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AN IMPROVED TRAP TO CAPTURE ADULT CONTAINER-INHABITING MOSQUITOES

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Abstract

Although dengue viruses are thought to be transmitted by *Aedes aegypti* in Puerto Rico, *Aedes mediovittatus*, the Caribbean tree hole mosquito, is also a potential vector. This species is native to the Greater Antilles and has been shown to be a competent vector of dengue viruses in the laboratory. Consequently, it has been suggested that *Ae. mediovittatus* could be acting as a secondary vector or virus reservoir. This study was part of an ongoing investigation into this, and it aimed to determine whether BG-Sentinel traps (BGS traps) could be used to collect adults of this mosquito and could be modified to increase the number of captures of this species in the field. We conducted experiments to test the relative attractiveness of BGS traps to *Ae. mediovittatus* and *Ae. aegypti* and explored the effects of chemical lures (BG-Lure, CO_2, octenol) and optical properties (color, size) on the capture rates of BGS traps in a large, outdoor cage in San Juan city, Puerto Rico. We also conducted field tests to compare modified BGS traps with the original traps in a rural community in Patillas municipality, Puerto Rico. Results obtained from the large, outdoor cage experiments indicated that trap captures of both mosquito species could be significantly enhanced by using black instead of white BGS traps combined with BG-Lure. Field experiments revealed that the modified traps captured a significantly greater number of *Ae. aegypti*, *Ae. mediovittatus*, and *Culex quinquefasciatus*, with greater sensitivity for the latter 2 species, and also captured a larger number of mosquito species and a smaller ratio of *Ae. aegypti* to *Ae. mediovittatus*, with greater than expected species co-occurrences.

Keywords

Aedes aegypti; *Aedes mediovittatus*; dengue; vector surveillance; BG-Sentinel traps

INTRODUCTION

The various species of mosquitoes that transmit dengue viruses are diurnal, anthropophilic biters, whose immature stages develop in both natural and artificial containers, and whose adult stages do not usually reach high densities (Moore et al. 1978, Gubler 1998). It has always been difficult to trap the adults of these mosquitoes (e.g., *Aedes aegypti* (L.), *Ae. albopictus* (Skuse), *Ae. polynesiensis* (Marks)) for surveillance purposes (Reiter and Gubler 1997). Traditionally, human bait catches and mechanical aspirators have been used indoors

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or outdoors to collect adults of these species. However, both methods have the disadvantage of being labor intensive and dependent on the skill and effort of the collector to trap mosquitoes. Several fan-operated traps have been specifically developed to capture *Ae. aegypti* adults (Fay 1968, Fay and Prince 1970, Wilton and Kloter 1985), which take advantage of the propensity of this species to be attracted to dark objects. Among these traps, the Fay-Prince trap has been the most widely used in field studies, but this trap has the disadvantage of being heavy and bulky, thus preventing its deployment in sufficient numbers to enable reliable estimation of mosquito populations. Jensen et al. (1994) showed that the bidirectional Fay-Prince trap (contrasting shiny black and white colors) captured more *Ae. aegypti* and *Ae. albopictus* than the modified omnidirectional Fay-Prince trap (dark color), the duplex cone trap (white and black colors; Freier and Francy 1991), or the Centers for Disease Control and Prevention (CDC) miniature light trap (white color; Sudia and Chamberlain 1962) in northern Florida. The Fay-Prince trap also collected numerous adult specimens of Culex quinquefasciatus Say, a mosquito species that is not efficiently collected in light traps (Smith et al. 2009).

The recently developed BG-Sentinel trap (BGS trap [Biogents, Regensburg, Germany]; Kröckel et al. 2006) is collapsible and lighter in weight than the Fay-Prince trap. It uses the same principle of visual attraction as the Fay-Prince trap (white and black color contrast): the exterior of the trap (cylindrically shaped) is white, and there is a black collecting cylinder on the top center of the trap, which is remarkably similar to Wilton and Kloter's (1985) ''Black Cylinder'' trap. There is a fan at the bottom of the cylinder that draws air, and mosquitoes that are sucked in are retained in a black nylon bag placed before the fan. Air drawn into the trap exhausts upward, through the white gauze that covers the top, so that volatile chemicals acting as olfactory lures inside the trap are carried over the trap to attract mosquitoes (Biogents 2013).

The developers of the BGS trap sell a disposable dispenser that contains a mixture of lactic acid, ammonia, and caproic acid (BG-Lure), all of which are found on human skin (Kröckel et al. 2006). The BG-Lure was developed as an alternative to the use of CO_2, which is an effective attractant for *Ae. aegypti* and sensitizes the females of this mosquito to human skin odors (Dekker et al. 2005), but is logistically difficult to use when many traps are deployed. BG-Sentinel traps with BG-Lure and CO_2 captured more *Ae. albopictus* than traps without CO_2 in northern Virginia (Meeraus et al. 2008), and BGS traps with BG-Lure plus CO_2 and octenol captured more females of this species than BGS without lures (Farajollahi et al. 2009). However, Williams et al. (2006a) found that 4 geographic strains of *Ae. aegypti* significantly differed in their attraction to BGS traps utilizing chemicals found on human skin; for example, they captured similar numbers of mosquitoes from a population in north Queensland in BGS traps with and without human skin lures and suggested that the visual properties of the trap were more important than the olfactory cues for this geographic strain. Laboratory experiments have shown that BGS traps without lures capture similar numbers of *Ae. aegypti* and *Ae. albopictus* as BGS traps with either CO_2 or octenol (Kawada et al. 2007).

BG-Sentinel traps have been shown to be more effective at capturing *Ae. aegypti* than CDC backpack aspirators, and also to collect adult females in a greater range of physiological states (Maciel-de-Freitas et al. 2006, Williams et al. 2006b, Ball and Richie 2010b). These traps have also been shown to be effective for collecting *Ae. albopictus* (Meeraus et al. 2008, Bahalala and Arias 2009, Farajollahi et al. 2009, Obenauer et al. 2010), *Ae. polynesiensis* (Schmaedick et al. 2008), and *Cx. quinquefasciatus* (Maciel-de-Freitas et al. 2006). These traps are being used to monitor the impact of vector control programs (Chambers et al. 2009, Rapley et al. 2009, Azil et al. 2011, Tan et al. 2011) and to investigate the spatial distribution and seasonal fluctuations of container-inhabiting mosquitoes (Azil et al. 2010, Roiz et al. 2010, Barrera 2011, Barrera et al. 2011, Mercer et al. 2012).

BG-Sentinel traps with BG-Lure have been used effectively to capture *Ae. aegypti* in urban, suburban, and rural areas of Puerto Rico, where this species shares the environment with *Ae. (Gymnometopa) mediovittatus* (Coq.), the Caribbean tree hole mosquito (Cox et al. 2007; Barrera 2011; Barrera et al. 2011, 2012; Little et al. 2011). *Aedes mediovittatus* is native to the Greater Antilles and is a competent vector of dengue viruses (DENV) (Gubler et al. 1985, Freier and Rosen 1988). These 2 mosquito species overlap in their use of water-filled container habitats near human dwellings, such as drums, tires, tree holes, and other miscellaneous containers (Garcia 1977, Moore 1983). *Aedes aegypti* is more closely associated with dense urban areas, whereas *Ae. mediovittatus* is more frequently associated with vegetated areas (Cox et al. 2007, Little et al. 2011). It is to be noted that *Ae. albopictus* has not been able to become established in Puerto Rico in spite of its presence in nearby islands and the active exchange with countries where this species is well established, such as the USA (Cox et al. 2007). Invading *Ae. albopictus* would likely totally overlap with native *Ae. mediovittatus* mosquitoes given their similar aquatic and terrestrial habitats (Barrera 1996).

We recently found that *Ae. mediovittatus* frequently feeds on people in rural areas (Barrera et al. 2012). Gubler et al. (1985) suggested that this mosquito could act as a reservoir for DENV during interepidemic periods, but its infection has not yet been found in the wild. To incriminate *Ae. mediovittatus* as a secondary DENV vector or reservoir, infected specimens will need to be found in the field. Areas of high overlap between *Ae. aegypti* and *Ae. mediovittatus* were recently identified using the BGS traps and remote sensing in southern Puerto Rico (Little et al. 2011). These traps captured 10 times fewer female *Ae. mediovittatus* than *Ae. aegypti*, but whether this reflected an actual difference in population size or trap bias is unknown. This study investigated whether the BGS traps could be used to collect *Ae. mediovittatus* and could be modified to increase the number of field captures of this species. The results showed that a simple modification made to the standard BGS trap significantly increased capture of *Ae. mediovittatus*, *Ae. aegypti*, and *Cx. quinquefasciatus*.

MATERIALS AND METHODS

Outdoor cage experiments

We conducted experiments (2 choices) to investigate the effects of chemical lures (BG-Lure, CO_2, and octenol) and optical properties (trap color and size) of BGS traps on the capture rate of *Ae. aegypti* and *Ae. mediovittatus* in a large, outdoor cage in San Juan city, Puerto

Rico (Table 1). The standard BGS trap is a collapsible device that takes the form of a white plastic cylinder (36 cm diam, 40 cm high; Fig. 1).

Female *Ae. aegypti* used in the outdoor cage experiments were F_2–F_5 generations that came from a colony established from eggs collected in Patillas municipality, Puerto Rico. Female *Ae. mediovittatus* came from a laboratory colony that was originally established from larvae collected in Bayamon municipality in 2009; this colony had been enriched with field-collected specimens every 4 months to avoid excessive inbreeding. Both mosquito species were reared at 26 ± 1.0°C, with 65–80% RH, and a photoperiod of 12:12 h (light:dark), in pans containing 150 larvae and 1 liter of dechlorinated water, and were fed increasing amounts of finely ground rabbit chow during development (0.7–1.6 mg per larva per day). Unfed females were 3- to 7-day-old specimens that were provided with 10% sucrose solution until the day before the experiments. Gravid females were 10- to 14-day-old specimens that were fed porcine blood daily for 5 consecutive days and then maintained for an additional 3 days prior to use in the experiments; a 10% sucrose solution was provided from adult emergence to 1 day prior to bloodfeeding and from the 4th day of bloodfeeding to the day before the experiment.

The outdoor cage was a geodesic dome tent (10 m diam, 3.4 m high; Shelter Systems, Santa Cruz, CA), with 2 0.2 × 2.5-m and 4 1.5 × 1.5-m screen windows. Two BGS traps were placed at fixed locations 1.6 m from the walls of the cage, with a minimum distance of 2.6 m between the traps. Equal numbers of 3- to 7-day-old female *Ae. aegypti* and *Ae. mediovittatus* (Table 1) were released into the cage at 4:30 p.m. and collected from the traps at 8:30 a.m. the following morning because the cage became too hot for trap operation outside these hours. Females that were not captured by either trap were collected with a net. Each experiment was replicated several times (Table 1). These replications were not always conducted on consecutive days but were completed over a 2-wk period. Different individual traps from a pool of traps were used for each replication, to avoid the accumulation of odors.

A series of experiments was conducted to evaluate the influence of changes to the olfactory and optical properties of the BGS trap on the numbers of female *Ae. aegypti* and *Ae. mediovittatus* captured, as outlined in Table 1. Commercial octenol dispensers (2 g sachet; BioQuip Products, Rancho Dominguez, CA) used in Experiment 4, and BG-lures (Biogents, Regensburg, Germany) used in Experiment 1 and Experiments 3–7, were placed on a petri dish at the bottom of the traps. The BG-lures were aged 1 day prior to use. Carbon dioxide was provided to traps in Experiments 2–6 through plastic tubing running from a 2-liter drink cooler containing 1 kg of dry ice; an empty cooler was used for the trap without CO_2. Cylindrical black trap covers were constructed from black shelf liner (Experiments 5–7), which was later replaced with black fabric for field testing (Fig. 1). We also tested whether the capture rates of female *Ae. aegypti* and *Ae. mediovittatus* could be enhanced by increasing the size of the BGS trap (Experiment 7). The large trap was made from a collapsible plastic container (48 cm diam, 61 cm high) covered with black shelf liner, into which a regular BGS trap was placed so that the top of the BGS trap was level with the top rim of the large container.

To evaluate whether altering the color of the BG sentinel trap differentially influenced the capture of female mosquitoes in different physiological states, we compared the capture rates of gravid (10- to 14-day-old) and unfed (3- to 7-day- old) female *Ae. aegypti* and *Ae. mediovittatus* in black and white BGS traps baited with BG-Lure (Experiment 8). We used 50 gravid and 50 unfed females of each species, and repeated the experiment 6 times. In preparation for the field experiment, we also compared the capture rates of unfed female *Ae. aegypti* in a BGS trap with BG-Lure covered with a cylinder of black shelf liner and a BGS trap with BG-Lure covered with a black fabric sleeve (Experiment 9).

Field experiment

We compared the effectiveness of black and white BGS traps for capturing adult mosquitoes using paired traps baited with BG-Lure in each of 32 randomly selected houses (64 traps) in Lamboglia, Patillas municipality, Puerto Rico. Houses were selected from a layer of all the houses in the community in a Geographical Information System (Little et al. 2011), using the random selection tool of ArcGIS (ArcGIS 9.2; Esri, Redlands, CA). In a previous study, both *Ae. aegypti* and *Ae. mediovittatus* were frequently collected around homes in this area (Little et al. 2011). Traps were operated for 4 consecutive days per week for 3 wk from September 12–29, 2011 (4 days × 3 weeks × 64 traps = 768 – 74 trap failures = 694 samples). The positions of paired black and white traps were switched every day of sampling to avoid possible bias as a result of position. Collection bags were replaced every day, and batteries were replaced after 2 days of operation. Captured adult mosquitoes were sorted by sex, identified to species, and counted.

Data analysis

Outdoor cage experiments—Experiments 1–7 were 2 (treatments) × 2 (species) full factorial designs; Experiment 8 was treated as a 2 (treatments) × 2 (species) × 2 (unfed, gravid females) full factorial design; and Experiment 9 was analyzed using a paired *t*-test (2 treatments). For Experiments 1–8, the dependent variable was the number of females of each species trapped in each trial, and generalized linear models (SPSS 19; IBM Corp., Armonk, NY) were used to analyze the effect of treatment, species, or physiological condition of the females and their interaction on the number of females captured per trap per day. We used a Poisson distribution and log link function.

Field experiment—We used a generalized linear mixed model (SPSS 19) to investigate the effect of BGS trap color on the number of female mosquitoes captured per trap per day, so that we could include repeated observations of the same traps in our analyses. The main effect was trap color (black, white); the covariates were the position of the trap in the house (left, right) and the sampling week (1–3); and the random effects variables were the house and the position of the trap in the house. The order of sampling (1–12) was used as the repeated samples indicator. Based on the variance:mean ratio, we used a negative binomial distribution and log link function for the analyses of *Ae. aegypti* and *Cx. quinquefasciatus*, and a Poisson distribution and log link function for *Ae. mediovittatus*. The position of the trap was not significant ($\alpha = 0.05$) for any species, so this variable was dropped from the final models.

We used tests of independence (2 × 2 contingency tables; Crosstabs, SPSS 19) to determine whether the color of the traps (black, white) affected the frequency of trap samples with or without each container mosquito species (*Ae. aegypti*, *Ae. mediovittatus*). The co-occurrence of *Ae. aegypti* and *Ae. mediovittatus* was also evaluated for each type of trap using a 2 (*Ae. aegypti*; present, absent) × 2 (*Ae. mediovittatus*; present, absent) contingency table, where the null hypothesis was that the species occurred independently of each other. The phi coefficient was calculated to test whether there was a significant (positive/negative) association between species.

RESULTS

Outdoor cage experiments

Experiment 1—There were significant effects of treatment (white BGS traps with and without BG-Lure; Wald $\chi^2 = 5.1$; $P < 0.05$) and species (Wald $\chi^2 = 49.8$; $P < 0.01$) on the number of females attracted to the experimental traps. In general, more females of *Ae. aegypti* were attracted to the BGS traps than *Ae. mediovittatus*, and the BG-Lure did not seem to affect the number of females of the latter species in the traps (Fig. 2).

Experiment 2—There were significant effects of treatment (white traps with and without CO_2; Wald $\chi^2 = 54.6$; $P < 0.001$) and species (Wald $\chi^2 = 22.0$; $P < 0.001$). Both species were captured in greater numbers in traps with CO_2, but fewer *Ae. mediovittatus* were captured than *Ae. aegypti* in both treatments (Fig. 3A).

Experiment 3—There were significant effects of treatment (white traps with CO_2 and BG-Lure versus white traps with BG-Lure only; Wald $\chi^2 = 7.5$; $P < 0.01$), species (Wald $\chi^2 = 137.6$; $P < 0.001$), and their interaction (Wald $\chi^2 = 4.2$; $P < 0.05$). The combination of CO_2 and BG-Lure was more effective at attracting females of *Ae. aegypti* than *Ae. mediovittatus* (Fig. 3B).

Experiment 4—There were significant effects of treatment (white traps baited with BG-Lure, CO_2, and octenol versus traps baited with only BG-Lure and CO_2; Wald $\chi^2 = 13.4$; $P < 0.001$), and their interaction (Wald $\chi^2 = 67.0$; $P < 0.001$). The addition of octenol resulted in fewer *Ae. aegypti* females but more *Ae. mediovittatus* females being captured (Fig. 3C).

Experiment 5—There were significant effects of treatment (traps with a black cover baited with BG-Lure and CO_2 versus white traps baited with BG-Lure and CO_2; Wald $\chi^2 = 267.7$; $P < 0.001$), species (Wald $\chi^2 = 39.5$; $P < 0.001$), and their interaction (Wald $\chi^2 = 73.6$; $P < 0.001$). The interaction effect seems to reflect the comparatively greater capture rate of *Ae. mediovittatus* than *Ae. aegypti* in black traps (Fig. 4A).

Experiment 6—There were significant effects of treatment (black traps baited with BG-Lure versus white traps baited with BG-Lure and CO_2; Wald $\chi^2 = 185.6$; $P < 0.001$), species (Wald $\chi^2 = 29.4$; $P < 0.001$), and their interaction (Wald $\chi^2 = 107.5$; $P < 0.001$). The significant interaction effect reflected a comparatively greater attractiveness of black traps to *Ae. mediovittatus* (Fig. 4B).

Experiment 7—There were no significant effects of treatment (large versus standard-size black BGS traps baited with BG-Lure; Wald $\chi^2 = 1.6$; $P > 0.05$), species (Wald $\chi^2 = 0.006$; $P > 0.05$), or their interaction (Wald $\chi^2 = 3.9$; $P = 0.05$). The marginal *P*-value of the interaction term reflects a slight increase in the numbers of *Ae. mediovittatus* and a slight reduction in the numbers of *Ae. aegypti* in larger traps (data not shown).

Experiment 8—There were significant effects of treatment (black traps with BG-Lure versus white traps with BG-Lure; Wald $\chi^2 = 324.1$; $P < 0.001$), species (Wald $\chi^2 = 8.2$; $P < 0.01$), physiological condition of the females (unfed versus gravid; Wald $\chi^2 = 15.3$; $P < 0.001$), and the interaction terms treatment × species (Wald $\chi^2 = 4.2$; $P < 0.05$), species × condition (Wald $\chi^2 = 7.1$; $P < 0.01$), and treatment × species × condition (Wald $\chi^2 = 5.0$; $P < 0.05$). These significant interactions reflect complex combined effects of trap color (most females were caught in black traps), physiological condition (more unfed females were trapped than gravid ones, mostly in black traps), and subtle, reversed responses of unfed and gravid females of each species (mostly in white traps; Fig. 5).

Experiment 9—There was no significant difference in the attractiveness of BGS traps with BG-Lure covered with a cylinder of black shelf liner or a black fabric sleeve to female *Ae. aegypti* (paired *t*-test; $t = 0.01$; $P > 0.05$).

Field experiment

Black BGS traps with BG-Lure captured 29% more adult specimens (11,277) and 42% more species of mosquitoes (17) than white BGS traps with BG-Lure (8,729 adults, 12 species) (Table 2). The most common species captured were *Cx. quinquefasciatus*, *Ae. aegypti*, *Ae. mediovittatus*, *Cx. janitor* Theobald, and *Cx. antillummagnorum* (Theobald). The BGS traps captured large numbers of males of *Cx. quinquefasciatus* and *Ae. aegypti* (Table 2). Males of other species were relatively underrepresented in trap captures.

There was a significant effect of trap color (black trap with BG-Lure versus white trap with BG-Lure; $F_{1,690} = 7.1$; $P < 0.01$) but no effect of sampling week (1–3 wk; $F_{2,690} = 0.4$; $P > 0.05$) on the number of *Ae. aegypti* females captured in BGS traps per day in the field, with black traps capturing 38% more *Ae. aegypti* females than white traps (3.87 ± 0.61 versus 2.79 ± 0.42 females [mean ± 95% confidence interval]; Fig. 6A). There were significant effects of trap color ($F_{1,689} = 13.3$; $P < 0.001$) and sampling week ($F_{2,689} = 4.7$; $P < 0.01$) on the number of *Ae. aegypti* males captured, with black traps capturing 52% more males than white traps (3.50 ± 0.65 versus 2.29 ± 0.45 males).

Trap color ($F_{1,689} = 5.1$; $P < 0.05$) and sampling week ($F_{2,689} = 12.3$; $P < 0.001$) significantly affected the number of *Ae. mediovittatus* females captured per day, with black traps capturing 79% more females than white traps (0.61 ± 0.14 versus 0.34 ± 0.08 females; Fig. 6B). No analyses were performed on *Ae. mediovittatus* males because they were too scarce (Table 2). The ratios of female *Ae. aegypti* to female *Ae. mediovittatus* observed in black and white traps were 6.39 and 8.14, respectively.

There were significant effects of trap color ($F_{1,690} = 13.3$; $P < 0.001$) and sampling week ($F_{2,690} = 20.8$; $P < 0.001$) on the number of *Cx. quinquefasciatus* females captured per day, with black traps capturing 15% more females than white traps (11.73 ± 1.54 versus 10.18 ± 1.43 females; Fig. 6C). Similarly, there were significant effects of trap color ($F_{1,689} = 48.9$; $P < 0.001$) and sampling week ($F_{2,689} = 32.6$; $P < 0.01$) on the number of *Cx. quinquefasciatus* males captured, with black traps capturing 22% more males than white traps (11.39 ± 1.56 versus 9.32 ± 1.54 males).

The occurrence (presence, absence) of female *Ae. aegypti* in BGS traps in the study area was independent of trap color ($\chi^2 = 0.1$; $P > 0.05$), with females being present in 77.5% and 76.7% of all black and white traps, respectively. In contrast, the occurrence of female *Ae. mediovittatus* in the traps was not independent of color ($\chi^2 = 6.8$; $P < 0.001$), with individuals being detected in 32.9% of black traps and 23.3% of white traps. The occurrence of *Cx. quinquefasciatus* females was also not independent of color ($\chi^2 = 10.3$; $P < 0.001$), with individuals being present in 92.1% of black traps and 84.2% of white traps. The cooccurrence of *Ae. aegypti* and *Ae. mediovittatus* was positive in black traps ($\chi^2 = 5.1$; Ø = 0.121; $P < 0.05$) but not in white traps ($\chi^2 = 0.5$; Ø = 20.04; $P > 0.05$), with both species being found together in 31.2% (110) of black trap samples but only in 19.9% (68) of white trap samples.

DISCUSSION

In this study, we investigated whether the BGS traps with BG-Lure were as effective at capturing female *Ae. mediovittatus* as they seem to be for *Ae. aegypti* (Kröckel et al. 2006) by using 2-choice experiments conducted in a large, outdoor cage. We also investigated whether we could modify the trapping system to significantly increase the capture rate of *Ae. mediovittatus* in the field, to facilitate future studies evaluating whether this species is acting as a secondary vector or reservoir of DENV in Puerto Rico, as suggested by Gubler et al. (1985).

The results showed that conventional (white) BGS traps with BG-Lure were biased against *Ae. mediovittatus*, and that trap captures in the outdoor cage and in the field were significantly improved by using BGS traps with BG-Lure and a black outer cover instead of the standard white exterior of the original trap. Black BGS traps with BG-Lure also captured significantly more male and female *Ae. aegypti* and *Cx. quinquefasciatus*, more mosquito species (17 versus 12), and yielded greater proportions of traps containing *Ae. mediovittatus* and *Cx. quinquefasciatus*. In general, *Ae. mediovittatus* responded more strongly to trap color than *Ae. aegypti*.

Black BGS traps with BG-Lure also attracted more unfed and gravid females of *Ae. aegypti* and *Ae. mediovittatus* than white BGS traps. A greater proportion of the released unfed females was captured in black BGS traps in comparison with gravid females, a response that was similar in both species. These results are comparable to those obtained by Maciel-de-Freitas et al. (2006) in Brazil where host-seeking *Ae. aegypti* females were captured in greater proportions in BGS than females in other physiological stages. Ball and Ritchie (2010b) observed a lower proportion of unfed, nulliparous and bloodfed parous *Ae. aegypti* females than other physiological states in BGS without lure in Australia. The authors interpreted that the nulliparous females were perhaps too young (1 to 2 days old) to seek hosts and that the recently fed, parous females were too heavy to engage in active flying.

We also found that the ratio of female *Ae. aegypti* to *Ae. mediovittatus* was lower in the modified BGS traps, suggesting that these traps provide a less biased assessment of the local abundance of *Ae. mediovittatus*. Interestingly, there was a significant positive association between these 2 species in the black BGS traps, whereas the species occurred independently in the white traps: *Ae. aegypti* and *Ae. mediovittatus* were found together in 110 out of 353 black BGS trap samples but in only 68 of 341 white BGS trap samples. We also found a positive relationship between pupae of *Ae. aegypti* and *Ae. mediovittatus* in containers in the study area (CDC, unpublished data). Thus, increased black trap sensitivity contributes to our understanding of the ecological patterns of distribution of these species.

Christophers (1960) showed that male and female adults of *Ae. aegypti* are attracted to dark objects. Consequently, the 1st traps specifically developed to monitor *Ae. aegypti* were black (Fay 1968, Wilton and Kloter 1985) or black and white (Fay and Prince 1970). The BGS trap also has black (top center) and white (outer surface) components. Our modification of the BGS trap involved changing the outer vertical surfaces from white to black, leaving the top of the trap white with a black center. It is possible that future experiments may find that a completely black BGS trap is even more attractive to these container mosquitoes; indeed, we found that completely black traps captured more female *Ae. aegypti* than white or black and white traps while developing an autocidal gravid ovitrap (Mackay et al. 2013). Furthermore, the influence of color should eventually be tested in the absence of BG-Lure or other baits. Williams et al. (2006a) showed that *Ae. aegypti* were captured at similar rates in BGS traps with and without BG-Lure, and demonstrated significant variations in the response of mosquitoes from different geographical origins. The BG-Lures are relatively expensive, and thus the ability to use traps without lures would bring substantial savings to mosquito surveillance programs. It is also worth noting that the black BGS traps captured significantly more *Cx. quinquefasciatus* than the white traps, despite this species being a typical nocturnal biter. These results are in agreement with previous observations made by Wilton and Kloter (1985), who noted large numbers of *Cx. quinquefasciatus* in their visual, black cylinder trap and in Fay and Prince (1970) traps in New Orleans. The high abundance of *Cx. quinquefasciatus* in the study area is a result of the presence of unsealed septic tanks, a phenomenon that is common in rural Puerto Rico (Barrera et al. 2008).

It has been shown that white BGS trap captures can be influenced by the presence and color of nearby objects, whereby black, low-reflectance objects lower the numbers of captured female *Ae. aegypti* (Ball and Ritchie 2010a). Although these results are difficult to extrapolate to black BGS traps, it is likely that the presence of competing dark objects in the vicinity of a black trap would induce a similar effect. Thus, to avoid this possible type of bias, it might be better to place the BGS traps away from dark objects in the field.

The addition of CO_2 to white and black BGS traps increased the capture rate of *Ae. aegypti* to a greater extent than it did for *Ae. mediovittatus*. This supports findings from previous studies, which have also shown that the use of CO_2 in various traps significantly improves the capture of *Ae. aegypti* (Canyon and Hii 1997, Russell 2004). In contrast, the use of octenol in combination with BG-Lure and CO_2 had a repellent effect on *Ae. aegypti*. Similarly, other studies have shown that octenol might not enhance trap captures of *Ae. aegypti* (Shone et al. 2003, Russell 2004) but repel the species (Canyon and Hii 1997). Captures of *Ae. mediovittatus* were slightly enhanced in the BGS traps with CO_2 alone or in combination with BG-Lure and octenol, but this effect was not sufficient to justify their use with black BGS traps.

In conclusion, it has been shown that modified, black BGS traps with BG-Lure can significantly increase the capture rate of *Ae. aegypti*, *Ae. mediovittatus*, and *Cx. quinquefasciatus* under field conditions. Therefore, we recommend testing this modification on other important container mosquito species, such as *Ae. albopictus* and *Ae. polynesiensis*.

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Fig. 1.
Original BG-Sentinel trap (white; left) and modified BG-Sentinel trap, showing the black outer cover (right).

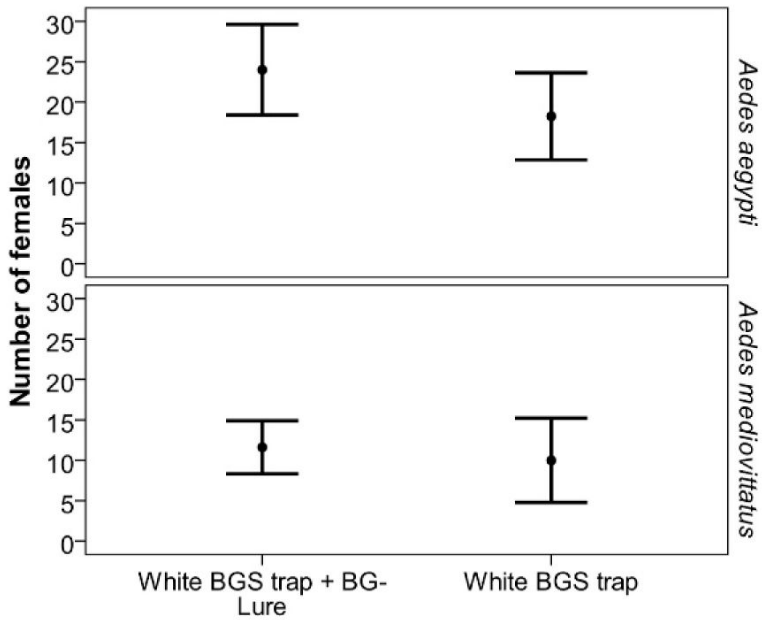

Fig. 2.

Average (± 95% confidence interval) number of female *Aedes aegypti* and *Ae. mediovittatus* captured per trap per day in white BG-Sentinel (BGS) traps with or without BG-Lure in 2-choice experiments conducted in an outdoor mosquito cage. Fifty females of each species were released into the cage, and the trial was repeated 8 times.

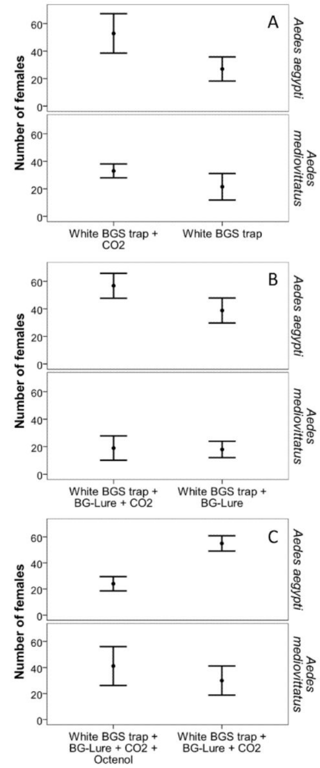

Fig. 3.

Average (± 95% confidence interval) number of female *Aedes aegypti* and *Ae. mediovittatus* captured in each trap per day in white BG-Sentinel (BGS) traps in 2-choice experiments conducted in an outdoor mosquito cage: (A) with or without CO_2; (B) with BG-Lure, and with or without CO_2; and (C) with BG-Lure and CO_2, and with or without octenol. Seventy-five females of each species were released into the cage in experiments A and B, and 80 females in experiment C. Each trial was repeated 6 times.

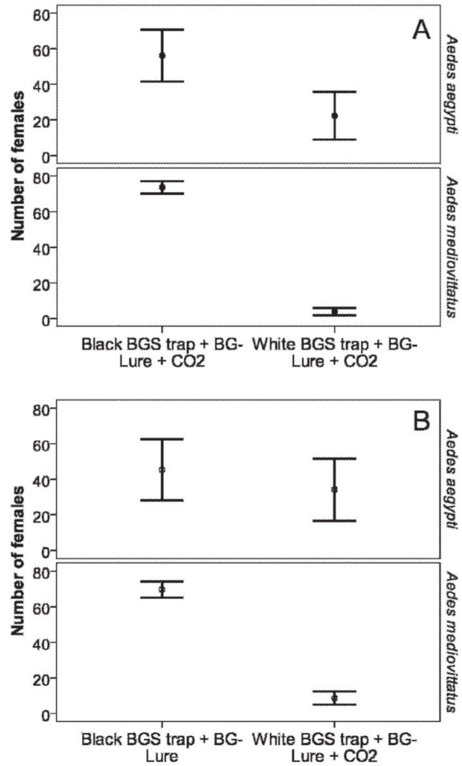

Fig. 4.

Average (± 95% confidence interval) number of female *Aedes aegypti* and *Ae. mediovittatus* captured per trap per day in BG-Sentinel (BGS) traps in 2-choice experiments conducted in an outdoor mosquito cage: (A) a black BGS trap with BG-Lure and CO_2 versus a white BGS trap with BG-Lure and CO_2; and (B) a black BGS trap with BG-Lure versus a white BGS trap with BG-Lure and CO_2. Eighty females of each species were released into the cage, and the trial was repeated 6 times.

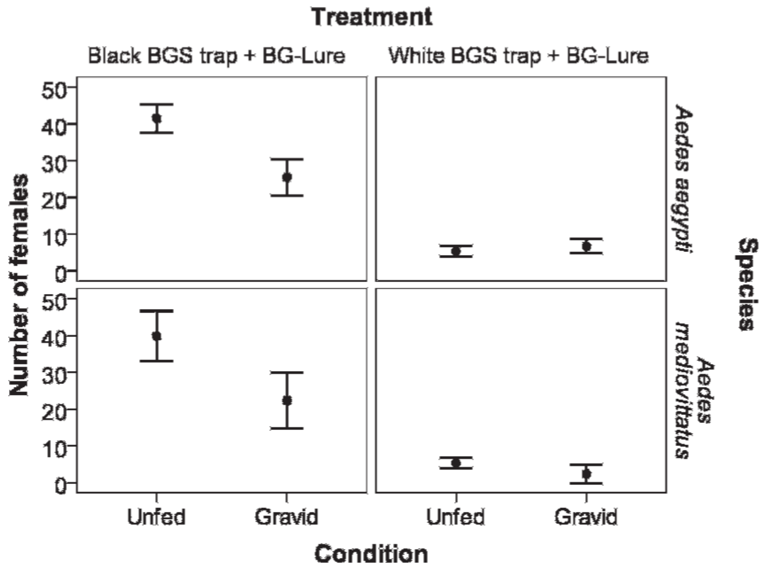

Fig. 5.

Average (± 95% confidence interval) number of unfed and gravid females of *Aedes aegypti* and *Ae. mediovittatus* captured per trap per day in black or white BG-Sentinel (BGS) traps with BG-Lure in 2-choice experiments conducted in an outdoor mosquito cage. Fifty unfed and 50 gravid females of each species were released into the cage, and the trial was repeated 6 times.

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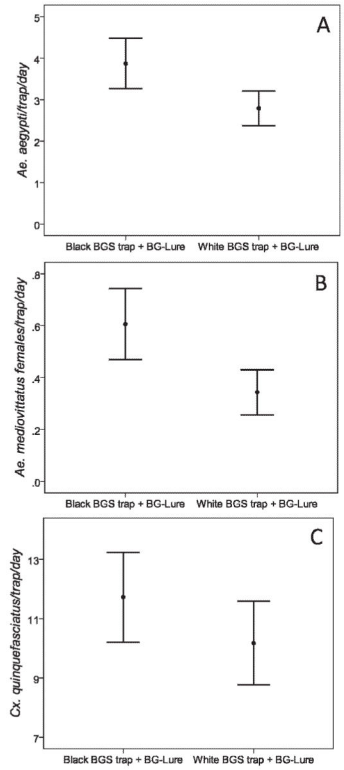

Fig. 6.

Average (± 95% confidence interval) number of (A) *Aedes aegypti*, (B) *Ae. mediovittatus*, and (C) Culex quinquefasciatus female mosquitoes per trap per day in paired black and white BG-Sentinel (BGS) traps baited with BG-Lure in 32 randomly selected houses of Lamboglia, Patillas municipality, Puerto Rico. Traps were operated for 4 consecutive days per week for 3 wk from September 12–29, 2011.

Table 1

Experiments (2 choices) testing the effects of chemical lures and optical properties of BG-Sentinel (BGS) traps on the capture rate of female *Aedes aegypti* and *Ae. mediovittatus* in a large, outdoor cage in San Juan city, Puerto Rico.

Experiment no.	Treatments		No. of unfed female *Ae. aegypti* released in the cage	No. of unfed female *Ae. mediovittatus* released in the cage	No. of trials
1	White BGS trap + BG-Lure	White BGS trap	50	50	8
2	White BGS trap + CO_2	White BGS trap	75	75	6
3	White BGS trap + BG-Lure and CO_2	White BGS trap + BG-Lure	75	75	6
4	White BGS trap + BG-Lure, CO_2, and octenol	White BGS trap + BG-Lure and CO_2	80	80	6
5	Black BGS trap + BG-Lure and CO_2	White BGS trap + BG-Lure and CO_2	80	80	6
6	Black BGS trap + BG-Lure	White BGS trap + BG-Lure and CO_2	80	80	6
7	Large black BGS trap + BG-Lure	Black BGS trap + BG-Lure	80	80	6

Table 2

Abundance of male and female adult mosquitoes of each species captured in black or white BG-Sentinel traps with BG-Lure in Lamboglia, Patillas municipality, Puerto Rico.

	Black + BG-Lure		White + BG-Lure		
Species	**Males**	**Females**	**Males**	**Females**	**Total**
Aedes aegypti	1,234	1,367	781	952	4,334
Ae. mediovittatus	22	214	9	117	362
Ae. tortilis	0	3	0	1	4
Anopheles albimanus	0	1	0	0	1
An. grabhamii	0	2	0	0	2
Culex antillummagnorum	5	44	4	34	87
Cx. atratus	0	1	1	0	2
Cx. chidesteri	1	7	0	5	13
Cx. habilitator	0	13	0	2	15
Cx. iolambdis	1	2	0	0	3
Cx. janitor	9	166	13	153	341
Cx. nigripalpus	0	3	0	2	5
Cx. quinquefasciatus	4,019	4,139	3,177	3,472	14,807
Cx. secutor	0	18	0	0	18
Deinocerites magnus	0	1	1	1	3
Toxorhynchites portoricensis	0	3	0	4	7
Uranotaenia lowii	0	2	0	0	2
Subtotal × sex	5,291	5,986	3,986	4,743	20,006
Subtotal × color	11,277		8,729		