Seasonal Genetic Changes of *Aedes aegypti* (Diptera: Culicidae) Populations in Selected Sites of Cebu City, Philippines

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ABSTRACT *Aedes aegypti* (L.) is the primary vector of dengue virus in the Philippines, where dengue is endemic. We examined the genetic changes of *Ae. aegypti* collected from three selected sites in Cebu city, Philippines, during the relatively wet (2011–2012) and dry seasons (2012 and 2013). A total of 493 *Ae. aegypti* adults, reared in the laboratory from field-collected larvae, were analyzed using 11 microsatellite loci. Seasonal variation was observed in allele frequencies and allelic richness. Average genetic differentiation ($D_{EST} = 0.018$; $F_{ST} = 0.029$) in both dry seasons was higher, due to reduced N_e , than in the wet season ($D_{EST} = 0.006$; $F_{ST} = 0.009$). Thus, average gene flow was higher in the wet season than in the dry seasons. However, the overall F_{ST} estimate (0.02) inclusive of the two seasons showed little genetic differentiation as supported by Bayesian clustering analysis. Results suggest that during the dry season the intense selection that causes a dramatic reduction of population size favors heterozygotes, leading to small pockets of mosquitoes (refuges) that exhibit random genetic differentiation. During the wet season, the genetic composition of the population is reconstituted by the expansion of the refuges that survived the preceding dry season. Source reduction of mosquitoes during the nonepidemic dry season is thus recommended to prevent dengue re-emergence in the subsequent wet season.

KEY WORDS *Aedes aegypti*, seasonal fluctuation, temporal genetics, Philippines, yellow fever mosquito

Dengue is the fastest emerging arboviral infection in the world (World Health Organization–Western Pacific Region Office [WHO-WPRO] 2014a). Its maximum burden is found in the Asia Pacific region where 75% or about 1.8 billion out of 2.5 billion people at risk reside (WHO-WPRO 2014a). Philippines has ranked fourth in the number of dengue cases among the 10 Association of Southeast Asian Nations (ASEAN; WHO-WPRO 2013), with direct medical costs of \$ 345 million (in 2012 US dollars; Edillo et al. 2015). *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse) (Diptera: Culicidae) are the primary and secondary vectors of dengue viruses (DENVs; *Flavivirus*) in the country, respectively. National antidengue programs such as massive dengue information campaigns, distribution and training for the use of ovicidal and larvicidal traps in schools as well as a multi-sectoral search and destroy of mosquito larval habitats using insecticides have been done in the country (Department of Science and Technology [DOST] 2013, Edillo and Madarieta 2012). Despite the control programs in place, the Department of Health–National Epidemiology Center (DOH-NEC)

reported 59,943 dengue cases from January 1 to September 6, 2014. However, these were 59.57% lower (148,279) than the same time period in 2013 (WHO-WPRO 2014b). Moreover, Cebu city ranked first in dengue cases in Central Visayas from 1997 to 2008 (Edillo and Madarieta 2012). Reasons include environmental risk factors, urbanization, human activities and movement, climate change, inadequate public health infrastructure, poor solid waste management, and lack of effective mosquito surveillance system (Beatty et al. 2011, Edillo and Madarieta 2012).

Understanding the genetic structure and gene flow among dengue mosquitoes is important for targeting management and control strategies (Endersby et al. 2009, Mendonca et al. 2014), particularly because the novel tetravalent dengue vaccine is only 56.5% efficacious among children in endemic Asian areas (Capeding et al. 2014), although development of tetravalent vaccines from various pharmaceutical companies is undergoing various stages of clinical tests (Center for Disease Control [CDC] 2013). Control of mosquito-borne diseases through introduction of antipathogen genes in mosquitoes depends highly on the genetic structure of populations. Monitoring temporal genetic changes is necessary in order to assess the influences of ecological and geographical variations in natural mosquito populations. Furthermore, population genetics can estimate the rate at which genes may spread within and between populations at various scales and can

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identify biological and physical features of the environment that may interfere with their movement (Lanzaro and Tripet 2003).

Distribution and relative abundance of insects are governed mostly by spatial scale climate (Andrewartha and Birch 1954, Sutherst and Maywald 1995). *Ae. aegypti* populations vary seasonally in Vietnam due to climatic, social factors, and insecticide use (Huber et al. 2002a,b), which may influence vector competence for DENV transmission (Failloux et al. 2002). In Manaus, Brazil, *Ae. aegypti* populations showed genetic homogeneity and extensive gene flow in the rainy season but were significantly structured in the dry season (Mendonca et al. 2014). Thus, seasonal genetic analyses are relevant in providing insights into the timing of vector control interventions particularly during interepidemic and epidemic periods of dengue transmission (Mendonca et al. 2014).

In the Philippines, there have been no studies on the population structure of *Ae. aegypti*. This study aimed to examine the seasonal genetic changes of *Ae. aegypti* subpopulations collected from selected sites in Cebu city, Philippines, in the wet season of 2011–2012 and dry seasons of 2012 and 2013.

Materials and Methods

Mosquito Collections. The study sites included three *barangays* (smallest government administrative unit) in Cebu city, Philippines, namely, Babag (BBG), Basak San Nicolas (BSN), and Poblacion Pardo (PRD; Fig. 1). These sites were among the top 10 *barangays* with highest case fatality rate (CFR) of dengue illnesses in Cebu city in 2010 (Cebu City Health Department [CCHD] 2010). The sites were chosen based on: 1) CFR of dengue cases, 2) elevation, and 3) type of settings (rural and urban). The rural mountainous site, BBG, is located 11 km north from the urban sites (PRD and BSN). The coastal BSN is 2 km away from PRD; both are 5 km and 7 km away from Cebu city's center, respectively. Third- and fourth-instar larvae and pupae of *Ae. aegypti* were collected monthly with equal sampling effort for each site in the following periods: November 2011–February 2012 (wet season), March–May 2012 (dry season), June–July 2012 (wet season), and in April–May 2013 (dry season; Table 1). The country has a tropical climate characterized by two main seasons: relatively wet (June–February) and dry seasons (March–May). The country's average temperature ranges from 25 to 32°C, with relative humidity around 77%.

Ae. aegypti females deposit eggs in several oviposition sites (Colton et al. 2003). To avoid sampling of siblings laid by a single female in the same oviposition containers, mosquito larvae and pupae were collected from multiple larval habitats (e.g., used rubber tires, plastic and metal drums, cemented water reservoirs, flower pots, and plastic water containers) in the field and around house premises. These larvae and pupae were placed in small rearing jars filled with distilled water (~150 ml) and were covered with fine mesh cloth. These were fed with fish food (0.02 g; Fwusow

Industry Co., Ltd., Sha Lu Taichung, Taiwan) until they metamorphosed into adult mosquitoes inside the laboratory at 23°C. Adult mosquitoes were fed on 4% sucrose solution for three days before storage. Adult *Ae. aegypti* samples were identified according to Belkin (1962). They were placed in 15-ml Falcon tubes with 70–100% alcohol (5–10 ml) and were stored at 7°C. Samples from field and house premises that were mostly collected from artificial containers were used for genotyping in the Powell Laboratory at Yale University, New Haven, CT.

Genetic Methods. *DNA Extraction.* Extraction of genomic DNA of *Ae. aegypti* was done using the DNeasy blood and tissue culture kit (Cat no. 69506; Qiagen, Hilden, Germany) following the manufacturer's protocol and by using RNase A (Cat no. 19101; Qiagen) after sample digestion for RNA removal.

Microsatellite Genotyping. Microsatellite fragments were amplified using 11 polymorphic loci that have been previously used by Brown et al. (2011) and Slotman et al. (2007) (Supp Table 1 [online only]) for population genetic studies. Each pair of nonoverlapping loci was amplified in a multiplex PCR reaction with a fluorescent M13 primer (Boutin-Ganache et al. 2001). PCR reactions (10 µl) contained 1x Type-it microsatellite PCR master mix (Cat no. 206243; Qiagen), 25 nM of each forward primer, 250 nM of each reverse primer, and 500 nM of fluorescently labeled M13 primer. The reaction mix was placed in a thermocycler (GeneAmp PCR System 9700, Applied Biosystems, CA) with the following conditions: preincubation at 94°C for 10 min following 35 cycles (94°C × 30", 54°C × 30", 72°C × 30") and a final incubation at 72°C for 5 min (Slotman et al. 2007, Brown et al. 2011). PCR products were sequenced using Applied Biosystems 3730xl DNA Genetic Analyzer with a GS 500 Rox allele size standard (Cat no. 4340060A; Applied Biosystems, CA). Genotypes were scored using GENEMAPPER software (Applied Biosystems, CA).

Genetic Analyses. Microsatellite allele frequency, Fisher's exact test to detect linkage disequilibrium (LD) for pairwise loci, estimates of observed (H_o) and expected heterozygosities (H_e), goodness-of-fit probability tests for Hardy–Weinberg equilibrium (HWE), and Weir and Cockerham's (1984) measure of inbreeding coefficient (F_{IS}) were performed using GENEPOP v4.2 (Raymond and Rousset 1995). Gene flow (Nm) by seasons were calculated using Arlequin v. 3.5 (Excoffier et al. 2006). Effective population size (N_e) was calculated based on molecular coancestry method (Nomura 2008) in NeEstimator v. 2.01 (Do et al. 2014). Mean allelic richness (A_R) and private allelic richness (P_{AR}) for unique alleles for each locus in each subpopulation were determined using HP-RARE (Kalinowski 2005). Estimate of genetic differentiation using Jost's D_{EST} (Jost 2008) was assessed with the web-based application SMOGD (Crawford 2010). Pairwise estimates of F_{ST} between subpopulations and their significance were determined using GENDIST software (Kalinowski 2005). In order to assign individual mosquitoes to genetic groups based on their genotypic

Fig. 1. Collection sites of *Ae. aegypti* in Babag, Basak San Nicolas, and Poblacion Pardo, Cebu city in Cebu province (right inset), Philippines (left inset).

Table 1. Data on genotyped microsatellite loci used for detecting genetic changes of *Ae. aegypti* population in Cebu city, Philippines

Locus ^a		Primer sequence	Number of alleles			Type of repeats	Source
			Rainy 2012	Dry 2012	Dry 2013		
AC1	For	TCCGGTGGGTTAAGGATAGA	8	10	7	Dinucleotide	Slotman et al. 2007
	Rev	ACTTCACGCTCCAGCAATCT					
AC2	For	AATACAACGCGATCGACTCC	8	6	4	Dinucleotide	Slotman et al. 2007
	Rev	AACGATTAGCTGCTCCGAAA					
AC4	For	GCGAATCGGTTCCCATAGTA	4	3	2	Dinucleotide	Slotman et al. 2007
	Rev	CTTTATCGATCGACGCCATT					
AC5	For	TGGATTGTTCTTAAACAAACACGAT	18	17	11	Dinucleotide	Slotman et al. 2007
	Rev	CGATCTCACTACGGGTTTCG					
AG1	For	AATCCCCACACAAACACACC	6	5	5	Dinucleotide	Slotman et al. 2007
	Rev	GGCCGTGGTGTTACTCTCTC					
AG2	For	TCCCCTTTCAAACCTAATGG	21	20	15	Dinucleotide	Slotman et al. 2007
	Rev	TTTGCCCTCGTATGCTCTCT					
AG5	For	TGATCTTGAGAAGGCATCCA	11	9	8	Dinucleotide	Slotman et al. 2007
	Rev	CGTTATCCTTTCATCACTTGTTTG					
CT2	For	CGCAGTAGGCGATATTCGTT	7	6	4	Dinucleotide	Slotman et al. 2007
	Rev	ACCACCAACACCAATTCT					
A1	For	GACGTAACCGAGTGGGAGA	7	6	5	Trinucleotide	Brown et al. 2011
	Rev	GCATTAAACCGCGCTAGAAC					
B2	For	GGAACACACTTGCAGGGACAT	8	7	5	Trinucleotide	Brown et al. 2011
	Rev	GCAGATGGTGGCAGTACTGA					
B3	For	GCAAGTTGCCAAAGTCTCAA	8	9	4	Trinucleotide	Brown et al. 2011
	Rev	ACCCACCGTTTGCTTTGTAG					

^a All forward primers were designed with a short M13 tail at the start (TCCCAGTCACGACGT)

similarity across 11 microsatellite loci, the Bayesian clustering method in STRUCTURE v2.3.4 (Pritchard et al. 2000) was used considering an admixture model and assuming that allele frequencies were independent of one another. This program does not require any a

Table 2. Effective population size (N_e) of *Ae. aegypti* samples from the wet season (2011–2012) and dry seasons of 2012 and 2013 based on the linkage disequilibrium (LD) model

Samples	LD	CI 95%
Wet Season 2011–2012		
BBG wet 2012	213.9	129.1–515.6
BSN wet 2012	19.5	15.8–24.4
PRD wet 2012	48.5	37.1–66.1
Total	281.9	182–606.1
Dry Season 2012		
BBG dry 2012	96.7	70.2–145.3
BSN dry 2012	18.4	13–27.6
PRD dry 2012	89.9	60.5–156.1
Total	205	143.7–329
Dry Season 2013		
BBG dry 2013	13.7	10.3–18.2
BSN dry 2013	22.6	11.6–73.7
PRD dry 2013	22.9	18–29.5
Total	59.2	39.9–121.4

CI, confidence interval.

Table 3. Mean allelic richness (A_R), mean private allelic richness (P_{AR}), and observed (H_O) and expected heterozygosities (H_E) of *Ae. aegypti* collected from three study sites in Cebu city, Philippines, in different seasons

Collection sites	Seasons	A_R^a	P_{AR}^a	H_O	H_E
BBG	Wet 2011–2012	5.10	0.84	0.5265	0.6044
BSN	Wet 2011–2012	5.36	0.99	0.5320	0.6018
PRD	Wet 2011–2012	4.96	0.58	0.5737	0.6099
Mean		5.14	0.80	0.5441	0.6054
BBG	Dry 2012	4.64	0.77	0.5456	0.6149
BSN	Dry 2012	4.80	0.84	0.5712	0.6280
PRD	Dry 2012	4.52	0.66	0.5351	0.6192
Mean		4.65	0.76	0.5506	0.6207
BBG	Dry 2013	3.68	0.47	0.5776	0.6104
BSN	Dry 2013	3.79	0.46	0.5739	0.5462
PRD	Dry 2013	3.83	0.58	0.6072	0.5667
Mean		3.77	0.50	0.5862	0.5744

^a A total of 109 genes were used for the rarefaction of A_R and P_{AR} .

Table 4. Two-way ANOVA on allelic frequencies, allelic richness (A_R), and private allelic richness (P_{AR}) of *Ae. aegypti* subpopulations in three study sites in Cebu city, Philippines, in the wet season of 2011–2012 and dry seasons of 2012 and 2013

Parameters	df	Sum of square	Mean square	F value	P value
Allele frequency					
Season	2	0.447	0.22341	6.639	0.00138*
Subpopulation	2	0.002	0.00108	0.032	0.96849
Residual	818	27.528	0.03365		
A_R					
Season	2	2.9091	1.454	104.143	0.00355*
Subpopulation	2	0.0781	0.039	2.795	0.173992
Residual	4	0.0559	0.014		
P_{AR}					
Season	2	0.15626	0.07818	4.201	0.104
Subpopulation	2	0.03696	0.01848	0.993	0.447
Residual	4	0.07444	0.01861		

* $P < 0.05$.

priori information regarding sampling locations and determines the underlying genetic population among a set of individuals genotyped at multiple loci assuming all samples came from different unknown populations. Genotypes were run with a burn-in value of 100,000 iterations followed by 500,000 replications. The number of clusters (K) was determined by conducting seven independent runs for three subpopulations, all samples in each season (2011–2012 wet season, 2012 and 2013 dry seasons), and the entire dataset. The optimal number of K clusters was determined using the Delta K method (Evanno et al. 2005) using the HARVESTER software (Earl and vonHoldt 2012); data were processed in CLUMPP (Jakobsson and Rosenberg 2007), and then visualized using DISTRUCT (Rosenberg 2004). Two-way ANOVA, multivariate analyses, and T-tests were performed in the R software v. 3.0.1. (R Core Team 2004) using the HSAUR package (Everitt and Hothorn 2006).

Results and Discussion

We observed a total of 274 alleles across the 11 polymorphic microsatellite loci (Slotman et al. 2007, Brown et al. 2011) across all three sites in Cebu city and season collections (Table 1). Average allele frequencies (Supp Table 1 [online only]) at each locus ranged from 0.03 to 0.20 in the wet season and 0.03–1.0 in the dry season, when certain alleles might have been fixed due to random genetic drift as a consequence of the reduction in N_e observed during the dry season (Table 2). Mean A_R and P_{AR} for all subpopulations in the wet season were higher than in the dry season (Table 3). Student's T-test showed that this difference in mean A_R was significant between seasons ($t = 3.97$, $df = 7$, $P = 0.005$) but mean P_{AR} was not significantly different ($t = 1.28$, $df = 7$, $P = 0.28$). Allelic frequencies were also significantly different between dry and wet seasons ($P < 0.05$; Table 4 and Fig. 2). Although H_O values were slightly lower than H_E in most sites for the two seasons, except in BSN and PRD during the dry season of 2013 (Table 3), multivariate analysis showed that they were not statistically different (sites: $F = 0.854$, $df = 8$, $P = 0.62$; seasons: $F = 2.759$, $df = 1$, $P = 0.07$).

Fig. 2. Microsatellite allele frequencies observed in Babag (A), Basak San Nicolas (B), and Poblacion Pardo (C) subpopulations of *Ae. aegypti* in different seasons for 11 microsatellite loci. Each colored bar represents allele frequency present in each locus.

No seasonal difference in H_E was detected either ($P > 0.05$).

Twelve deviations from HWE were detected in five (A1, AC2, AC5, AG2, AG5) of the 11 loci within the subpopulations after Bonferroni's multiple correction

($P < 0.0009$; [Supp Table 2](#) [online only]). Eight of these 12 deviations from HWE occurred within the subpopulations of the dry season and four deviations were detected in the wet season. Separate LD analyses by seasons showed that only one out of 165 (0.6%) comparisons had

Table 5. Genetic differentiation using F_{ST} ^a (below diagonal) and Jost's D_{EST} ^a (above diagonal) of *Ae. aegypti* subpopulations between study sites in Cebu city, Philippines, and between different seasons (wet season of 2011–2012 and dry seasons of 2012 and 2013)

Sites: season	BBG wet 2011–2012	BSN wet 2011–2012	PRD wet 2011–2012	BBG dry 2012	BSN dry 2012	PRD dry 2012	BBG dry 2013	BSN dry 2013	PRD dry 2013
BBG wet 2011–2012		0.007	0.007	0.002	0.014	0.013	0.008	0.003	0.041
BSN wet 2011–2012	0.0104		0.006	0.012	0.003	0.008	0.012	0.023	0.011
PRD wet 2011–2012	0.0066	0.0117		0.013	0.007	0.005	0.011	0.008	0.021
BBG dry 2012	0.0035	0.0183	0.0115		0.023	0.016	0.005	0.010	0.022
BSN dry 2012	0.0204	0.0079	0.0102	0.0315		0.008	0.017	0.026	0.014
PRD dry 2012	0.0187	0.0115	0.0181	0.0245	0.0166		0.018	0.013	0.017
BBG dry 2013	0.0192	0.0258	0.0195	0.0197	0.0271	0.0395		0.029	0.021
BSN dry 2013	0.0141	0.0309	0.0197	0.0139	0.0288	0.0273	0.0343		0.025
PRD dry 2013	0.042	0.0277	0.0244	0.0384	0.0295	0.0381	0.0371	0.0366	

^a Harmonic mean of D_{EST} and F_{ST} . High mean estimates of D_{EST} and F_{ST} are in bold but showed little genetic differentiation (0.0–0.05).

Table 6. Gene flow (N_m) of *Ae. aegypti* subpopulations in study sites in Cebu city, Philippines, collected in the wet season of 2011–2012 and dry seasons of 2012 and 2013

Sites: season	BBG wet 2011–2012	BSN wet 2011–2012	PRD wet 2011–2012	BBG dry 2012	BSN dry 2012	PRD dry 2012	BBG dry 2013	BSN dry 2013	PRD dry 2013
BBG wet 2011–2012	-	57.52588	61.00787	34.34916	60.51996	25.55448	13.53011	37.98311	6.6963
BSN wet 2011–2012	-		16.35781	16.83407	136.03319	25.91268	14.54606	12.66568	10.00955
PRD wet 2011–2012	-			53.18637	28.59893	10.88164	22.0622	10.1007	7.40195
BBG dry 2012	-				13.76186	14.82996	16.10138	8.97948	7.69826
BSN dry 2012	-					29.83588	5.50338	10.74357	5.78398
PRD dry 2012	-						9.11656	7.47741	4.79892
BBG dry 2013	-							5.50338	8.73182
BSN dry 2013	-								6.13272
PRD dry 2013	-								

significant difference between AC2 and AG2 loci in PRD in the wet season (2011–2012), whereas three out of 166 (1.8%) comparisons, namely, between AC2 and B2 loci and between AC2 and AG1 loci in PRD; and between AC2 and B2 loci in BBG in the dry season of 2013. The loci did not display LD across all subpopulations and seasons, thus, implying that they were not physically linked (Mendonca et al. 2014) in concordance with previous marker validation by Brown et al. (2011). Deviations from HWE are generally attributed to differential selection, LD, and non-random mating that may lead to heterozygous deficiency or inbreeding. The overall F_{IS} per locus for each site in different seasons ranged from -0.018 (A1 locus) in 2013 dry season to 0.551 (AG2 locus) in 2012 dry season (Supp Table 3 [online only]), suggesting some degree of selection in the population. However, the seasonal average F_{IS} estimates detected only incipient inbreeding in the 2011–2012 wet season and 2012 dry season and none in the 2013 dry season.

Overall genetic differentiation ($D_{EST}=0.014$ and $F_{ST}=0.02$) in the current study was low (Table 5). Gene flow based on N_m values (Table 6) in the 2011–2012 wet season ranged from 61.0 to 16.4 (average = 45.0) effective migrants per generation, suggesting higher dispersal rate of *Ae. aegypti* among subpopulations than those in the dry seasons of

2012 (range = 29.8–13.8; average = 19.5) and 2013 (range = 8.7–5.5; average = 6.8). Nevertheless, the subpopulations were not completely isolated from each other at any time, as the number of migrants was still significant in the dry seasons. This finding was also supported by the absence in genetic structure after Bayesian clustering analysis (Fig. 3). Clustering with a $K=6$ implies genetic homogeneity for all subpopulations in different seasons (Fig. 3C). With a $K=2$ (Fig. 3A) indicates that all *Ae. aegypti* samples could be grouped by two seasons, or by $K=3$ (Fig. 3B) would imply that all samples could be geographically differentiated regardless of seasonal difference but did not detect any significant pattern.

Results of the current study are consistent with previous studies (Whitlock and Barton 1997, Huber et al. 2002a, Mendonca et al. 2014) that observed seasonal genetic variation in *Ae. aegypti* populations. In Ho Chi Minh city, Vietnam, genetic differentiation of *Ae. aegypti* decreases in the wet season when larval habitats are abundant and it increases in the dry season when larval habitats are dramatically reduced, thus, restricting gene flow (Huber et al. 2002b). The same seasonal trend was found in Manaus, Brazil, where samples collected from the dry season are significantly structured and not in the rainy season due to their reduced population size (Mendonca et al. 2014). In

Fig. 3. Genetic structure of *Ae. aegypti* subpopulations from three study sites in Cebu city, Philippines, collected in wet season of 2011–2012 and dry seasons of 2012 and 2013. STRUCTURE bar plots: the height of each color represents the probability of assignment to each K cluster. Each vertical bar represents a single individual. (A) K = 2; (B) K = 3; (C) K = 6 for all samples in all subpopulations in different seasons.

contrast, Costa-Ribeiro et al. (2006) reported higher genetic structure of *Ae. aegypti* in the rainy season in Rio de Janeiro, Brazil. The differences could be due to the different sensitivity or usefulness of the markers (Lanzaro et al. 1995) or different ecological settings of the populations. Moreover, the Bayesian STRUCTURE method used in this study did not find evidence of genetic structure among our subpopulations between seasons (Fig. 3). However, we were able to detect a significant increase in allelic richness during the wet season relative to the dry season. It is likely that the decrease in allelic richness observed during the dry season is a consequence of the genetic drift experienced when the population size is considerably reduced (Table 2). The proliferation of larval habitats suitable for development of larvae and pupae during the wet season may subsequently lead to the increased number of alleles as the population expands and the ability of *Ae. aegypti* eggs to resist desiccation in the dry season (Trpis 1972). This may quickly reestablish the genetic diversity despite undergoing a period of harsh conditions. Our data are consistent with this hypothesis. The absence of genetic structure, expected during the dry season, can be explained by the persistence of considerable gene flow among subpopulations. Although migration among sites decreased in the dry

season, the number of detected migrants was still significant.

We have shown that the *Ae. aegypti* population in Cebu city, Philippines, experienced temporal genetic changes due to genetic drift. The N_e showed greater variation between seasons (Table 2). The wet season (2011–2012) had the highest total N_e of 281.9 *Ae. aegypti* individuals. Total N_e was reduced in the 2012 dry season with 205 individuals and the least occurred in 2013 dry season with 59.2 individuals only. Campos et al. (2012) suggested that females tend to lay their eggs in fewer larval habitats and possibly closer to resting sites during the dry season. Although dengue cases in Cebu city in the wet season consistently increased from year-to-year (Edillo and Madarieta 2012), the reduction of genetic diversity during drift in mosquito population in the dry season did not seem to significantly affect recolonization in the next subsequent wet season. This might be attributed to reports that *Ae. aegypti* has developed evolutionary adaptability during extreme seasonal fluctuations (Cheng et al. 2012). *Ae. aegypti* eggs have been shown to survive desiccation by entering diapause or quiescence. Survival rates after a 120-d desiccation period range from 7 to 43% (Trpis 1972). In Tanzania, eggs deposited in rubber tires survive the entire dry season and hatch once larval habitats become available in the wet season (Trpis 1972).

Resistance to desiccation may be the crucial factor for stabilizing population genetic diversity (Mendonça et al. 2014) during recolonization in the wet season. In Cebu city, artificial containers (drums and plastic containers) serve as the key repositories of pupal productivity that maintain *Ae. aegypti* population (Edillo et al. 2012). In temperate regions, *Ae. aegypti* populations survive the cold season at least partially in the egg stage. Cold-season mortality of *Ae. aegypti* eggs is lower (30.6%) in Buenos Aires, Argentina, than those from tropical regions during the dry season (Fischer et al. 2011).

The little genetic differentiation of *Ae. aegypti* subpopulations between rural (BBG) and urban sites (BSN and PRD) in Cebu city might be explained by human-related activities that facilitate the dispersal of the vector and the relative spatial proximity of the subpopulations. Subpopulation in the rural mountainous site did not appear to impede gene flow with the urban subpopulations in BSN and PRD in any of the seasons. We did not find evidence of a founder effect in any of subpopulations, as there was little genetic differentiation among sites in the dry season. Similarly, Brown et al. (2013) did not find spatial genetic variation of *Ae. aegypti* in the Florida Keys, USA.

A massive mosquito larvicide campaign has been continuously conducted in the Philippines (DOH 2011, DOST 2013). The yearly increase and decrease of dengue cases in the wet and dry seasons in Cebu (Edillo and Madarieta 2012), respectively, might be linked to temporal genetic changes in *Ae. aegypti* subpopulations but also could have been a consequence of antidengue campaigns such as insecticide use, or of temporal changes that are innate to virus ability to infect the hosts (Zhang et al. 2013). Paupy et al. (2005) and Yébakima et al. (2004) showed that intensive insecticide use and social factors (i.e., human density) have caused distinct patterns in the genetic structure of dengue mosquitoes.

In conclusion, *Ae. aegypti* subpopulations in Cebu city, Philippines, are under intense selection in the dry season when larval habitats are scarce. During the wet season, genetic composition of the population is reconstituted by hatching of eggs that have survived the dry season, acting as reservoir for genetic diversity. Overall, *Ae. aegypti* population in Cebu city had little genetic differentiation although gene flow was higher in the wet season than in both years' dry seasons. We recommend intensified vector control during the nonepidemic dry season to prevent population re-emergence and increased dengue transmission in the following wet season. Conducting temporal genetic studies in a geographically broader scale in the country should also be helpful in future dengue control efforts.

Supplementary Data

Supplementary data are available at *Journal of Medical Entomology* online.

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References Cited

- Andrewartha, H. G., and L. C. Birch. 1954. The distribution and abundance of animals, p. 88. University of Chicago Press, Chicago.
- Beatty, M. E., P. Beutels, M. I. Meltzer, D. S. Shepard, J. Hombach, R. Hutubessy, D. Dessis, L. Coudeville, B. Dervaux, O. Wichmann, et al. 2011. Health economics of dengue: A systematic literature review and expert panel's assessment. *Am. J. Trop. Med. Hyg.* 84: 473–88.
- Belkin, J. N. 1962. The mosquitoes of the south pacific (Diptera, Culicidae), 2nd ed. University of California Press, Berkeley, CA.
- Boutin-Ganache, I., M. Raposo, M. Raymond, and C. F. Deschepper. 2001. M13-tailed primers improve the readability and usability of microsatellite analyses performed with two different allele-sizing methods. *Biotechniques* 31: 24–26.
- Brown, J. E., C. S. McBride, P. Johnson, S. Ritchie, C. Paupy, H. Bossin, J. Lutomiah, I. F. Salas, A. Ponlawat, A. J. Cornel, et al. 2011. Worldwide patterns of genetic differentiation imply multiple 'domestications' of *Aedes aegypti*, a major vector of human diseases. *Proc. R. Soc. B* 278: 2446–2454.
- Brown, J. E., V. Obas, V. Morley, and J. R. Powell. 2013. Phylogeography and spatio-temporal genetic variation of *Aedes aegypti* (Diptera: Culicidae) populations in the Florida Keys. *J. Med. Entomol.* 50: 294–299.
- Capeding, M. R., N. G. Tran, S. R. Hadinegoro, H. I. Muhammad Ismail, T. Chotpitayasunond, M. N. Chua, C. Q. Luong, K. Rusmil, D. N. Wirawan, R. Nallusamy, et al. 2014. Clinical efficacy and safety of a novel tetravalent dengue vaccine in healthy children in Asia: a phase 3, randomised, observer-masked, placebo-controlled trial. *Lancet* 384: 1358–1365.
- (CDC) Center for Disease Control. 2013. Current status of dengue vaccine development. SAGE/Immunization Meeting. National Center for Emerging and Zoonotic Infectious Diseases. (http://www.who.int/immunization/sage/meetings/2013/april/2_Roehrig_Dengue_SAGE_April2013.pdf) (accessed 19 March 2015).
- Cheng, C., B. J. White, C. Kamdem, K. Mockaitis, C. Costantini, M. W. Hahn, and N. J. Besansky. 2012. Ecological genomics of *Anopheles gambiae* along a latitudinal cline: A population re-sequencing approach. *Genetics* 190: 1417–1432.
- (CCHD) Cebu City Health Department. 2010. Cebu city epidemiology statistics and surveillance unit: Dengue fever surveillance 2010 report. CCHD, Cebu City, Philippines.
- Crawford, N. G. 2010. SMOGD: software for the measurement of genetic diversity. *Mol. Ecol. Resour.* 10: 556–557.
- Colton, Y. M., D. D. Chadee, and D. W. Severson. 2003. Natural skip oviposition of the mosquito *Aedes aegypti* indicated by codominant genetic markers. *Med. Vet. Entomol.* 17: 195–204.
- Costa-Ribeiro, M.C.V., R. Lourenco-de-Oliveira, and A. B. Failloux. 2006. Geographic and temporal genetic patterns of *Aedes aegypti* populations in Rio de Janeiro, Brazil. *Trop. Med. Int. Health* 8: 1276–1285.
- Do, C., R. S. Waples, D. Peel, G. M. Macbeth, B. J. Tillett, and J. R. Ovenden. 2014. NeEstimator V2: re-implementation of software for the estimation of contemporary effective population size (N_e) from genetic data. *Mol. Ecol. Res.* 14: 209–214.
- (DOST) Department of Science and Technology—Dengue Vector Surveillance. 2013. (<http://oltrap.pchrd.dost.gov.ph>) (accessed 4 November 2014).
- (DOH) Philippine Department of Health. 2011. Dengue surge in Luzon. (<http://dev1.doh.gov.ph/content/dengue-surge-luzon>) (accessed 4 November 2014).

- Earl, D. A., and B. M. von Holdt. 2012. STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conserv. Genet. Resour.* 4: 359–361.
- Edillo, F., and S. Madarieta. 2012. Trends of dengue infections (1997–2008) in Cebu Province, Philippines. *Dengue Bull.* 36: 37–49.
- Edillo, F., N. D. Roble, and N. D. Otero 2nd. 2012. The key breeding sites by pupal survey for dengue mosquito vectors, *Aedes aegypti* (Linnaeus) and *Aedes albopictus* (Skuse), in Guba, Cebu City, Philippines. *Southeast Asian J. Trop. Med. Public Health* 43: 1365–1374.
- Edillo, F. E., Y. Halasa, F. M. Largo, J.N.V. Erasmo, N. B. Amoin, M.T.P. Alera, I.-K. Yoon, A. C. Alcantara, and D. S. Sheppard. Economic cost and burden of dengue in the Philippines. 2015. *Am. J. Trop. Med. Hyg.* 92: 360–366.
- Endersby, N. M., A. A. Hoffmann, V. L. White, S. Lowenstein, S. Ritchie, P. H. Johnson, L. P. Rapley, P. A. Ryan, V. S. Nam, N. T. Yen, et al. 2009. Genetic structure of *Aedes aegypti* in Australia and Vietnam revealed by microsatellite and exon primed intron crossing markers suggests feasibility of local control options. *J. Med. Entomol.* 46: 1074–1083.
- Excoffier, L., G. Laval, and S. Schneider. 2006. An integrated software package for population genetics data analysis, version 3.01. Computational and Molecular Population Genetics Lab. Institute of Zoology, University of Berne, Switzerland.
- Evanno, G., S. Regnaut, and J. Goudet. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. *Mol. Ecol.* 14: 2611–2620.
- Everitt, B. S. and T. Hothorn. 2006. A handbook of statistical analyses using R. Chapman and Hall/CRC Taylor and Francis Group Boca Raton, FL.
- Failoux, A. B., M. Vazeille, and F. Rodhain. 2002. Geographic genetic variation in populations of the dengue vector *Aedes aegypti*. *J. Mol. Evol.* 55: 653–663.
- Fischer, S., I. S. Alem, M. S. De Majo, R. E. Campos, and N. Schweigmann. 2011. Cold season mortality and hatching behavior of *Aedes aegypti* (Diptera: Culicidae) eggs in Buenos Aires city, Argentina. *J. Vector Ecol.* 36: 94–99.
- Huber, K., L. L. Loan, T. H. Hoang, T. K. Tien, F. Rodhain, and A. B. Failoux. 2002a. Temporal genetic variation of *Aedes aegypti* populations in Ho Chi Minh city (Vietnam). *Heredity* 89: 7–14.
- Huber, K., L. L. Loan, T. H. Hoang, S. Ravel, F. Rodhain, and A. B. Failoux. 2002b. Genetic differentiation of the dengue vector, *Aedes aegypti* (Ho Chi Minh city, Vietnam) using microsatellite markers. *J. Mol. Ecol.* 11: 1629–1635.
- Jakobsson, M. and N. A. Rosenberg. 2007. CLUMPP: A cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics* 23: 1801–1806.
- Jost, L. 2008. GST and its relatives do not measure differentiation. *Mol. Ecol.* 17: 4015–4026.
- Kalinowski, S. 2005. HP-Rare: A computer program for performing rarefaction on measures of allelic diversity. *Mol. Ecol. Notes* 5: 187–189.
- Lanzaro, G. C., and F. Tripet. 2003. Gene flow among populations of *Anopheles gambiae*: A critical review, pp. 109–132. In W. Takken and T. W. Scott (eds.), *Ecological Aspects for Application of Genetically Modified Mosquitoes*: Kluwer Academic Press, Dordrecht (Netherlands).
- Lanzaro, G. C., L. Zheng, Y. T. Toure, S. F. Traore, F. C. Kafatos, and K. D. Vernick. 1995. Microsatellite DNA and isozyme variability in a west African population of *Anopheles gambiae*. *Insect Mol. Biol.* 4: 105–112.
- Mendonça, B.A.A., A.C.B. de Sousa, A. P. de Souza, and V. M. Scarpassa. 2014. Temporal genetic structure of major dengue vector *Aedes aegypti* from Manaus, Amazonas, Brazil. *Acta Trop.* 13: 80–88.
- Nomura, T. 2008. Estimation of effective number of breeders from molecular coancestry of single cohort sample. *Evol. Appl.* 1: 462–474.
- Paupy, C., N. Chanthha, J. M. Reynes, and A. B. Failloux. 2005. Factors influencing the population structure of *Aedes aegypti* from the many cities in Cambodia. *Heredity* 95: 144–147.
- Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using Multilocus genotype data. *Gen. Soc. Am.* 155: 945–959.
- R Core Team. 2004. R: A language and environment for statistical computing. R foundation for statistical computing. Vienna Austria.
- Raymond, M., and F. Rousset. 1995. GENEPOP (version 4.2): Population genetics software for exact tests and ecumenism. *J. Hered.* 86: 248–249.
- Rosenberg, N. A. 2004. Distruct: A program for the graphical display of population structure. *Mol. Ecol. Notes* 4: 137–138.
- Slotman, M. A., N. B. Kelly, L. C. Harrington, S. Kittawee, J. W. Jones, T. W. Scott, A. Caccone, and J. R. Powell. 2007. Polymorphic microsatellite markers for studies of *Aedes aegypti* (Diptera: Culicidae), the vector of dengue and yellow fever. *Mol. Ecol. Notes* 7: 168–171.
- Sutherst, R. W., G. F. Maywald, and D. B. Skarrat. 1995. Predicting insect distributions in a changed climate, p. 59–61. In R. Harrington and N. E. Stork (eds.), *Insects in a changing environment: 17th symposium of the Royal Entomological Society of London, 7–10 September 1993*. Academic Press, Rothamsted Experimental Station, London.
- Trpis, M. 1972. Dry season survival of *Aedes aegypti* eggs in various breeding sites in the Dar es Salaam area, Tanzania. *Bull. World Health Org.* 47: 433–437.
- Weir, B. S., and C. C. Cockerham. 1984. Estimating F-statistics for the analysis of population structure. *Evolution* 38: 1358–1370.
- Whitlock, M. C., and N. H. Barton. 1997. The effective size of a subdivided population. *Genetics* 146: 427–441.
- (WHO-WPRO) World Health Organization–Western Pacific Region Office. 2013. Emerging disease surveillance and response: Dengue Situation Updates. WPRO 2014. (http://www.wpro.who.int/emerging_diseases/DengueSituationUpdates/en/)
- (WHO-WPRO) World Health Organization–Western Pacific Region Office. 2014a. Emerging disease surveillance and response. (www.wpro.who.int/emerging_diseases/Dengue/en/) (accessed 4 November 2014).
- (WHO-WPRO) World Health Organization–Western Pacific Region Office. 2014b. Dengue in the Philippines. (http://www.wpro.who.int/philippines/areas/communicable_diseases/dengue/continuation_dengue_area_page/en/) (accessed 4 November 2014).
- Yébakima, A., C. Charles, L. Mousson, M. Vazeille, M. M. Yp-Tcha, and A.-B. Failoux. 2004. Genetic heterogeneity of the dengue vector, *Aedes aegypti*, in Martinique. *Trop. Med. Int. Health* 9: 582–587.
- Zhang, X., J. Sheng, P. Plevka, R. J. Kuhn, M. S. Diamond, and M. G. Rossman. 2013. Dengue structure differs at the temperatures of its human and mosquito hosts. *Proc. Natl. Acad. Sci. USA* 110: 6795–6799.

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