Male accessory gland substances from *Aedes albopictus* affect the locomotor activity of *Aedes aegypti* females

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Dengue is one of the world's most important mosquito-borne diseases and is usually transmitted by one of two vector species: Aedes aegypti *or* Aedes albopictus*. These two diurnal mosquitoes are frequently found coexisting in similar habitats, enabling interactions between adults, such as cross-mating. The objective of this study was to assess cross-mating between* Ae. aegypti *females and* Ae. albopictus *males under artificial conditions and evaluate the locomotor activity of* Ae. aegypti *virgin females injected with male accessory gland (MAG) homogenates to infer the physiological and behavioural responses to interspecific mating. After seven days of exposure, 3.3-16% of* Ae. aegypti *females mated with* Ae. albopictus *males. Virgin* Ae. aegypti *females injected with conspecific and heterospecific MAGs showed a general decrease in locomotor activity compared to controls and were refractory to mating with conspecific males. The reduction in diurnal locomotor activity induced by injections of conspecific or heterospecific MAGs is consistent with regulation of female reproductive activities by male substances, which are capable of sterilising female* Ae. aegypti *through satyrisation by* Ae. albopictus*.*

Key words: *Aedes aegypti* - *Aedes albopictus* - cross-mating - locomotor activity - laboratory

Dengue is one of the most important mosquito-borne diseases in the world, being endemic in approximately 112 countries, with an estimation of more than 300 million cases per year (Bhatt et al. 2013). The etiologic agent is a RNA Flavivirus, that is typically transmitted to humans by the mosquito *Aedes (Stegomyia) aegypti* (Linnaeus, 1762). Another vector of dengue of increasing importance worldwide is *Aedes (Stegomyia) albopictus* (Skuse, 1894) (Consoli & Lourenço-de-Oliveira 1994).

Originating from Asia, *Ae. albopictus* invaded the United States of America (USA) and Brazil in the 1980s and, despite its high susceptibility to dengue virus infection under laboratory conditions (Miller & Ballinger 1988, Lourenço-de-Oliveira et al. 2003, de Castro et al. 2004), it is still considered a potential dengue vector in Brazil, although dengue viruses have been isolated by pools of larvae in nature (Serufo et al. 1993, Degallier et al. 2003, Figueiredo et al. 2010).

Although *Ae. aegypti* is usually associated with urban areas and *Ae. albopictus* prefers habitats with more vegetation (Consoli & Lourenço-de-Oliveira 1994, Braks et al. 2003, Lima-Camara et al. 2006, Rey et al. 2006), these species may be sympatric in urban and suburban areas, where they may coexist in the same larval habitats (Braks et al. 2003, Honório et al. 2009). In addition, *Ae. aegypti* and *Ae. albopictus* adults frequently show bimodal diurnal activity periods under natural and artificial conditions, flying, mating and blood-feeding preferentially during the morning and afternoon hours (Hartberg 1971, Gubler & Bhattacharya 1972, Lima-Camara 2010).

The invasion of *Ae. albopictus* in the USA in the 1980s led to declines in distribution and abundance of *Ae. aegypti* in the southeast of that country (Lounibos 2002). Among the explanations proposed to explain the displacement of *Ae. aegypti* populations by *Ae. albopictus,* the most commonly cited is asymmetric interspecific larval competition, which usually favours *Ae. albopictus*, under conditions of limited resources commonly encountered in their container habitats (Juliano 1998, Daugherty et al. 2000, Braks et al. 2004).

Nevertheless, in areas where *Ae. aegypti* and *Ae. albopictus* are sympatric, these species swarm and mate during the same morning and afternoon peaks of activity (Nasci et al. 1989, Honório et al. 2009), which could facilitate asymmetric mating interference between them, a phenomenon called satyrisation (Ribeiro & Spielman 1986, Ribeiro 1988, Tripet et al. 2011). *Ae. aegypti* and *Ae. albopictus* females are usually inseminated only once by males of their own species, because accessory gland (AG) substances transferred from males make mated females refractory to subsequent inseminations (Craig Jr 1967). Thus, cross-mating interference could sterilise a female that cannot produce viable offspring (Leahy & Craig Jr 1967).

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Satyrisation between these species has been documented under laboratory conditions (Nazni et al. 2009) and in the field (Nasci et al. 1989, Tripet et al. 2011). Under laboratory conditions, Nazni et al. (2009) reported successful bidirectional cross-mating between *Ae. albopictus* and *Ae. aegypti*, which was followed by oviposition of sterile eggs. Results of cage experiments by Nasci et al. (1989) and Bargielowski et al. (2013) suggested that *Ae. aegypti* females are more receptive to insemination by *Ae. albopictus* males than *Ae. albopictus* females are receptive to insemination by *Ae. aegypti* males. In addition, implants of heterologous male AGs (MAGs) resulted in oviposition of sterile eggs by *Ae. aegypti* females, but no oviposition by *Ae. albopictus* females (Leahy & Craig Jr 1965). Tripet et al. (2011) also injected heterologous MAG in *Ae. aegypti* and *Ae. albopictus* virgin females and reported sterilisation for the former species, but no effect on the ability of *Ae. albopictus* to mate with their own species. Tripet et al. (2011) therefore proposed asymmetric mating interference as a potential neglected explanation for the observed displacement of *Ae. aegypti* populations in areas where *Ae. albopictus* invaded, such as the southeastern USA and Bermuda (Lounibos 2007, Kaplan et al. 2010).

Locomotor activity analysis of mosquitoes can be useful for testing the effect of external or internal factors, such as dengue infection on adult female performance (Lima-Camara et al. 2011). Moreover, previous studies have shown that mating has a significant effect on flight activity of many mosquito species, including *Ae. aegypti* (Clements 1999). Indeed, the AGs of male mosquitoes may produce substances that are transferred to females during mating and alter their physiology and behaviour. The effects of such substances include the inhibition of female remating, stimulation of oviposition and preoviposition behaviours and the inhibition of host-seeking behaviour (Klowden 1996, 1999).

The objective of this study was to verify the frequency of cross-mating between *Ae. aegypti* females and *Ae. albopictus* males under artificial conditions and evaluate the locomotor activity of *Ae. aegypti* virgin females injected with conspecific vs. heterospecific AGs to infer their physiological and behavioural responses to interspecific mating.

MATERIALS AND METHODS

Mosquito rearing - *Ae. aegypti* and *Ae. albopictus* eggs were obtained from colonies at Laboratory of Transmitters of Hematozoa, at Oswaldo Cruz Foundation, state of Rio de Janeiro (RJ), Brazil. Both *Ae. aegypti* and *Ae. albopictus* colonies are maintained with eggs collected from areas of sympatry in RJ since 1987. Larvae were reared in plastic trays with tap water and fish food (Tetramin®). Trays were kept in an incubator with photoperiod of light/dark (LD) 12:12, at 27ºC and 80% relative humidity. Pupae of both species were isolated individually to ensure virgin males and females after emergence.

Dissection of MAGs for injection of Ae. aegypti females - Dissected AGs of 25 virgin four-six day-old *Ae. aegypti* and 25 *Ae. albopictus* males (Consoli & Lourenço-de-Oliveira 1994) were stored in 50 µL of saline. Dissections in saline were made under a stereoscopic microscope. Each injecting solution was prepared with 50 pairs of AGs in 50 µL of saline. Prior to injections, this AG solution was sonicated for 1 min and centrifuged for 1 h at 13,000 *g*.

Five-seven-day-old virgin *Ae. aegypti* females were individually injected by intrathoracic inoculation either with 0.28 µL of saline (control group), 0.28 µL of AG solution of *Ae. aegypti* males or 0.28 µL of AG solution of *Ae. albopictus* males (MAG groups) using a Nanoject microinjector (Drummond Scientific). Since each solution contained one AG/µL, we injected more than a quarter (0.28) of an AG into each *Ae. aegypti* female. This dose is sufficient for females to respond as if they have been inseminated, since each MAG apparently contains enough active material to sterilise at least 64 females (Craig Jr 1967).

Analysis of locomotor activity - The activity of *Ae. aegypti* and *Ae. albopictus* in treatment and control groups was recorded using a larger version of the Drosophila Activity Monitor (TriKinetics) as described in Lima-Camara et al. (2011). After inoculation with saline or AGs, each *Ae. aegypti* female was individually placed in a glass tube (1 cm x 7 cm) with a cotton plug soaked in 15% sucrose solution and the tube was placed in the Activity Monitor inside a Precision Scientific Model 818 Incubator under a constant temperature of 25ºC and a photoperiod of 12 h of light and 12 h of dark (LD 12:12). For each mosquito, the total locomotor activity during 30 min-intervals was recorded continuously for six days after inoculation. We calculated the William's mean (Wm) as an estimate of the central tendency of activity during each time interval (Gentile et al. 2009, Brito et al. 2013). Wm is a modified geometric mean that accommodates frequent zero values (Williams 1937). Only data from individuals that lived until the fourth day after inoculation were retained and the data analysis was carried out comparing the activity of control and AG-injected groups from 24 h (1st day) to 96 h (4th day) after inoculation. At the end of the locomotor activity experiment, all live *Ae. aegypti* females of each group were transferred to 17 cm x 17 cm cages and exposed to conspecific males for 48 h in a ratio of 1 female:2 males. After 48 h, all three spermathecae were dissected from live females for detection of *Ae. aegypti* sperm.

Cross-mating experiment - To assess the frequency of cross-mating between *Ae. aegypti* females and *Ae. albopictus* or *Ae. aegypti* males under conditions in our laboratory, approximately 30 virgin *Ae. aegypti* females were placed in a 17 cm x 17 cm cage, with 40 virgin *Ae. aegypti* or *Ae. albopictus* males. Three-five-day-old females and three-five-days-old males were placed together in these cages in two different combinations (*Ae. aegypti* females x *Ae. aegypti* males and *Ae. aegypti* females x *Ae. albopictus* males) for seven days. Each pairing was replicated three times, for a total number of six cages. Cotton soaked in a 15% sucrose solution was continuously available for all mosquitoes as a sugar source.

After seven days of exposure to *Ae. aegypti* or *Ae. albopictus* males, spermathecae of all live *Ae. aegypti* females were removed and examined under a compound microscope at 100X magnification.

Statistical analysis - For statistics analysis, we used locomotor activity values of all mosquitoes transformed to log+1. The daily locomotor activity of control and treatment groups was summarised by five indices: (i) the mean total activity, (ii) the mean diurnal activity, (iii) the mean diurnal activity without lights-on, i.e., the mean activity during the photophase except for the first 30 min, which corresponds to the morning peak, (iv) the mean lights-on, which corresponds to the first 30 min just after the lights-on and (v) the mean nocturnal activity. Two-way ANOVA (in conjunction with Tukey test) was applied to test differences in the locomotor activity, measured by these indices, between control (saline injected) vs. MAG groups (injections with *Ae. aegypti* male's AG or *Ae. albopictus* male's AG). The second factor in the two-way ANOVA is "block", since the locomotor study was carried out twice. All statistics were done in R Program version 2.15.1 (R Core Team 2012).

RESULTS

In both control and treatment groups, *Ae. aegypti* females showed a bimodal rhythm, with peaks at the lights-on and the lights-off (Figs 1, 2). The lights-on peak was higher in the control group whereas, in general, *Ae. aegypti* females injected with heterospecific MAG substance showed the highest peak at lights-off (Figs 1, 2). *Ae. aegypti* females injected with MAGs showed a general decrease in all activity indices compared to controls (Table I), showed by lower arithmetic means.

The total activity of the three tested groups showed a significant effect of status (injected with saline vs. injected with AG) [$F = 10.536$; degree of freedom (df) = 2; $p < 0.001$], but no significant block effects ($F = 0.292$; df = 1; $p = 0.590$) or interaction between block and status ($F = 1.699$; df = 2; $p = 0.186$). Tukey test (Fig. 3) showed a significantly reduced activity of *Ae. aegypti* virgin females injected with conspecific MAG as compared to the control group ($p < 0.001$). The total locomotor activity of females injected with *Ae. albopictus* MAG was lower than the control group as well (Table I), but the difference was not statistically significant ($p = 0.156$), remaining higher than those injected with conspecific MAG (Fig. 3). When the locomotor activity is stratified according to time of day, it becomes evident that only the diurnal behaviour is affected by MAG (Fig. 3). Considering the lights-on peak, that is, the transition between dark and light provides the sharpest discrimination among groups with both MAG-injected groups showing significantly less activity than the control group, with conspecific MAG inducing similar decrease of heterospecific MAG.

At the end of the locomotor activity experiments, live *Ae. aegypti* females of all the three tested groups were exposed to conspecific males for 48 h. Most *Ae. aegypti* females injected with saline (control) had positive spermathecae (84%), that is, they were inseminated by conspecific males within 48 h. On the other hand, no *Ae. aegypti* females injected with conspecific MAG were inseminated by conspecific males (Table II). Similar results were observed for *Ae. aegypti* females injected with *Ae. albopictus* MAG, among whom 97.3% (36/37) were unmated after 48 h, despite the heterospecific AG injection (Table II).

Cages 1-3 of the spontaneous mating experiment contained 30 *Ae. aegypti* females and 40 *Ae. aegypti* males each and, respectively, 90% (27/30) and 100% (27/27) of *Ae. aegypti* females in cages 1-3 were inseminated by their conspecific males. In cage 2, 35% (8/23) of *Ae. aegypti* females were inseminated by their conspecific males.

Cages 4-6 exhibited limited cross-mating between 30 *Ae. aegypti* females and 40 *Ae. albopictus* males, but most *Ae. aegypti* females remained uninseminated after seven days: 96% (24/25) in cage 4, 96.7% (29/30) in cage 5 and 83.3% (20/24) in cage 6 (Table III).

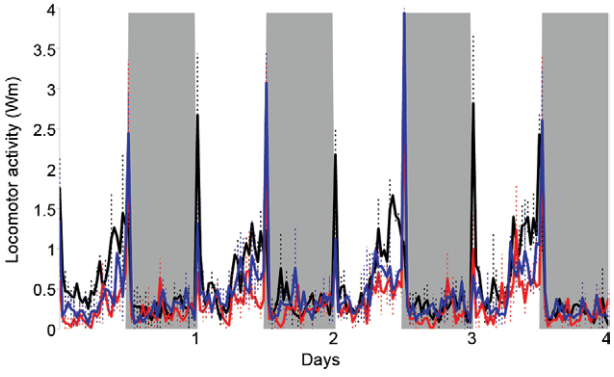

Fig. 1: William's mean (Wm) of locomotor activity of *Aedes aegypti* females injected with saline (black line-control group), accessory gland (AG) of *Ae. aegypti* males (red line) and AG of *Aedes albopictus* males (blue line) exposed to four days of 12 h of light (white columns) and 12 h of dark (grey columns) (light/dark: 12:12) at 25ºC. Dotted lines represent the standard error of the Wm.

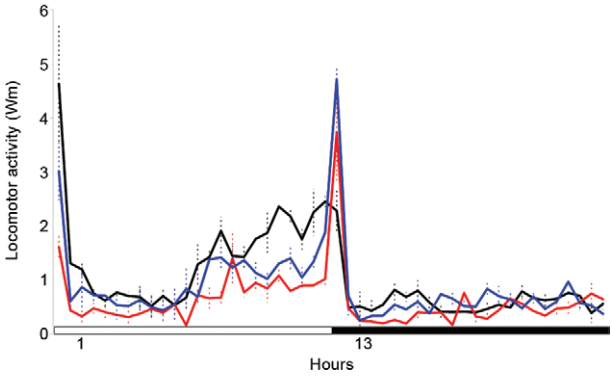

Fig. 2: William's mean (Wm) of locomotor activity of *Aedes aegypti* females injected with saline (black line-control group), accessory gland (AG) of *Ae. aegypti* males (red line) and AG of *Aedes albopictus* males (blue line) under (light dark: 12:12) at 25ºC. Lines represent the 30 min mean activity (± standard error) of control and male AGs injected females in the four tested days.

DISCUSSION

In this study, the consequences of mating interactions between two important arboviruses vectors - *Ae. aegypti* and *Ae. albopictus* - were reported. This is the first time that the locomotor activity of *Ae. aegypti* females injected with saline, conspecific and heterospecific MAG extracts has been evaluated.

Both conspecific and heterospecific MAGs induced a reduction in the intensity of diurnal activity while keeping the overall bimodal pattern, with peaks of activity at lights-on and lights-off. In agreement with our results, Jones (1981) reported similar patterns of flight activity in virgin and inseminated *Ae. aegypti* females under LD 12:12. Activity reduction associated with MAG injection suggests that females interpreted the injected materials as a signal of insemination. Previous studies suggest that inseminated *Aedes* females show changes in their activity patterns, because MAGs produce several specific proteins that are transferred to females during mating, influencing reproductive and feeding behaviour (Klowden 1996, 1999, Gillott 2003). Changes in the pattern of activity of inseminated females have also been described for mosquitoes of other genera, such as *Anopheles gambiae* (Jones & Gubbins 1977, 1978) and *Anopheles stephensi* (Rowland 1989).

Activity reduction after heterospecific MAG injection suggests that *Ae. albopictus* males may induce behavioural changes in *Ae. aegypti* through mating. However, a few differences were observed between the influences of *Ae. aegypti* and *Ae. albopictus* MAGs. The total mean activity of *Ae. aegypti* injected with *Ae. albopictus* MAG extract did not show a significant decrease compared with the control group whereas *Ae. aegypti* injected with conspecific MAG extract showed a significant decrease. Similar activity reduction in both conspecific and heterospecific injection groups is observed during the lights-on peak. These differences may be explained by small differences in MAG composition between species or differences in the female receptor sites. In fact, *Ae. aegypti* MAG seems to produce several non-homologous proteins that are not found in other Diptera males, such as *Culex pipiens*, *An. gambiae*, *Drosophila melanogaster* (Sirot et al. 2008) and, probably, *Ae. albopictus*. This deserves future investigations.

Further evidence of the successful mating signal induced by *Ae. albopictus* MAG in *Ae. aegypti* females comes from the mating experiments. After exposing saline injected *Ae. aegypti* females to conspecific males for 48 h, most control females successfully mated, while those injected either with *Ae. aegypti* or *Ae. albopictus* MAGs remained sperm-negative, indicating that MAG injections from males of either *Aedes* species were capable of making the *Ae. aegypti* female refractory to further insemination. Our results agree with Craig Jr (1967) who implanted several *Ae. aegypti* male tissues in the thorax of virgin *Ae. aegypti* females and, after 24 h of recovery, exposed them to conspecific males for an additional 24 h. The author dissected the spermathecae for sperm detection and, as in our study, most (at least 85%) virgin *Ae. aegypti* females injected either with saline, testis and gut tissues were inseminated while MAG injected females showed no sign of insemination after 8 h of exposure to conspecific males. Interestingly, when females were exposed to males immediately after injection, 26% were inseminated, suggesting that several hours are required before the sterilising effect of MAG is accomplished (Craig Jr 1967).

Shutt et al. (2010) injected virgin *Ae. aegypti* and anopheline females (*An. stephensi* and *An. gambiae* S and M molecular forms) with their conspecific MAG homogenates and reported a drastic reduction on the likelihood of subsequent mating with conspecific males.

TABLE I

Arithmetic means (± standard error) of total, diurnal, nocturnal, diurnal without lights-on and light-on means of *Aedes aegypti* females injected with saline, accessory gland (AG) of *Ae. aegypti* males and AG of *Aedes albopictus* males

	Saline (n = 64)	*Ae. aegypti* male AG (n = 56)	*Ae. albopictus* male AG (n = 70)	F test[a]
Total mean	3.01 ± 0.30	1.75 ± 0.24 (-41.86)	2.63 ± 0.27 (-12.6)	F = 10.536; df = 2; p < 0.001
Diurnal mean	3.72 ± 0.37	2.00 ± 0.32 (-46.23)	3.17 ± 0.34 (-14.78)	F = 18.443; df = 2; p < 0.001
Nocturnal mean	2.30 ± 0.39	1.50 ± 0.28 (-34.78)	2.09 ± 0.31 (-9.13)	F = 1.712; df = 2; p = 0.183
Diurnal mean without lights-on	3.49 ± 0.37	1.87 ± 0.32 (-46.42)	2.97 ± 0.35 (-14.9)	F = 16.519; df = 2; p < 0.001
Light-on mean	9.06 ± 1.69	4.84 ± 1.56 (-46.58)	7.64 ± 1.76 (-15.67)	F = 14.702; df = 2; p < 0.001

a: result of ANOVA testing variation among three groups. Transformed log + 1 values were used for statistics. Numbers inside parenthesis represent the percentage of decrease (-) of means of *Ae. aegypti* females injected with *Ae. aegypti* and *Ae. albopictus* AG in relation to the control group (saline). df: degrees of freedom.

Moreover, the authors cross-injected MAG extracts from M molecular form from males into S molecular form females and *vice-versa* in order to test the divergence in MAG proteins for refractoriness to further mating. These authors found refractoriness for further mating in cross-injected *An. gambiae* M and S molecular forms, which suggests that these forms have not diverged in relation to sex peptides responsible for female monogamy.

Our data also corroborates the results of Tripet et al. (2011). These authors cross-injected *Ae. aegypti* and *Ae. albopictus* females and reported that the majority of *Ae. albopictus* females injected with *Ae. aegypti* MAG subsequently mated with conspecific males. In contrast, *Ae. aegypti* and *Ae. albopictus* females injected with conspecific MAG, as well as *Ae. aegypti* females injected with *Ae. albopictus* MAG, were refractory when exposed to conspecific males.

Although multiple reproductive barriers isolating *Ae. aegypti* and *Ae. albopictus* have been identified (Leahy & Craig Jr 1967), cross-mating between these two species is reported in the field, although at a low frequency (Tripet et al. 2011). Under laboratory conditions in the absence of conspecifics, Bargielowski et al. (2013) reported a high cross-mating frequency (> 30%) between *Ae. aegypti* females and *Ae. albopictus* males when they were exposed for three weeks. In our study, using cage

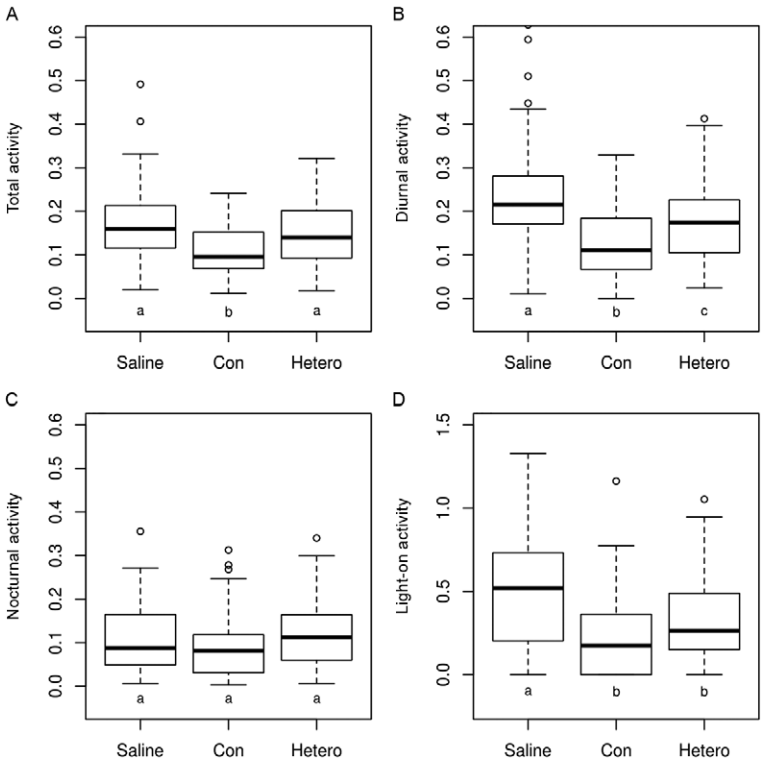

Fig. 3: boxplots of means of *Aedes aegypti* females injected with saline (n = 64), accessory gland (AG) of *Ae. aegypti* males (Con) (n = 56) and AG of *Aedes albopictus* males (Hetero) (n = 70). A: total activity (diurnal + nocturnal); B: diurnal activity; C: nocturnal activity; D: lights-on activity. Different letters identify groups that were statistically different ($p < 0.05$) according to the Tukey test.

TABLE II

Number and percentage of positive and negative dissected spermathecae of *Aedes aegypti* females injected with saline, accessory glands (AGs) of *Ae. aegypti* males and AGs of *Aedes albopictus* males and exposed to conspecific males for two days

Injection	Dissected females (n)	Positive spermathecae n (%)	Negative spermathecae n (%)
Saline (control)	25	21 (84)	4 (16)
Ae. aegypti male's AGs	26	0 (0)	26 (100)
Ae. albopictus male's AGs	37	1 (2.7)	36 (97.3)
Total	88	22 (25)	66 (75)

TABLE III

Number and percentage of negative and positive spermathecae of *Aedes aegypti* females after being exposed to *Ae. aegypti* and *Aedes albopictus* males for seven days

	Ae. aegypti ♀ x *Ae. aegypti* ♂		
	Inseminated n (%)	Uninseminated n (%)	Total n
Cage 1	27 (90)	3 (10)	30
Cage 2	8 (36.4)	14 (63.6)	22
Cage 3	27 (100)	0 (0)	27
	Ae. aegypti ♀ x *Ae. albopictus* ♂		
	Inseminated n (%)	Uninseminated n (%)	Total
Cage 4	1 (4)	24 (96)	25
Cage 5	1 (3.3)	29 (96.7)	30
Cage 6	4 (16.7)	20 (83.3)	24

each cage previously contained 30 virgin *Ae. aegypti* females and 40 virgin *Ae. aegypti* (3 cages) or *Ae. albopictus* males (3 cages). All live *Ae. aegypti* females had their spermathecae dissected after the seven days.

experiments, we found a range of 3.3-16% *Ae. aegypti* females inseminated by *Ae. albopictus* males. This is low compared with the conspecific mating rate (> 90% in all cages, but cage 2 with 35% insemination). It is important to point out that even with the relatively low mating frequency between *Ae. aegypti* females and *Ae. albopictus* males observed in our study (3.3-16%) under laboratory conditions, mathematical models of satyrisation predict that parapatry or extinction can occur under conditions of 5% or less of interspecific matings (Ribeiro 1988).

Since a higher cross-mating frequency (> 30%) between *Ae. aegypti* females and *Ae. albopictus* males have been reported under laboratory conditions when adults were exposed for three weeks (Bargielowski et al. 2013), we might infer that the lower cross-mating frequency (3.3-16%) reported in this study might be associated with the number of days *Ae. aegypti* females were exposed to *Ae. albopictus* males or cage size or previous history of the *Ae. aegypti* population, which is capable of evolving resistance to satyrisation (Harper & Paulson 1994, Bargielowski et al. 2013).

Matings between wild *Ae. albopictus* and transgenic *Ae. aegypti* have been observed under laboratory conditions (Lee et al. 2009). In homologous mating between *Ae. aegypti* female/*Ae. aegypti* male and *Ae. albopictus* female/*Ae. albopictus* male, the authors reported a frequency of 87.78% and 85.56% of inseminated females, a total of 2,310 and 2,780 eggs laid and 98% and 97% of hatched eggs, respectively. Nevertheless, for reciprocal mating between *Ae. aegypti* female and *Ae. albopictus* male, there was two inseminated females (2.2%) and 590 unviable eggs laid (0% hatched) (Lee et al. 2009). This insemination frequency (2.2%) was similar with the frequencies we verified in our experiments (3.3% and 3.6%). Also using natural cage mating technique, Nazni et al. (2009) reported that cross mating between *Ae. aegypti* females and *Ae. albopictus* males produced more eggs than that between *Ae. albopictus* females and *Ae. aegypti* males, although none of them was viable. Similar results were reported earlier by Leahy and Craig Jr (1965).

For the first time, it was demonstrated that *Ae. albopictus* MAG decreases the diurnal locomotor activity of *Ae. aegypti* females in a similar way of *Ae. aegypti* MAG. In addition, this study confirmed that *Ae. albopictus* MAG makes *Ae. aegypti* females refractory to mating with conspecific males. Cross-mating between these two dengue vectors could partly explain the displacement of *Ae. aegypti* by *Ae. albopictus* and the high frequency of unviable eggs that has been observed in some areas of RJ. Moreover, we might speculate that this mating error in nature could reduce the vector capacity of an infected *Ae. aegypti* female by eliminating virus transmission to its offspring (vertical transmission).

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