB, Pizaari T (2007) Ageing and sexual conflict. Science 316: 383–384.

35. Jones TM, Featherston R, Paris DBBP, Elgar MA (2007) Age-related sperm transfer and sperm competitive ability in the male hide beetle. Behav Ecol 18: 251-258.

36. Bloem KA, Bloem S (2000) SIT for codling moth eradication in British Columbia, Canada. Area-Wide Control of Fruit Flies and other Insect Pests. International Conference on Area-Wide Control of Insect Pests and the Fifth International Symposium on Fruit Flies of Economic Importance , Penang, Malaysia.

37. Rendón P, McInnis D, Lance D, Stewart J (2004) Medfly (diptera: tephritidae) genetic sexing: large-scale field comparison of males-only and bisexual sterile fly releases in Guatemala. J Econ Entomol 97: 1547-1553.

38. Dame DA, Curtis CF, Benedict MQ, Robinson AS, Knols BG (2009) Historical applications of induced sterilisation in field populations of mosquitoes. Malar J 8 Suppl 2: S2.

39. Cosgrove JB, Wood RJ, PetriÄ‡ D, Evans DT, Abbott RH (1994) A convenient mosquito membrane feeding system. J Am Mosq Control Assoc 10: 434-436.

40. Oliva CF, Maier MJ, Gilles J, Jacquet M, Lemperiere G, et al. (2013) Effects of irradiation, presence of females, and sugar supply on the longevity of sterile males Aedes albopictus (Skuse) under semi-field conditions on Reunion Island. Acta Trop 125: 287-293.

41. Mains JW, Mercer DR, Dobson SL (2008) Digital image analysis to estimate numbers of Aedes eggs oviposited in containers. J Am Mosq Control Assoc 24: 496-501.

42. Fried M (1971) Determination of sterile-insect competitiveness. J Econ Entomol 64: 869-872.

43. Mahmood F, Reisen WK (1982) Anopheles stephensi (Diptera: Culicidae): changes in male mating competence and reproductive system morphology associated with aging and mating. J Med Entomol 19: 573-588.

44. Mahmood F, Reisen WK (1994) Anopheles culicifacies: effects of age on the male reproductive system and mating ability of virgin adult mosquitoes. Med Vet Entomol 8: 31-37.

45. Verhoek BA, Takken W (1994) Age effects on the insemination rate of Anopheles gambiae s.l. in the laboratory. Entomol Exp Appl 72: 167-172

46. Gary Jr RE, Cannon III JW, Foster WA (2009) Effect of Sugar on Male Anopheles gambiae Mating Performance, as Modified by Temperature, Space and Body Size. Parasit Vectors 2: 19-31.

47. Kassim NF, Webb CE, Russell RC (2012) The importance of males: larval diet and adult sugar feeding influences reproduction in Culex molestus. J Am Mosq Control Assoc 28: 312-316.

48. Bellini R, Puggioli A, Balestrino F, Brunelli P, Medici A, et al. (2014) Sugar administration to newly emerged Aedes albopictus males increases their survival probability and mating performance. Acta Trop 132 Suppl: S116-123.

49. Damiens D, Vreysen MJ, Gilles JR (2013) Anopheles arabiensis sperm production after genetic manipulation, dieldrin treatment, and irradiation. J Med Entomol 50: 314-316.

50. Oliva CF, Damiens D, Vreysen MJ, Lemperière G, Gilles J (2013) Reproductive strategies of Aedes albopictus (Diptera: Culicidae) and implications for the sterile insect technique. PLoS One 8: e78884.

51. Armbruster P, Conn JE (2006) Geographic variation of larval growth in North American Aedes albopictus (Diptera: Culicidae). Ann Entomol Soc Am 99: 1234-1243.

52. Lounibos LP, Escher RL, Lourenço-de-Oliveira R (2003) Asymmetric evolution of photoperiodic diapause in temperate and tropical invasive populations of Aedes albopictus (Diptera: Culicidae). Ann Entomol Soc Am 96: 512-518.

53. Leisnham PT, Sala LM, Juliano SA (2008) Geographic variation in adult survival and reproductive tactics of the mosquito Aedes albopictus. J Med Entomol 45: 210-221.

54. O'Donnell D, Armbruster P (2009) Evolutionary differentiation of fitness traits across multiple geographic scales in Aedes albopictus (Diptera: Culicidae). Ann Entomol Soc Am 102: 1135-1144.

55. Leisnham PT, Lounibos LP, O'Meara GF, Juliano SA (2009) Interpopulation divergence in competitive interactions of the mosquito Aedes albopictus. Ecology 90: 2405-2413.

56. Maïga H, Damiens D, Niang A, Sawadogo SP, Fatherhaman O, et al. (2014) Mating competitiveness of sterile male Anopheles coluzzii in large cages. Malar J 13: 460.

57. Yamada H, Vreysen MJ, Gilles JR, Munhenga G, Damiens DD (2014) The effects of genetic manipulation, dieldrin treatment and irradiation on the mating competitiveness of male Anopheles arabiensis in field cages. Malar J 13: 318.

58. Ignatowicz S, Wesolowska B, Zaedee IH (1994) Detection of Irradiated Insect Pests in Stored Products: Locomotor Activity of Irradiated Adult Beetles. 6th International Working Conference on Stored-Product Protection, Australia.

59. Bhakthan NMG, Nair KK (1972) Fine structural damage in the somatic tissues of gamma-irradiated house fly 1 Flight muscles. Ann Entomol Soc Am 65:504-508.

60. Weldon CW, Prenter J, Taylor PW (2010) Activity patterns of Queensland fruit flies (Bactrocera tryoni) are affected by both mass-rearing and sterilization. Physiol Entomol 35:148-153.

61. Lees RS, Gilles JRL, Hendrichs J, Vreysen MJB, Bourtzis K (2015) Back to the future: the sterile insect technique against mosquito disease vectors. Curr Opin Insect Sci 10:156 –162.

62. Zhang D, Lees RS, Xi Z, Gilles JRL, Bourtzis K (2015) Combining the Sterile Insect Technique with Wolbachia - Based approaches: II- a safer approach to Aedes albopictus population suppression programmes, designed to minimize the consequences of inadvertent female release. PLos One 7.

63. Bouyer J, Lefrançois T (2014) Boosting the sterile insect technique to control mosquitoes. Trends Parasitol 30: 271-273.

64. Benelli G (2015) Research in mosquito control: current challenges for a brighter future. Parasitol Res 114: 2801-2805.

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