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Why is *Aedes aegypti* Linnaeus so Successful as a Species?

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Abstract

Diseases transmitted by mosquitoes impose enormous burden towards human morbidity and mortality. Over the last three decades, Brazil has suffered from severe Dengue epidemics. In September 2014, this situation is further complicated by the introduction of two other viruses, Zika and Chikungunya, placing Brazil in a triple epidemic. In this article, we discuss the biology of *Aedes aegypti* Linnaeus, and the principal initiatives currently used to control mosquito populations and the diseases they transmit. *Aedes aegypti* has broad global distribution and is involved in the transmission of various arboviral diseases such as Dengue, Zika, and Chikungunya. Several factors contribute to the success of the species, particularly behavioral plasticity, rapid development, desiccation-resistant eggs, resistance to the principle insecticide classes currently available on the market, preference for the urban environment, and proximity to humans. Vector control programs are the best way to reduce the burden of mosquito-borne diseases. Chemical control is most commonly used in recent times, and unfortunately, the results have not been satisfactory but instead, there is increased vector dispersal and, subsequently, the spread of disease epidemics. Investigations of alternative control methods such as release of *Wolbachia*-infected mosquitoes for blocking vector-borne pathogens, release of transgenic mosquitoes carrying a lethal gene for offspring, and the use of insecticide-dispersing mosquitoes are under way in Brazil, and some have shown promising results. Special emphasis should be placed on integrated management of all available tactics, so as to maximize efforts towards mosquito control. Finally, we emphasize that continuous actions and community participation control initiatives are critically important for success.

Introduction

Diseases transmitted by mosquitoes impose enormous burden towards human morbidity and mortality. In the last three decades, Brazil has suffered severe Dengue epidemics caused by widespread circulation of the four viral serotypes. This situation in Brazil was further complicated in September 2014 by the introduction of Zika and Chikungunya, causing a triple epidemic. Further, neurological complications likely related to the Zika virus worsened public health threats. The Ministry of Health (MS) declared an Emergency Situation of National Importance in Public Health in November 2015 due to the growing incidence of microcephaly. The emergency status was maintained by the MS until the end of 2016 (Brazil 2015; SVS 2016).

In early 2016, the World Health Organization (WHO) declared a Public Health Emergency of International Importance due to the wide dispersal of the Zika virus and its potential consequences, with autochthonous transmission occurring in 24 countries in the Americas and an estimated 2.5 billion people at risk of exposure (WHO 2016). In addition to the staggering increase in Zika cases highlighted by the media, Brazil continues to experience high incidence of Chikungunya and Dengue infections. Dengue undoubtedly remains among the most important arboviruses affecting humans in terms of morbidity and mortality (Fares *et al* 2015). In the last year alone, Brazil reported about 1,600,000 cases and approximately 800 deaths, with 65% of all cases reported in the southeastern region (SVS 2015).

At the center of this triple epidemic is the mosquito *Aedes* (*Stegomyia*) *aegypti* Linnaeus, a vector of all three viral diseases. This species is present in all states of the Brazilian federation and approximately 4500 municipalities. It is a cosmopolitan species, found mainly in tropical and subtropical regions where climatic and social conditions are favorable to development (David *et al* 2009; Kraemer *et al* 2015).

Climatic factors contribute to the successful establishment of this vector, including adequate temperature for vector development and a rainy season, which favors production of breeding sites. Other factors contribute to vector success in dispersal, particularly the lack of urban infrastructure and basic sanitation, irregular occupation of areas, deficiency in garbage collection, irregular distribution of water, and lack of community awareness and participation. These factors also favor mosquito population growth and compromise vector control programs. David *et al* (2009) demonstrated that the degree of human organization and anthropogenic environmental modifications, local basic sanitation conditions, community industrialization, and even cultural habits can have a direct influence on *Ae. aegypti* occurrence and population density.

In this context, this work discusses aspects of the biology and success of the mosquito *Ae. aegypti*, in addition to the current main initiatives regarding control of this vector and the diseases it transmits.

Aedes aegypti

Aedes aegypti is a mosquito species native to Africa and currently widely distributed throughout the world (Fig 1). Some authors have suggested raising *Stegomyia* to the genus level, which would change the classification of this species to *Stegomyia aegypti*. This proposal has not yet been adopted as a general consensus; thus, we use the nomenclature *Ae. aegypti* in this manuscript (Reinert *et al* 2004; Polaszek 2006; Wilkerson *et al* 2015). We also suggest that modification of the taxonomic status of an insect with great importance in public health and for which the general population is well informed may cause confusion, potentially hampering community support in vector control programs.

Aedes aegypti is the most synanthropic species of the Culicidae, always cohabiting with humans. It is preferentially diurnal and tends to be most active at dawn and dusk, thus avoiding the hottest periods of the day. Despite these clear behavioral preferences, this is an extremely opportunistic species that will bite in the day or night, and is known to occupy environments with conditions outside of the preferred range (Lourenço de Oliveira 2015).

Adults have black coloration with white markings and silver scales; however, they may present strong variation in tonality, sometimes with light brown coloration. The clypeus presents two prominent tufts of silver scales. The thorax is covered by dark and light scales, with silver scales forming longitudinal lines with a lyre-like shape. The external contour of this shape is formed by broader scales, while the central part, near the lyre-shaped pattern, formed by finer and more delicate lines (Forattini 2002).

Males are generally smaller than females; however, body size is not the ideal parameter for the sexual differentiation in this species because size is influenced by other external factors (e.g., food availability, larval density in breeding sites). Instead, prominent dimorphic features are present in antennal morphology (i.e., feathery in males and bristly in females) and structures of the buccal apparatus (i.e., long palps in males and short palps in females) (Consoli & Oliveira 1994; Forattini 2002; Eiras 2011).

Vector Biology

The *Aedes* life cycle comprises four distinct phases: egg, larva, pupa, and adult (Fig 2). The eggs are small (about 0.5 mm), elliptical, light colored at the time of oviposition, and darkening over time. Embryogenesis is complete in 2 or 3 days on average. When immersed in water eggs may hatch in minutes, but in the absence of water, high resistance to desiccation allows eggs to remain viable for weeks, months, or even over a year (Farnesi *et al* 2009; Faull & Williams 2015).

We note not only that the number of viable eggs in the same location likely decreases over time but that the eggs that remain viable may be sufficient for local maintenance of the species when conditions are unfavorable for mosquito development (Juliano *et al* 2010). Silva & Da Silva (1999) demonstrated that for *Ae. aegypti* kept in an environmental chamber at 28°C with mean humidity of 80%, after 121 days, the hatch rate was 97.2%. After 492 days, egg viability fell to 0.2%.

Egg eclosion gives rise to larvae, which undergo four developmental instars comprising the only feeding stage for immatures and the only growth stage in the life cycle. The larvae of *Ae. aegypti* are aquatic and feed on suspended organic matter adhered to the walls or sediment at the bottom of the reservoirs. Larval feeding habits are non-selective, thus in addition to food particles larvae may readily ingest

Fig 1 Global distribution of *Aedes aegypti* (data from 1960 to 2014). Adapted by Kraemer *et al* (2015). Link: http://www.nature.com/articles/sdata201535.

chemical or biological insecticides (Lourenço de Oliveira *et al* 2015).

Aedes aegypti larvae breathe atmospheric air through spiracle openings on the siphon, located on the eighth abdominal segment. This behavior is a crucial consideration for vector control programs, as larvae must rise to the surface regularly to breathe. While on the surface, they become more detectable and, thus, more susceptible to control agents.

The development time for *Ae. aegypti* larvae is influenced by many factors, primarily temperature, food availability, and larval density in the breeding site. Under optimal conditions, larvae may complete all instars and enter the pupal stage in 4 to 5 days; however, the developmental period may be considerably prolonged under adverse conditions. The pupal phase is a non-feeding stage in which the mosquito undergoes a series of internal transformations that will culminate with adult formation. Pupae remain on the surface of the water almost continuously to breathe, diving to the bottom of the reservoir only when a threat is perceived. Under optimal temperature conditions (about 27°C), the pupal phase has an average duration of 2 days; however, this period may be prolonged at lower temperatures. Pupal mortality rate is extremely low; thus, it is suggested that the number of pupae found in a reservoir corresponds directly to the number of adults that will emerge (Lourenço de Oliveira *et al* 2015).

Adults emerge slowly and remain on the water surface for several minutes, after which they fly in search of shelter. They typically seek humid areas that are protected from light and wind and, often, those near potential breeding sites. During this time, the exoskeleton hardens in both sexes, and rotation of genital structures occurs in males to facilitate

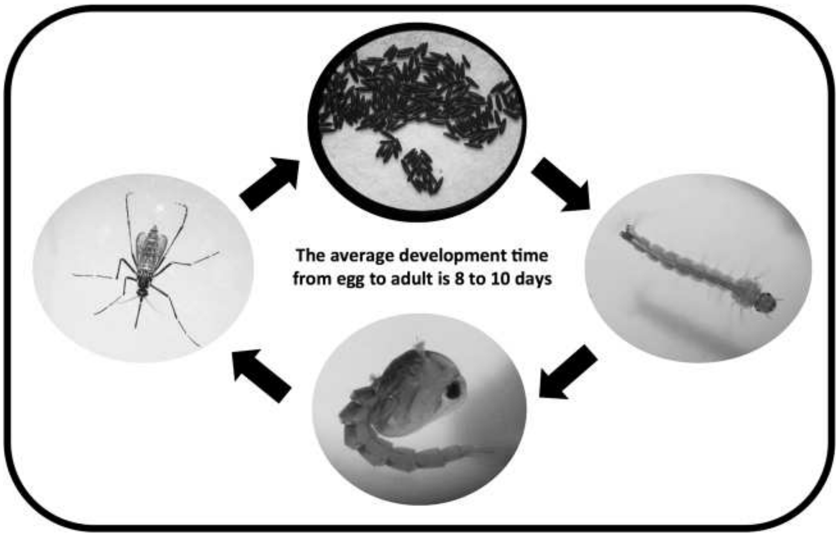

Fig 2 *Aedes aegypti* life cycle.

copulation. After copulation, females store seminal fluid in a structure called the spematheca, which allows sperm to remain viable indefinitely. Females may then produce fertile eggs for the remainder of their lifetime with no need for additional copulation (Forattini 2002; Lourenço de Oliveira *et al* 2015).

Adult males and females feed on sugary substances, mainly of vegetable origin. Females also require blood meals for maturation of the ovaries and consequent formation of eggs. Gravid females seek areas with accumulated standing water, preferably clean (with little decomposing organic matter), in which to lay eggs. Eggs are deposited individually by attachment to the wall of the reservoir, in a humid location just above the water surface. Some authors have also reported eggs placed directly on the surface of the water in field traps (Wermelinger *et al* 2015). When the water level in the reservoir rises, the eggs become inundated and hatch, reinitiating the cycle.

The general developmental cycle of *Ae. aegypti* consists of two phases: terrestrial and aquatic. Ecologically speaking, this is advantageous for the insect, as niche differentiation restricts intraspecific competition between adult and immature phases. Another important aspect is that immature stages are restricted to the oviposition site and unable to migrate; they are more vulnerable to control measures in these stages; control methods should thus preferably focus on the aquatic phase.

Why *is Aedes aegypti* so Successful?

Although *Ae. aegypti* does demonstrate behavioral and habitat preferences (e.g., active period in the day, temperature, water quality), they also display opportunistic behavior. This species frequently occurs in areas with suboptimal environmental conditions, will feed at any time of day, and can utilize water with large amounts of decomposing organic matter. They exhibit a high degree of plasticity, and can quickly adapt to different environmental conditions. These factors, in addition to having eggs that are highly resistant to desiccation, ensure the maintenance of the life cycle even in periods that are unfavorable for vector development.

The adaptation of this mosquito to areas with unfavorable water quality was recently been demonstrated by Paploski *et al* (2016), in a study carried out in four regions of Salvador, Bahia. *Aedes aegypti* larvae and adults were found in sewers in two of the four study regions; during the evaluations, almost 50% of the sewers had standing water and about 40% contained *Ae. aegypti* larvae. Researchers also found *Aedes albopictus* Skuse and *Culex quinquefasciatus* Say in these environments. The importance of sewerage sites for reproduction in *Aedes* has already been described in other countries such as Colombia and Mexico (Andrade & Cabrini 2007). This highlights the need for investigation of these types of breeding places in other areas of Brazil.

Aedes aegypti is well adapted to urban environments and prefers to occupy human dwellings. Data from the Ministry of Health of Brazil show that about 80% of the breeding sites are found in these urban domestic environments. *Aedes aegypti* also prefers man-made, artificial containers, bringing it in closer contact with humans (Brasil 2014). Generally, this species prefers containers that are colored or with dark interiors, typically in shady areas. This behavior is likely related to breeding success in these types of habitats, in addition to light aversion or photophobia of immature phases. This should be taken into consideration during home inspections, since larval occupation of these dark, shaded containers can make detection difficult for vector control services and residents.

Another important consideration for on-site inspection is that even very small containers or other breeding habitats cannot be disregarded as potential breeding sites (e.g., plastic cups, PET bottles, aluminum cans, small plant pots); *Ae. aegypti* readily uses such sites, often transitionally, for reproduction. This is possible due to rapid development in this species, which allows adults to emerge before the water evaporates, avoiding larval mortality. Further, these types of water-holding containers are often involved in the passive dispersal of *Ae. aegypti*, because they are easily transported among locations and may retain eggs attached to inner surfaces. The focus on these breeding sites does not, however, diminish the importance of perennial breeding sites such as water tanks, since breeding habitats with higher water-holding capacity can continuously produce larger populations of mosquitoes.

The high reproductive capacity of *Ae. aegypti* is another factor that contributes to its success. A single female can lay approximately 100 eggs per gonotrophic cycle, making it difficult to control the vector in a given area (CDC 2016). One characteristic that, for instance, hinders control of *Ae. aegypti* is their habit of "skip oviposition." Females routinely distribute eggs among several breeding sites, making detection and complete elimination of breeding sites and eggs a nearly impossible task. Abreu *et al* (2015) demonstrated that a single female distributes eggs among an average of 4 to 6 breeding sites, and when conditions permit, will use up to 11. This is an important characteristic, as even a small number of eggs originating from a few adults may be enough to permit local persistence of mosquitoes and mosquito-borne diseases throughout the year.

Aedes aegypti has great epidemiological importance because in addition to being intimately associated with urban environments, it is highly anthropophilic, i.e., it prefers humans as a blood meal source (McBride *et al* 2014). Transovarial transmission may also occurs in this species, in which females transfer virus to their offspring. Although the contribution of vertical transmission is quite controversial (Zeidler *et al* 2008; Buckner *et al* 2013; Thangamani *et al* 2016), it might also contribute to the maintenance of circulating virus in the population.

The resistance of *Ae. aegypti* to them main chemical insecticides used for control is alarming, and makes control measures even more difficult. In Brazil, organophosphates were the only method used for many years to control *Ae. aegypti*. Intensified use following the 1986 epidemic and extending to the beginning of 2000 resulted in a strong selective pressure for resistance, and consequent decrease in the effectiveness of this group of insecticides. After 2000, organophosphates were replaced by pyrethroids for adult control. Unexpectedly, mosquito resistance to this new class of insecticides occurred extremely rapidly, with detected resistance in some populations as early as 2002 and 2003 (Valle *et al* 2015).

We emphasize that insecticides do not create resistant individuals, but that mutant alleles already exist naturally in populations. The widespread use of insecticides selects these resistant alleles, while eliminating those that are susceptible. This is the genetic basis of resistance. A major concern with respect to resistance is the intensity of selection pressure, which is caused mainly by the continuous use of a particular class of insecticide without frequent rotation and evaluation. This may have an irreversible effect on some populations, through the complete elimination of susceptible individuals (Crow 1957). Some authors also report risks involved in the use of domestic insecticides by the general population (Oliveira *et al* 2015a), noting that these products may also play a role in the selection of resistant mosquito populations.

Main Control Strategies for *Aedes aegypti*

According to the Ministry of Health National Guidelines for the Control of the Dengue Epidemic, *Ae. aegypti* in Brazil is primarily combatted using chemical, mechanical, and biological control methods (Brasil 2009). Some authors highlight the importance of adopting alternative measures for mosquito control (Maciel-de-Freitas *et al* 2012; Araújo *et al* 2015; Zara *et al* 2016). Currently, less conventional initiatives are being investigated to control the vector and the diseases it transmits, such as the use of *Wolbachia* bacteria to block transmission and the release of sterile transgenic mosquitoes to suppress wild populations.

Chemical control

Chemical control consists of the application of chemical compounds that can act against immature (larvicides) and adult phases (adulticides). It is and has been the main form of control for mosquitoes and other insect pests over centuries. Although widely used to combat *Ae. aegypti* to the present day, chemical control has not been demonstratively effective in reducing vector populations. On the contrary, we have seen an increase in locations with mosquito infestation. Much of this problem is related to the misuse of chemicals, which in times of epidemics are used in larger quantities or over shorter intervals than recommended. Another problem is the use of the same product for several consecutive years, which is typically a result of management problems and the acquisition of a large quantity of product by control organizations. These and other factors have a direct impact upon the profile of mosquito resistance to these products.

The use of chemical insecticides carries other unintended negative consequences, such as the elimination of non-target species, environmental contamination, accumulation of these products in the food chain, food contamination, and dangers to animal and human health. Misuse of these products may also lead to the “loss” of chemical molecules, and the exhaustion of possibilities for chemical control of these insects. The success of a chemical control program should be based on the monitoring of resistance profiles by location, on product rotation, and strategies to be used.

The chemical insecticides currently used for control of insect vectors of public health importance vary in mechanisms of action. Adults and larvae can be targeted using neurotoxic insecticides, while developmental regulators can be used against larvae. Organophosphates (larvae and adults), pyrethroids (adults), chitin synthesis inhibitors (larvae), and juvenile hormone analogues (larvae) are the most commonly used insecticide classes (Braga & Valle 2007; Valle *et al* 2015).

In addition to traditional forms of insecticide application by fogging (ultralow volume) and direct application in reservoirs, other forms of product dispersal have been investigated. Among them, we highlight the use of mosquito dispersers of insecticides, devices with insecticides, and the use of insecticide-impregnated clothing and fabrics.

The use of mosquitoes as insecticide dispersers is a strategy that consists in attracting female *Ae. aegypti* to small containers treated with the insecticide pyriproxyfen, called “dissemination stations.” When they come in contact with the insecticide particles in these reservoirs, the insecticide particles attach to their bodies and are passively transported to new oviposition sites. This contaminates the water in the reservoirs with insecticide, making the environment unfit for larval development and preventing adult emergence (Devine *et al* 2009; Abad-Franch *et al* 2015). According to this study, females can transport the insecticide to breeding sites up to 400 m from the dissemination station. The authors found a larval mortality rate of approximately 90% in breeding sites after the establishment of dissemination stations. Pyriproxyfen is an insect development regulator analogous to juvenile hormone, so it is non-toxic to humans. It is currently used by the Brazilian Ministry of Health as a conventional larvicide to combat dengue across the country (Abad-Franch *et al* 2015).

Another strategy that has already been evaluated is the use of devices with continuous release of insecticides. Studies performed in a domiciliary environment demonstrated that this strategy was effective at preventing mosquito bites, as well as to kill females. Mosquito biting behavior was nearly completely inhibited when exposed to 5 to 10% solutions of metofluthrin. This is a pyrethroid insect repellent, commonly used in devices known as emanators. Exposed females become disoriented and consequently seek shelter and rest. According to the authors, roughly 85% of the mosquitoes exposed to the device die in less than an hour. This device has a lifetime of approximately 20 days. Among the limitations of this technique, we highlight the difficulty of use in very large environments and the need for replacement after insecticidal effects cease (Rapley *et al* 2009; Ritchie & Devine 2013).

Another common method of chemical insecticide use is impregnation of clothes and fabrics. This method is already used in military uniforms to avoid insect bites in the wild, and is now being adapted for use with school uniforms. This strategy aims to prevent mosquito bites in students, mainly during the class period, reducing the need for use of conventional repellents. Among the limitations of this method are continuous contact with the product, the difficulty of manufacturing and maintaining these uniforms, and that students are only protected on school days, during the class period (Wilder-Smith *et al* 2011, 2012; Tozan *et al* 2014).

Screens can also be impregnated with these products, in which case deltamethrin is typically used. These screens can be installed on doors and windows of homes, schools, and health facilities. Generally, this strategy has prioritized sites close to regions with the highest number of reported mosquito-borne infections. There are some limitations to this method, such as the difficulty of large-scale use and costs of screen installation and maintenance (Baly *et al* 2011, 2012, 2016). It is important to note that practically all of these methods can contribute to the selection of insecticide-resistant mosquito populations.

Mechanical control

In general, mechanical control of mosquitoes consists of modifying the environment with the objective of eliminating, reducing, or avoiding the conditions necessary for population increase. For *Ae. aegypti*, this form of control should only be used in theory, because in practice, there is great difficulty in achieving widespread elimination of breeding sites and populations.

In general, the preferred breeding sites of *Ae. aegypti* are found inside houses, or in peridomiciliary areas near houses. Breeding sites are usually found in shady locations, in reservoirs with dark coloration, and in accumulation of clean water. Very large collections of water (such as lakes) are avoided by this species, with most breeding sites being small to medium in size and often transitional. In the vast majority of cases, *Ae. aegypti* prefers to inhabit man-made containers. When potential breeding sites are encountered, the following question should be proposed: is it necessary? The answer to this question is often “no,” in which case the breeding site should be disposed of. If the answer is “yes,” then the necessary modifications should be made to eliminate the potential of the site for mosquito breeding.

Actions to limit breeding site potential include maintenance of water reservoirs (which should remain 100% closed), potted plant trays (which should preferably be removed), tires (which should be discarded in a suitable place or kept in places that are protected from water), gutters (which must be kept clean and in perfect working order), and various containers, such as bottles, cans, jars, cups, and others (which should be disposed of properly). Special care should be taken with less obvious breeding sites, such as the drains in places seldom used (in which mosquitoes access should be blocked by screening or closure), trays behind some refrigerators (which must be screened or cleaned weekly), and any forgotten areas or structures in the home that are not frequently used and may hold water (they should be eliminated).

In periods of drought or in locations where water supply is irregular, it is of utmost importance to maintain water storage reservoirs. They must be kept 100% closed or screened, and openings in screens should be repaired to prevent entry and exit of mosquitoes. Valença *et al* (2013) concluded that this type of container may serve as a breeding site for mosquitoes in some regions. It is important to note that a poorly enclosed container can become a higher quality breeding site than a fully opened container, because partial closure (i.e., with holes or tears) protects and shades the environment while permitting mosquito entry and exit, and ideal situation for vector development.

Natural reservoirs are not of strong epidemiological importance in the maintenance, reproduction, and dispersal of *Ae. aegypti*; however, they should not be disregarded as some authors have reported encountering larvae in these sites (Varejão *et al* 2005; Gonçalves & Messias 2008). In Brazil, *Ae. aegypti* larvae have been found in natural reservoirs such as bromeliads, tree holes, bamboos, and even rock holes; however, these encounters are extremely rare when compared to the preferential reservoir types (Consoli & Lourenço de Oliveira 1994). Priority should thus be given to artificial reservoirs for detection and control of *Ae. aegypti*.

A study carried out in the Botanical Garden of Rio de Janeiro indicates that bromeliads do not constitute preferential foci of *Ae. aegypti*. A total of 120 bromeliads from ten different species were evaluated over the course of 1 year. Out of three thousand collected mosquito larvae and pupae, there were only two *Ae. aegypti* (0.07%) and five *Ae. albopictus* (0.18%) (Mocellin *et al* 2009). The authors emphasized that the Botanic Garden represents a transition area between the urban and the wild environment, and lies adjacent to the districts of Rio de Janeiro that are considered endemic for Dengue.

One proposal for mechanical control that deserves recognition is the "10 Minutes Against Aedes" campaign. This is an initiative that requests community participation in vector control by committing to spend 10 min per week identifying and removing possible breeding sites in the home. Weekly intervention eliminates adult production, because a week is typically not enough time for the mosquito to complete development from egg to adult, even under optimal conditions (which usually require 8 to 10 days). This proposal was conceived by researchers at the Oswaldo Cruz Foundation (Fiocruz), and is currently part of the control program in several states, including Rio de Janeiro and Minas Gerais (Fiocruz 2016a).

Biological control

Biological control employs natural enemies, including pathogens, predators, or parasitoids, with the potential to reduce populations of target species. Biological control of insect vectors such as *Ae. aegypti* has not been very successful despite the existence of a range of predators of immature stages, such as fish (Cavalcanti *et al* 2007) and larvae of other arthropods (Albeny *et al* 2011). This is because the success of biological control programs requires not only that the control agent feeds on the target prey but that the conditions are present to ensure efficient targeting of prey by natural enemies (e.g., seasonal synchronization with prey, adaptation to the same climatic conditions, selectivity, and use of the same habitat). Production of the control organism must also be practically feasible (e.g., high reproductive potential, ease of large-scale breeding) (Parra *et al* 2002; Van Lenteren 2009).

Predators such as fish can be used in the biological control of *Ae. aegypti* in some special cases, such as in small garden ponds, abandoned swimming pools, tanks for collecting rainwater, and other reservoirs that cannot be eliminated. This method cannot, however, be extended to the main breeding sites of the mosquito, such as plant dishes, water boxes, tires, gutters, and discarded drinking containers; its use is thus limited and impractical on a large scale.

In India, fish are commonly used to control mosquito vectors of diseases such as malaria (Chandra *et al* 2008; Kant *et al* 2013). Among the most common species are *Poecilia reticulata*, *Xiphophorus maculatus*, and *Gambusia affinis*, all exotic species. In the municipality of Alfenas, Minas Gerais, *P. reticulata* and *X. maculatus* reduced or eliminated aquatic phases of *Ae. aegypti* in the evaluated reservoirs, with predation rates up to 100% for larvae and pupae (Brasil 2016). However, some authors warn of the risks of introducing non-native fish such as *Poecilia* spp. in natural environments, such as risk of predation on native species and subsequent decline, as well as the potential to transmit diseases to native fish species (Shulse *et al* 2013; Azevedo-Santos *et al* 2016). The potential for lack of efficiency in the control of culicids should also be considered when assessing the costs and benefits of introduction.

The primary methods of biological control for *Ae. aegypti* utilize pathogens. Among the most common is the entomopathogenic bacteria *Bacillus thuringiensis* var. Israelis (Bti). These bacteria are commonly found in nature, and form spores and crystals that carry toxins with strong insecticidal activity. Bti has very high specificity, and is practically innocuous to humans and non-target fauna. Several studies have evaluated the action of Bti against *Ae. aegypti*, many with satisfactory results (Boyce *et al* 2013). Further, the combined action of the toxins produced by Bti is a positive factor for reducing the risk of acquired resistance in target populations (Andrade & Cabrini 2007). Recently, a biolarvicide based on Bti was developed by the Oswaldo Cruz Foundation in partnership with the Brazilian company BR3. DengueTech® is a product capable of eliminating *Ae. aegypti* larvae in breeding sites in less than 24 h, with residual action up to about 60 days (Fiocruz 2016b).

Among the limitations of Bti, environmental factors may interfere in insecticidal activity. Silva *et al* (2014) demonstrated that solar radiation, high temperature, and heavy rainfall negatively affected the efficiency of the commercial product VectoBac WG, a Bti-based biolarvicide. The authors concluded that the product was more effective in breeding sites protected from environmental factors, such as reservoirs in peridomiciliary areas. These results also indicate lower product effectiveness in regions with warmer climates.

Use of wolbachia bacterium

Wolbachia is a gram-negative Alphaproteobacteria from the order Rickettsiales that exhibits symbiotic relationships with its hosts (O'Neill *et al* 1992; Dumler *et al* 2001; Werren *et al* 2008). *Wolbachia* was first discovered in the reproductive tissues of *Culex pipiens* Linnaeus (Hertig & Wolbach 1924) in 1924, and was subsequently named *Wolbachia pipientis*. However, due to the large number of strains and taxonomic uncertainties of the species, the scientific community today refers to it simply as *Wolbachia* (Lo *et al* 2007).

It is an obligate intracellular bacterium, maternally transmitted from mother to offspring. It is present in about 65% of the insects, including butterflies, moths, ants, crickets, locusts, termites, beetles, flies, and mosquitoes, among others

(Hilgenboecker *et al* 2008; Oliveira *et al* 2015b). *Wolbachia* occurs in other non-insect invertebrates, such as arachnids (Gotoh *et al* 2003), crustaceans (Almerão *et al* 2012), and nematodes (Bandi *et al* 1998, 2001). It has never been found in vertebrates, and cannot be transmitted to humans (Popovici *et al* 2010).

One of the control strategies with *Wolbachia* involved the introduction of the bacterium into *Ae. aegypti*, a species in which the bacterium does not naturally occur, in order to block pathogens such as Dengue, Zika, and Chikungunya viruses. The potential of *Wolbachia* to block these and other pathogens has been described in a number of studies (Teixeira *et al* 2008; Hedges *et al* 2008; Moreira *et al* 2009; Walker *et al* 2011; Dutra *et al* 2016; Aliota *et al* 2016; Kamtchum-Tatuene *et al* 2016).

This line of research began in Australia with the successful transfer of *Wolbachia* from the fruit fly *Drosophila melanogaster* Meigen to *Ae. aegypti* using microinjection technique. This accomplishment took about 4 years and thousands of experimental attempts. After successful mosquito infection and establishment inside mosquito cells, *Wolbachia* stably persists in several mosquito tissues (Mcmeniman *et al* 2009).

After establishment of *Wolbachia*-infected *Ae. aegypti* colonies, trials were conducted in Australia in field and semi-field conditions to evaluate the potential for transfer to wild mosquitoes. A few weeks after release of these mosquitoes in the field, the proportion of mosquitoes with *Wolbachia* infection was nearly 100% of the test population (Walker *et al* 2011; Hoffmann *et al* 2011). Further, after roughly 5 years in the field with no further interventions, nearly 100% of the population still host *Wolbachia*, and the bacteria continues to block Dengue virus infection (Hoffmann *et al* 2014; Frentiu *et al* 2014), which demonstrates the safety of the method.

This invasion of infected mosquitoes is possible due to the reproductive manipulation of their hosts by *Wolbachia*, called cytoplasmic incompatibility (CI). Infected females always generate infected offspring, regardless of the infection status of male mates. When uninfected females mate with infected males, the fertilized eggs die and there is no generation of offspring. Thus, 75% of the possible crosses favor *Wolbachia*, either directly through generation of infected offspring or indirectly through preventing production of uninfected offspring (Fig 3).

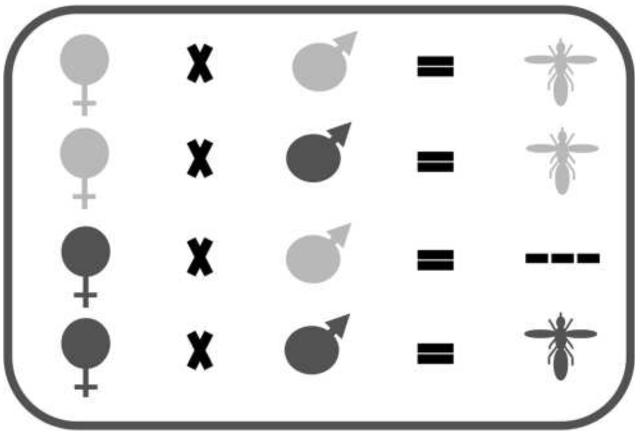

Fig 3 The simplified representative scheme of possible genetic crosses and the mechanism of cytoplasmic incompatibility in *Aedes aegypti* mosquitoes. *Light gray symbols* represent mosquitoes infected with *Wolbachia*, *dark gray symbols* represent uninfected mosquitoes, and the *dashed line* indicates no production of offspring.

An international non-profit initiative called *Eliminate Dengue: Our Challenge* has been trialing in several countries and involves the release of *Ae. aegypti* containing *Wolbachia* to replace wild mosquito populations and, therefore, reduce the transmission of arboviroses. In Brazil, the project is conduct by the Oswaldo Cruz Foundation and supported by the Ministry of Health (*"Eliminar a Dengue: Desafio Brasil"*). *Wolbachia*-containing *Ae. aegypti* were brought from Australia and backcrossed with Brazilian mosquitoes for several generations in order to insert *Wolbachia* into lab colonies while maintaining the genetic characteristics of local populations, following a model proposed by Yeap *et al* (2011). Field tests to evaluate the dispersal of mosquitoes with *Wolbachia* into wild populations in Brazil are being carried out in areas of Rio de Janeiro. Results indicate that a few weeks after release of a small number of infected mosquitoes, field populations reached infection averages near 90%. In addition to Australia and Brazil, Vietnam and Indonesia have been testing release of *Wolbachia*-infected mosquitoes and are finding similar results. Experiments are also under way in Medellín, Colombia (Callaway 2016).

There are a few points on the safety of the technique that should be highlighted. First, *Wolbachia* is an intracellular bacterium that cannot survive outside the insect cell, i.e., when the mosquito dies, it also dies. Researchers have determined that *Wolbachia* is not present in *Ae. aegypti* saliva. This is because the bacterium is not able to cross the narrow salivary duct of the mosquito, since mosquito cells are on the scale of approximately 10 μm, while the buccal apparatus of the insect salivary duct has a width of only 1 μm. Another factor that should be considered is that humans have been in contact with generations of *Wolbachia*-infected *C. quinquefasciatus* and *Ae. albopictus*, and thus far, no negative effects from bites have ever been reported. Moreover, researchers from the Eliminate Dengue Program in Australia voluntarily offered themselves as blood hosts for a *Wolbachia*-infected colony over a 5-year period, resulting in thousands of mosquito bites among them; there were no detectable negative results. Finally, it is worth highlighting that authorization by (competent) governing organizations in each country was required prior to initiation of field trials, and this bolsters the safety of the technique. Thoughtful engagement of community members is also carried out to gain majority support of the community before the release of infected mosquitoes. The greatest challenge regarding the use of *Wolbachia* as a strategy to block disease transmission by *Ae. aegypti* is expanding the proposal for use in large areas (Dutra *et al* 2015; Caragata *et al* 2016).

Another line of *Wolbachia* research proposes the release of infected males with the aim of suppressing wild mosquitoes through the mechanism of cytoplasmic incompatibility. To this end, studies are being carried out in the USA, Singapore, China, and French Polynesia for potential suppression of *Ae. aegypti* and *Ae. albopictus* (Callaway 2016).

The use of *Wolbachia* strategy to replace mosquito population has the potential of significantly decrease the transmission of several arboviroses in one given area and can easily be used in an integrated manner with other strategies. It has been recently reported that temperature can reduce *Wolbachia* load in mosquitoes (Ross *et al* 2017), which in turn could potentially reduce its blocking potential. It is important to emphasize that *Ae. aegypti* mosquitoes live inside dwellings and preferably stay in cooler environments where temperatures are much lower than the outside environmental temperature. Therefore, it is important to study the variance of *Wolbachia* density in mosquitoes as close as to the mosquito living habitats, instead of stable temperature incubators. Furthermore, the risk of reduced blocking effect by a fraction of the released *Wolbachia* mosquito population has been shown through mathematical modeling based on vector competence data from dengue patients (Ferguson *et al* 2015). *Wolbachia* can reduce from 66–75% the R0 for dengue, which, in a normal epidemic setting, would eliminate the disease transmission.

Sterile insect technique

This technique is based on the sterilization of male insects by irradiation. When released in the field, they mate with females and the cross produces inviable offspring, suppressing the population. The irradiation consists of minimal doses of X-rays or gamma rays, after which random chromosomal rearrangements occur that are capable of provoking sterilization in males. Studies involving male sterilization by irradiation began in the 1960s, and the first targets of this technique were insect agricultural pests (Knipling 1955), such as the fruit fly *Ceratitis capitata* Wiedemann.

Despite the success of sterile insect technique (SIT) in agricultural pests, some authors suggest that the use of this technique in *Ae. aegypti* is difficult to practically impossible. Ferreira *et al* (2008) argue that this is mainly due to heterogeneous spatial distribution of breeding sites and mosquito breeding behavior. In addition, Valle *et al* (2015) point out that mosquitoes are very small, delicate insects which are not very resistant to irradiation and difficult to manipulate, making them unsuitable organisms for this methodology. Despite these limitations, some authors support the possibility of SIT application as a method of control for mosquito vectors, and suggest this alternative as a strategy to avoid or decrease the use of chemical insecticides (Alphey *et al* 2010, 2013).

Genetically modified mosquitoes carrying lethal gene— RIDL

Another technique that is based on the use of sterile males, known as an "evolution" of SIT, is the release of insects carrying a dominant lethal gene (RIDL). The method consists of release of sterile male *Ae. aegypti* carrying a lethal gene for offspring, which functions by producing a protein that prevents offspring from reaching adulthood. In addition to the lethal gene, these mosquitoes also have a fluorescent gene for identification under ultraviolet light (Oliveira *et al* 2011). This technology was developed in 2002 by researchers at the University of Oxford in the UK, and was realized through microinjection of the two specific genes into *Ae. aegypti* DNA (producing the OX513A strain). This mosquito is produced in the laboratory by the British company Oxitec, separated, and released in large quantities in the field. Males carrying the lethal gene mate with wild females, which then generate inviable offspring. A large number of mosquitoes must be released because OX513A males have reduced fitness compared to wild mosquitoes, and may only survive about 5 days. To allow these mosquitoes to reproduce and generate viable offspring in the lab, an antidote is deployed (tetracycline antibiotic) via supplementing along with insect feed (Fig 4).

Tests with these transgenic mosquitoes in Brazil were initiated in Bahia state in the cities of Jacobina and Juazeiro, and resulted in an 80–95% reduction of wild mosquito populations (Carvalho *et al* 2015; Oxitec Brasil 2016). In 2015, the

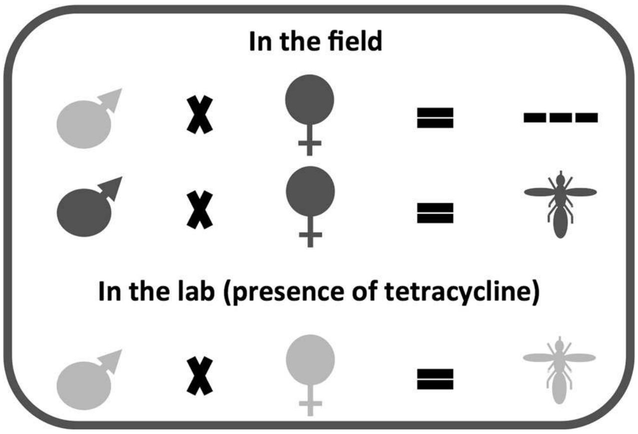

Fig 4 Simplified representative scheme of crosses involving transgenic *Aedes aegypti* mosquitoes. *Light gray symbols* represent transgenic mosquitoes carrying the lethal gene, *dark gray symbols* represent mosquitoes without the lethal gene, and the *dashed line* indicates inviable offspring.

project started in the city of Piracicaba, São Paulo. According to Oxitec, preliminary results showed an 82% reduction of larvae in the wild *Ae. aegypti* population. In addition, in the same period, a 91% drop in dengue cases in this region was reported. Similar results were also found following the release of OX513A in the Cayman Islands in 2010, with 80% suppression of the wild mosquito population (Harris *et al* 2011, 2012). These studies demonstrate the potential of this technology for suppression of wild *Ae. aegypti* populations.

Several difficulties have been encounter in the use of transgenic *Aedes*. First, the large number of mosquitoes necessary for release is prohibitive. For transgenic mosquitoes to be effective, an estimated 10 transgenic mosquitoes should be released for each wild mosquito, which requires release of approximately 20 million mosquitoes per week in a small area (e.g., a town of about 10,000 inhabitants) in the first 4 to 6 months of the protocol. The constant release events are non-sustainable due to high cost, and because separation of sexes is not perfect, a small number of females may be released (less than 0.05%). Further, about 5% of the mosquitoes can survive the lethal gene, and the general population is hesitant to support transgenic mosquito release (Oxitec Brasil 2016; Wallace 2013).

Final Considerations

The success of *Ae. aegypti* with respect to development and reproduction, diversity of breeding sites, high dispersal capability, colonization of urban environment, and the transmission of diseases is becoming increasingly clear. This highlights the importance and necessity of using all available tools for controlling *Ae. aegypti* populations and, consequently, the diseases they transmit. The community support of initiatives as well as the interconnection among the various tools available are crucial for successfully combating this vector, particularly in light of the triple epidemic that Brazil is currently experiencing.

Several factors should be considered to enhance effectiveness of current control programs, including compatibility with other techniques, safety of the proposal, sustainability, selectivity, cost-effectiveness, and feasibility for large-scale production and implementation. Issues such as basic sanitation, adequate residual and solid waste management, regular water supply, health education, globalization, tourism, and the intense human movement are factors that should be prioritized, and these issues should be addressed within and linked to public health policy as a strategy for forming sound proposals that include all sectors of society.

There is also an obvious need for alternative methods of mosquito vector control. In February 2016, the Ministry of Health published a technical note suggesting that pilot projects using the release of *Wolbachia*-infected and transgenic mosquitoes should be carried out in Brazil. This has also been suggested by the World Health Organization.

Our attention should be focused on the possibility of new epidemics, as well as the entry of other pathogens into the country. We are currently on alert with the possible entry of a new virus or strain of the Mayaro virus in South America. Although the disease is transmitted primarily by wild mosquitoes of the genus *Haemagogus*, there is a great deal of concern about the possibility of viral adaptation to urban vectors such as *Ae. aegypti*.

Finally, the fight against *Aedes* must be continuous, with integrated control actions executed steadily through all seasons, not only during periods of abundant rain and high heat. The fight against this medically important vector should be maintained by the government and should attain year-round community support. With our participation, we can reduce the adaptive success of *Ae. aegypti*, thereby reducing the impact of arboviruses on human populations.

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