Mosquitoes do play an important role in many ecosystems, as crucial pollinators for many plants and food for a range of species [7]. However, they also constitute a threat to a large percentage of the world's population, and it has been convincingly argued that the benefits of their extinction would vastly outweigh the ecological scars they would leave behind [8]. The burden of the diseases caused by mosquito-borne pathogens undoubtedly justifies all efforts to understand the complex biology and ecology of mosquitoes [1] as well as to constantly improve and implement mosquito-control programs [9]. It certainly warrants the firm warning made by Bill Gates – one of the people who has done the most for the fight against malaria and other neglected tropical diseases [10] – about the danger that these small creatures represent[vi]. Nevertheless, from a strictly technical point of view, mosquitoes cannot be accused of murder any more than any person unwillingly and unwittingly transmitting *Mycobacterium tuberculosis*, HIV, or severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) to another could be subjected to a similar accusation. Thus, while placing mosquitoes at the top of the 'most-wanted' list may seem understandable in view of the suffering they indirectly cause, one can hardly fail to question whether their reputation as the world's deadliest animals is entirely fair.

Resources

[i] www.sciencealert.com/what-are-the-worlds-15-deadliest-animals

[ii] https://en.wikipedia.org/wiki/List_of_deadliest_animals_to_humans

[iii] www.cnet.com/pictures/the-24-deadliest-animals-on-earth-ranked/24/

[iv] www.cdc.gov/niosh/topics/outdoor/mosquito-borne/default.html

[v] www.who.int/neglected_diseases/vector_ecology/mosquito-borne-diseases/en/

[vi] www.gatesnotes.com/health/most-lethal-animal-mosquito-week

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Forum

Yes, Irradiated Sterile Male Mosquitoes Can Be Sexually Competitive!

Jérémy Bouyer[1,2,3,*] and Marc J.B. Vreysen[1]

Adequate sexual competitiveness of sterile males is a prerequisite for genetic control methods, including the sterile insect technique. During the past decade several semi-field and open-field trials demonstrated that irradiated male mosquitoes can be competitive.

Although the biological quality or competitiveness (Box 1) of released sterile male insects is essential to ensure success in genetic insect pest-control programmes, in most cases this is rarely assessed [1]. Entomological effectiveness in all male release programmes – including irradiated, *Wolbachia*-infected, and transgenic males – that use colonized insects can only be proven by calculating the competitiveness of the released males. Here, we argue that a reduction in quality of the produced sterile male insects is mostly related to the mass-rearing, handling, marking, and release processes, rather than radiation per se. As an example, in the sterile insect technique (SIT) programmes against tsetse flies, it was demonstrated that chilling and transport of sterile male pupae were the main factors impacting their quality [2].

The competitiveness *C* of a sterile male is the odds of a wild female being mated with a sterile male compared with a wild male when exposed to both in equal numbers. A *C* value of 1 indicates that sterile and wild males are equally competitive. A *C* value of 0.5 indicates that females are two times more likely to be mated with wild males. The SIT is a genetic control tactic used for the management of selective insect pests and relies on the release of mass-produced male insects that are sterilized by ionizing radiation. The mating of a wild female insect with a sterile male will result in no offspring. Insemination of the oocyte with sperm that contains numerous dominant lethal mutations will cause embryonic arrest and death. In the case of SIT, an appropriate irradiation dose must be selected that ensures adequate sterility without impairing *C*.

Insights from Semi-field Trials

Eleven studies with four mosquito species (*Aedes aegypti, Ae. albopictus, Anopheles arabiensis*, and *An. coluzzii*) were analysed, and in most cases the irradiation treatment was given during the pupal stage (see Table S1 in the supplemental information

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Box 1. What is Competitiveness?

Competitiveness, C, is often estimated using Fried's index following the formula: $C = \frac{(Ha-E)/(E-Hs)}{R}$, where Ha is the natural fertility of females, E the observed fertility rate under a given ratio R of sterile over wild males and Hs the residual fertility of males [14]. Hs is often neglected when the residual fertility of males is below 1% [10]. Ha is estimated as the percentage of fertile eggs in a cage with nonirradiated males (or a control site), E the percentage of fertile eggs from a cage with a given ratio R (or a release site), and Hs the hatch rate of eggs from females mated with sterile males. To estimate a reference value of C, it is suggested to use a sterile male–wild male–wild female ratio of 1:1:1. Increased sterile-to-wild male ratios will increase competition between sterile males themselves, and this may reduce their quality. Under field conditions, the release site must be isolated, or R below 4, to reduce the impact of fertile female immigration [15] on the underestimation of C (Figure I).

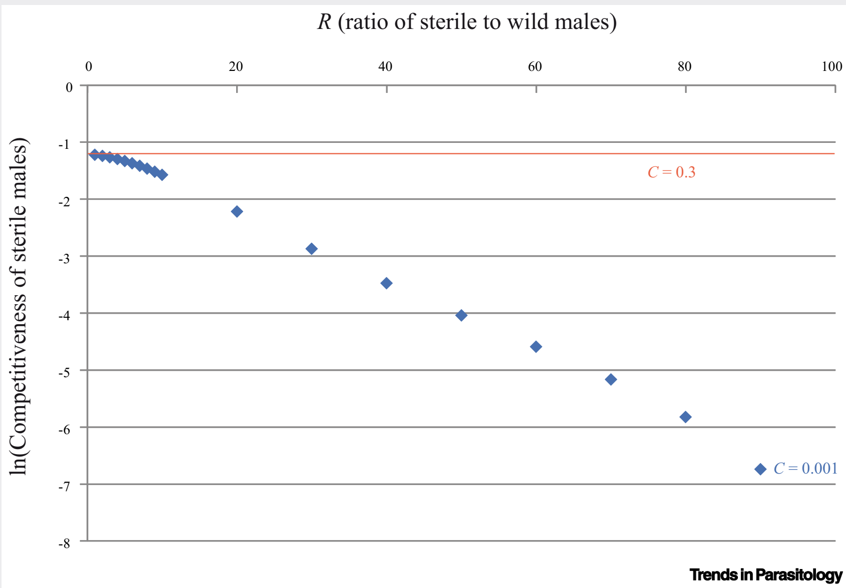

Figure I. Impact of the Ratio (R) of Sterile to Wild Males on the Observed Competitiveness (C) of Sterile Males in Field Conditions Assuming Immigration of Fertile Females in the Release Site. Red line, real C; blue points, observed C. See Table S2 for details.

online). The studies used different doses, type of irradiators, and male age when irradiated and when offered to the females for mating – all of which can affect the amount of dominant lethal mutations in the sperm [3] and, hence, the C value. Also, the ratio R of sterile to wild males was variable, which can impact the observed C (Box 1). In general, *Anopheles* species required a higher dose to obtain the same level of sterility as compared with *Aedes* species, and the irradiation dose was negatively correlated with the C values (Figure 1A). However, this was not consistent, and C was sometimes very variable at the same dose, for example, a C value between 0.4 and 1 for *Ae. albopictus* irradiated with a dose of 35 Gy. However, the C value of *An. arabiensis*, treated as adults with 75 to 120 Gy, was similar and overall better than irradiating them as pupae [4]. In addition to improved C values, irradiating adults resulted in better insemination rates as compared with irradiating pupae. These data indicate that irradiating pupae induced higher levels of somatic cell damage than irradiating adults, probably because more stem cells were negatively affected in pupae. Similar observations were made with other insect species [1]. Moreover, contrary to adult irradiation, irradiation of pupae can result in partial recovery of fertility with age [5].

In all studies, the C value was above 0.2, which is considered to be the lower threshold for cost-effective projects. A lower value would require an asymptomatic increase in the amount of sterile males to be released, as was indicated by two independent modelling studies [6,7] (Figure 1C,D).

Insights from Recent Field Trials

Although, world-wide, there are 34 SIT mosquito trials presently implemented [8], only four have reported an estimation of the C value – all on *Aedes* species (Figure 1B). These projects reported a C value above the 0.2 threshold, resulting in the successful suppression of *Ae. albopictus* in China [9], Italy [10], and Mauritius [11]. A combined SIT-incompatible insect technique (IIT) approach was used in the trial in China, releasing triple *Wolbachia*-infected male *Ae. albopictus* that were irradiated with only 40 Gy. The low radiation dose was enough to sterilize accidentally released female mosquitoes, and the males conferred the conditional sterility created by the *Wolbachia* symbiont; this resulted in a higher C value. The main objective of the trial in Brazil was to test a release system mounted on an unmanned aerial vehicle (drone) for the aerial release of sterile male *Ae. aegypti*. During three successive releases, ~2500 sterile males were dispersed per ha and this resulted in a maximum sterile-to-wild male ratio of 0.8:1 in the release area [12]. The proportion of unviable eggs collected in the release area increased by >50% as compared with that of a neighbouring control area where no sterile mosquitoes were released; this corresponded to a C value of 0.26.

Concluding Remarks and Perspectives

All of these studies confirm that irradiated male mosquitoes can be competitive with the native mosquito population after release. Recent advances in the R&D of mosquito SIT have made all the

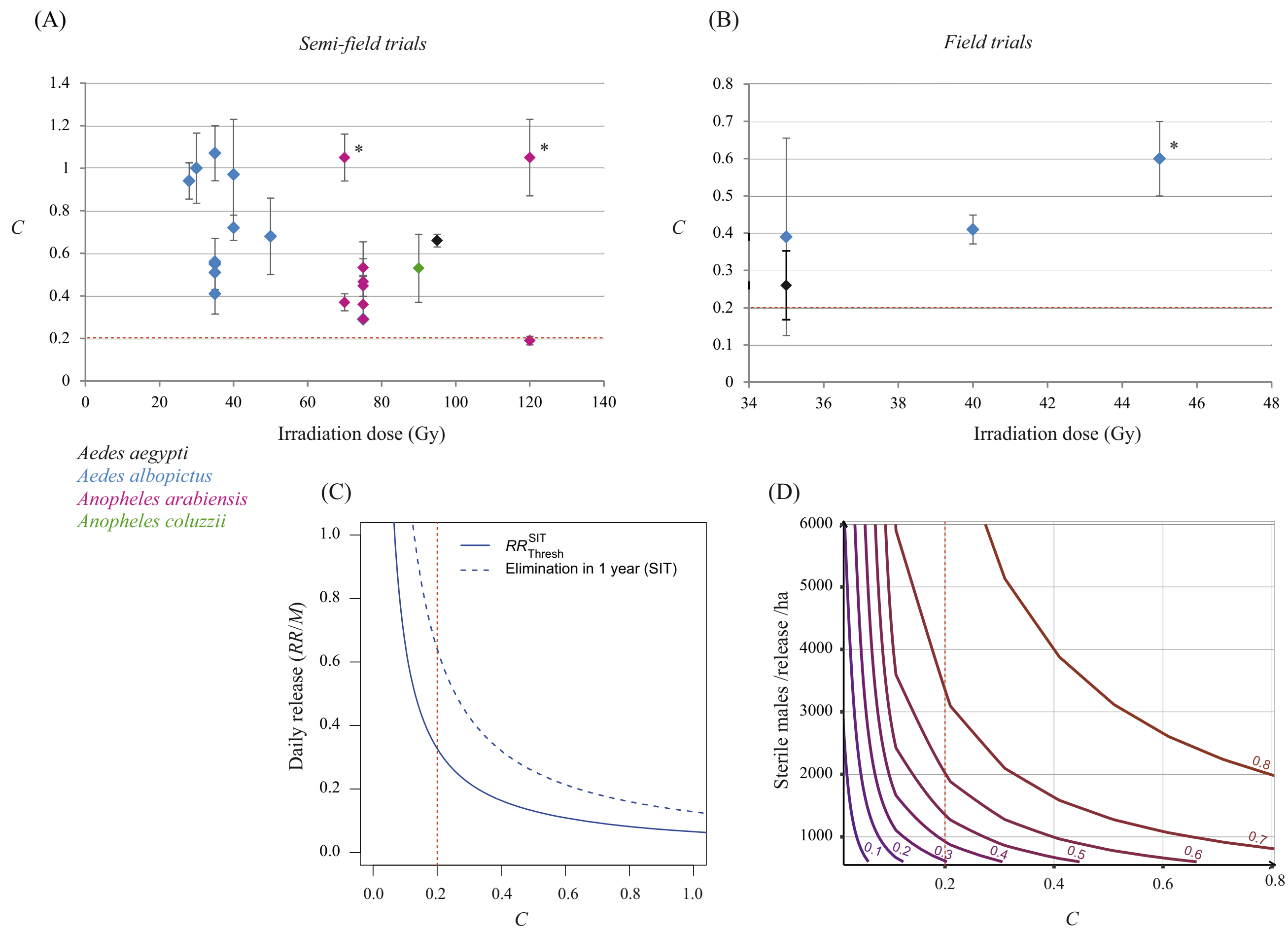

Figure 1. Competitiveness of Irradiated Male Mosquitoes and Impact on the Necessary Release Rates in Operational Programmes. Competitiveness (*C*) of irradiated sterile male mosquitoes was measured in semi-field trials (A) and field trials (B), as a function of the irradiation dose (see Table S1 for details). Values marked with a '*' correspond to sterile males irradiated as adults whereas all others were irradiated as pupae. The value marked with a '#' corresponds to a combination between the incompatible and the sterile insect technique [9]. (C) Relationship between the competitiveness and the daily release rate of sterile males to obtain eradication of a local population of *Aedes albopictus* in 1 year. The release rate (*RR*), expressed as a proportion of the initial male population (*M*), is increasing asymptomatically for a competitiveness below a threshold of 0.2. Source: modified from [6]. (D) Relationship between the competitiveness and the daily release rate (sterile males per ha per week) to obtain a given suppression rate of female mosquitoes. The suppression rate of adult females is indicated along the isoclines from 0.1 to 0.8. Source: modified from [7]. In all panels, the red broken lines represent a competitiveness of 0.2.

components of the SIT package more effective; this has contributed to increased and adequate competitiveness of the released male mosquitoes that made these trials a success [13]. Male quality-reducing processes (mass-rearing, handling, marking and release) are not specific to the SIT and also affect programmes using *Wolbachia*-induced sterility or transgenic males. It is, of course, obvious that treating insects with excessive high radiation doses will impair their competitiveness, but in most programmes it has been possible to select a trade-off dose obtaining >99% male sterility without significantly reducing their biological quality[i].

To increase the probability of success, a phased conditional approach (PCA) was proposed for the management of mosquito populations using the SIT. In this approach, support for the next phase is conditional on the completion of activities in the previous phase, and the scope, expense, and commitment increase along the process [8]. An assessment of the *C* value is part of the baseline data collection phase (I) and should be conducted first under laboratory conditions, followed by studies under semi-field and field conditions before commencing pilot trials (phase II). Having knowledge of *C* values is considered to be a crucial and mandatory milestone, and a necessity that allows estimating the required sterile-to-wild male ratios to suppress the target population.

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As many factors, other than irradiation, can cause a loss of quality of sterile males, this estimate of the *C* value in the field must be obtained using the same protocols of production, handling, irradiation, and release as those that will be used in the operational programme. This should be done through a specific mark-release-recapture protocol where the ratio of sterile to wild males is measured using adult traps in the same sites simultaneously with assessing induced sterility in the native female population using ovitraps. An IAEA guideline is available in this respect[ii] and can be used with technologies based on *Wolbachia*-infected or transgenic mosquitoes, but such estimates are rare in the literature. As an example, the Verily team carried out an impressive suppression trial in California using *Wolbachia*-infected *Ae. aegypti* male mosquitoes, but no competitiveness metrics were measured, which significantly reduces the value of this study. The ground release trial with engineered RIDL® (Release of Insects carrying a Dominant Lethal) mosquitoes was a notable exception, but the *C* value obtained was less than 0.06 (cited in [12]). This value is much lower than any estimate obtained for irradiated mosquitoes, and to date, no other genetic control method has provided higher estimates of *C* than those of irradiated males, despite the general inappropriate dogma in the literature that irradiated male mosquitoes cannot be competitive.

Ground releases are very ineffective (giving point releases), labour and transport intensive, and they require prolonged detrimental chilling of the sterile males to cover the targeted areas [2]. Therefore, it will be important to improve aerial distribution of sterile male mosquitoes in the future to enhance their quality [12]. Improved aerial dispersal systems would also make the technology more cost effective as it would reduce the time and staff necessary to treat a given area [9].

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Supplemental Information

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Resources

[i]www-naweb.iaea.org/nafa/ipc/public/Guidelines-for-MRR-Aedes_v1.0.pdf

[ii]www.iaea.org/sites/default/files/aedes-who-iaea-2020.pdf

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Forum

Antibody Therapy Goes to Insects: Monoclonal Antibodies Can Block *Plasmodium* Transmission to Mosquitoes

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Malaria eradication is a global priority but requires innovative strategies. Humoral immune responses attack different parasite stages, and antibody-based therapy may prevent malaria infection or transmission. Here, we discuss targets of monoclonal antibodies in mosquito sexual stages of *Plasmodium*.