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Yearly variations of the genetic structure of *Aedes aegypti* (Linnaeus) (Diptera: Culicidae) in the Philippines (2017–2019)

Frances Edillo^{a,*}, Rhoniell Ryan Ymbong^a, Maureen Mathilde Cabahug^a, Dinesse Labiros^a, Mark Windy Suycano^a, Louis Lambrechts^b, Anavaj Sakuntabhai^c

^a Mosquito Research Laboratory, Department of Biology, University of San Carlos - Talamban campus, Cebu city 6000, Philippines

^b Insect-Virus Interactions Unit, Institut Pasteur, UMR2000, CNRS, Paris, France

^c Functional Genetics of Infectious Diseases Unit, Institut Pasteur, UMR2000, CNRS, Paris, France

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ABSTRACT

Dengue is the fastest emerging arboviral disease in the world, imposing a substantial health and economic burden in the tropics and subtropics. The mosquito, *Aedes aegypti*, is the primary vector of dengue in the Philippines. We examined the genetic structure of *Ae. aegypti* populations collected from the Philippine major islands (Luzon, Visayas and Mindanao), each with highland (Baguio city, Cebu city mountains and Maramag, Bukidnon, respectively) and lowland sites (Quezon city; Liloan, Cebu and Cagayan de Oro [CDO] city, respectively) during the wet (2017–2018 and 2018–2019) and dry seasons (2018 and 2019). Mosquitoes ($n = 1800$) were reared from field-collected eggs and immatures, and were analyzed using 12 microsatellite loci. Generalized linear model analyses revealed yearly variations between highlands and lowlands in the major islands as supported by Bayesian clustering analyses on: 1) stronger selection (inbreeding coefficient, $F_{IS} = 0.52$) in 2017–2018 than in 2018–2019 ($F_{IS} = 0.32$) as influenced by rainfall, 2) the number of non-neutral loci indicating selection, and 3) differences of effective population size although at $p = 0.05$. Across sites except Baguio and CDO cities: 1) F_{IS} varied seasonally as influenced by relative humidity (RH), and 2) the number of non-neutral loci varied as influenced by RH and rainfall indicating selection. Human-mediated activities and not isolation by distance influenced genetic differentiations of mosquito populations within ($F_{ST} = 0.04$) the major islands and across sites (global $F_{ST} = 0.16$). Gene flow (Nm) and potential first generation migrants among populations were observed between lowlands and highlands within and across major islands. Our results suggest that dengue control strategies in the epidemic wet season are to be changed into whole year-round approach, and water pipelines are to be installed in rural mountains to prevent the potential breeding sites of mosquitoes.

1. Introduction

Dengue is the fastest emerging arboviral infection world-wide (WHO, 2019a, 2019b). Approximately, over 390 million infections occur annually world-wide, of which more than 95 million are clinically apparent (Bhatt et al., 2013). As of March 2021, the Philippines had 13,699 dengue cases including 50 deaths (case fatality rate = 0.4%); which were 68% lower compared to 42,584 cases during the same period in 2020 (WHO, 2021). Cheng et al. (2018) estimated that by 2016–2020, the annual average hospitalized (401,191) and ambulatory dengue cases (239,497) in the Philippines resulted to USD 139 million (PhP 5.9 billion) and USD 19 million (PhP 827 million), respectively.

A. aegypti (L.) and *Aedes albopictus* (Skuse) (Diptera: Culicidae) are the primary and secondary mosquito vectors, respectively, of dengue viruses (DENVs; *Flaviviruses*) with four serotypes in the Philippines. These medically important species transmit not just DENVs but potentially also chikungunya and Zika viruses (Mckenzie et al., 2019; Ryan et al., 2019; Souza-Neto et al., 2019).

Climate change affects the survival and dispersion of *Ae. aegypti* and *Ae. albopictus* and transmission rates of viral pathogens (Ryan et al., 2019). Artificial environments sustain the geographic range limit of these species (Capinha et al., 2014). Dengue outbreaks occurred in Tokyo, Japan in 2014 after 70 years without dengue (Kutsuna et al., 2015); Madeira, Portugal in 2012 (Lourenço and Recker, 2014) and

* Corresponding author.

E-mail addresses: feedillo@usc.edu.ph (F. Edillo), rrjymbong@usc.edu.ph (R.R. Ymbong), louis.lambrechts@pasteur.fr (L. Lambrechts), anavaj.sakuntabhai@pasteur.fr (A. Sakuntabhai).

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Baguio, Philippines recently (The City Government of Baguio, 2020). If mosquito range shifts track optimal temperature ranges for transmission (21.3–34.0 °C for *Ae. aegypti*; 19.9–29.4 °C for *Ae. albopictus*), poleward shifts in *Aedes*-borne virus distributions are expected with available domestic environments in the new areas (Ryan et al., 2019). It is estimated that 3.9 billion people are at risk of infection with dengue (Brady et al., 2012). Dhimal et al. (2015) noted that the expanded distribution of *Ae. aegypti* in previously non-endemic areas and in highly elevated mountainous region calls for urgent actions to protect the health of the local people and travelers. However, there have been no studies associating temperature and elevation on the genetic structure of *Ae. aegypti*, to our knowledge thus far.

Only a few studies on the genetic structure of dengue mosquitoes were conducted in the Philippines. In Metro Manila, Carvajal et al. (2020) reported fine-scale population genetic structure of *Ae. aegypti*, weak genetic drift, and long-distance dispersal via human-mediated mobility. Based on the fine-scale genetic structure of *Ae. aegypti*, Manila roads are potential barriers to mosquito dispersal (Regilme et al., 2021). In central-western Philippines, Fonzi et al. (2015) found considerable gene flow of *Ae. aegypti* due to anthropic factors (cargo ships and human population density), while geographical distance is not correlated to genetic differentiation. Moreover, the genetic structure of *Ae. aegypti* varies seasonally (Huber et al., 2002; Mendonca et al., 2014; Sayson et al., 2015), which are primarily associated with climate, insecticide use (Scarpassa and Conn, 2007) and social factors. In Cebu city, Sayson et al. (2015) reported that intense selection of *Ae. aegypti* in the dry season (2012–2013) caused a reduced population size that favored heterozygotes, leading to small pockets of mosquitoes (refuges) that exhibited random and increased genetic differentiation ($F_{ST} = 0.029$). In the wet season (2011–2012), the mosquitoes' genetic composition was reconstituted by refuges' expansion that survived the preceding dry season and contributed to lower genetic differentiation ($F_{ST} = 0.009$). Moreover, the Philippine summer solstice reached 13-h and 21 min on June 21, 2017 and 13-h on June 21, 2018 based on Philippine Atmospheric, Geophysical and Astronomical Services Administration (PAG-ASA) data. These harsh environmental conditions (i.e., hot, humid and longer day length) in the dry season pose another adaptation challenge for dengue mosquitoes. Mendonca et al. (2014) also found more significantly structured *Ae. aegypti* populations in the dry season due to reduced effective population size than those in the wet season in Manaus, State of Amazonas, Brazil.

Integrated vector management, that includes a combination of various methods such as vector control and education of the public, is extremely relevant (WHO, 2012). The Philippine Department of Health (DOH, 2020) has been advocating the enhanced 4S strategy as the main focus for dengue prevention and control program. The enhanced 4S strategy stands for: 1) seek and destroy mosquito-breeding sites, 2) seek early consultation if one develops dengue-associated symptoms, 3) employ self-protection measures, and 4) say "no" to indiscriminate fogging and implement fogging only during outbreaks in hotspot areas. Understanding the spatio-temporal genetic structure among dengue mosquitoes, particularly in the current global warming, is very important for targeting management and control strategies of dengue and other *Aedes*-borne diseases, and for assessing the influences of ecological and geographical variations in natural mosquito populations. However, there has been no study on the population structure of *Ae. aegypti* in the entire Philippine archipelago with the current global warming. Thus, this study aimed to examine the spatial (i.e., elevation and major islands) and temporal (i.e., seasonal and yearly) genetic changes of *Ae. aegypti* populations in selected highland and lowland sites in the Philippine major islands (Luzon, Visayas and Mindanao) during the wet and dry seasons of 2017–2019 using 12 microsatellite loci.

2. Materials and method

2.1. Study sites and period

The study sites in the Philippine (Fig. 1) were chosen based on: 1) elevation to represent highland (>350 m ASL) and lowland sites (<100 m ASL) (Table 1), 2) latitudes to represent the three major islands (Luzon, Visayas and Mindanao), 3) high dengue incidence (DOH, 2017), and 4) similar biotype according to modified Corona's climate classification (Corporal-Lodangco and Leslie, 2017). The country has a tropical climate characterized by two seasons: relatively wet (June–February) and dry seasons (March–May); summer solstice occurs in late June. The highland sites of Luzon, Visayas and Mindanao included Baguio city (BG), Cebu city (CC) mountains, and Maramag, Bukidnon (BUK), respectively, whereas the lowland sites included Quezon city (QC); Liloan (LIL), Cebu; and Cagayan de Oro (CDO) city, respectively (Fig. 1; Table 1). Each highland or lowland site had three subsites; half- to three kilometer-distance was established between subsites. BG subsites from wet season (2017–2018) until dry season (2018) were replaced by new subsites from December 1, 2018 until May 2019 for the wet season (2018–2019) and dry season (2019) collections due to change of collectors and their non-access to previous subsites (Table 1).

2.2. Mosquito collections and rearing

Aedes eggs were collected monthly by modified ovidial/larvicidal (O/L) trap method from Luzon and Mindanao sites during the wet and dry seasons (June 2017–June 2019). Immatures were collected by net method from Visayas sites during the wet (2017–2018) and dry (2018) seasons only; the rest were collected by modified O/L trap method. Briefly, black plastic O/L trap (DOST, Manila) was filled with tap water and filter paper-wrapped wooden paddle was put diagonally in it to serve as the substrate for female *Aedes* to lay eggs on it. Each O/L trap was placed outside human dwelling under the roof gutter and was inspected daily for possible deposition of *Aedes* egg cohorts. Thirty to 40 O/L traps were placed randomly in each of the three subsites per highland or lowland site of each big island for each monthly collection. Each egg paper was air-dried and put inside a plastic cup for shipment with permission by the Bureau of Quarantine, Cebu city, Philippines.

Field-collected parental *Aedes* eggs and subadults were reared in the insectary with controlled conditions of 25 °C, 75–80% relative humidity (RH) and 12-h light:12-h dark (12 L:12D) photoperiod. Only one individual for each O/L trap was randomly chosen from the 30–40 O/L traps placed per subsite in each monthly collection. Weather data sets from 2017 to 2019 nearest to the study sites were obtained from PAG-ASA through Freedom of Information (FOI) site of the Philippine government for all sites except for Liloan (World Weather Online, 2022). *Aedes* subadults were placed in plastic transparent cups covered with fine-mesh cloth, were allowed to emerge into adults, and were sorted out by sex and species (Belkin, 1962) because *Ae. albopictus* sometimes were present. *Ae. aegypti* males have bushy-like bristles on their antennae, while females have scanty bristles. Emerged adult samples were stored at an ultralow freezer (–80 °C).

2.3. DNA extraction

Extraction of genomic DNA of wet season-collected *Ae. aegypti* was done by 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, Hilden, Germany) after sample digestion for RNA removal. Twenty-five DNA extract-samples from each of the three subsites ($n = 75$ from each highland or lowland site in each of the three major islands) for each season from June 2017 to June 2019 were processed for microsatellite analyses, and sibling samples were excluded.

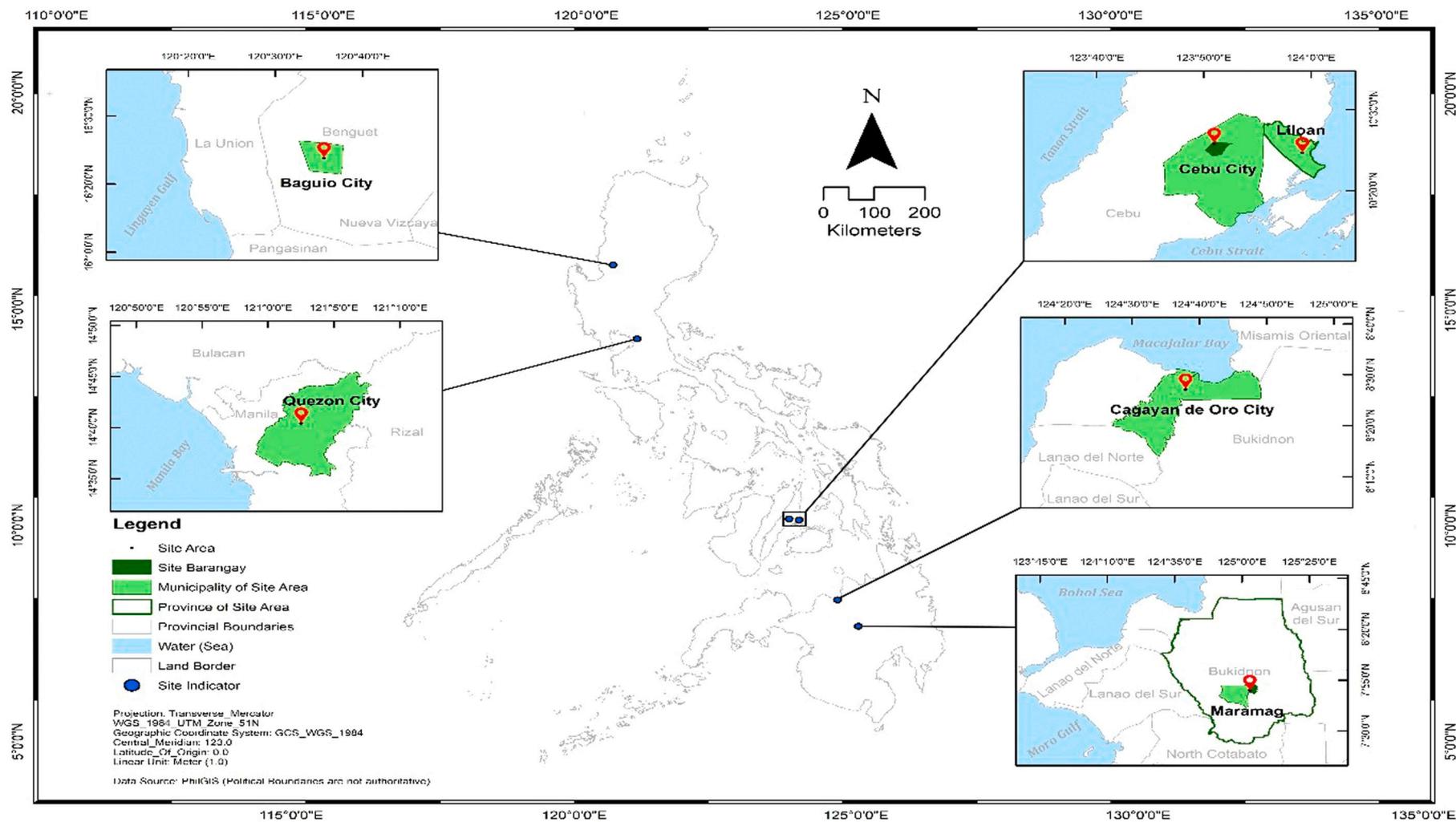


Fig. 1. The Philippine study sites in Luzon (Baguio and Quezon cities), Visayas (Cebu city mountains and Liloan, Cebu), and Mindanao (Maramag, Bukidnon and Cagayan de Oro city).

Table 1
Highland and lowland study sites within Philippine big islands (Luzon, Visayas and Mindanao) with coordinates, subsites and elevations.

Sites	Coordinates	Subsites	Elevation (m ASL)
Luzon Baguio city (BG)	16.402°N; 120.596°E	UP-Baguio ¹	1489.00
		Trancoville ¹	1417.00
		Cabinet Hill ¹	1474.00
		Sta. Scholastica ²	1391.00
		Country club ²	1473.00
		Assumption Road ²	1412.00
Quezon city (QC)	14.676°N; 121.044°E	NIMBB,UP-Diliman ³	75.00
		Daan Tubo	65.00
		Payatas B	62.00
		Taptap	661.74
Visayas Cebu city (CC) mountains	10.317°N; 123.891°E	Babag 2	404.66
		Tabunan, Cantipla	873.42
Liloan (LIL), Cebu	10.4121°N; 123.986°E	Poblacion	16.63
		Yati	20.52
		Catarman	16.17
Mindanao Maramag, Bukidnon (BUK)	7.8592°N; 125.0515°E	Lumbo	458.00
		Sentro, Dologon	371.00
		Musuan, Dologon	386.00
		Gusa	14.00
Cagayan de Oro city (CDO)	8.454°N; 124.632°E	Cugman	21.00
		Bugo	20.00

m ASL: meter above sea level.

¹ First set of subsites (wet season of 2018 up to November 2018).

² Second set of subsites (December 1, 2018 up to dry season of 2019).

³ National Institute of Molecular Biology and Biotechnology, University of the Philippines, Diliman.

2.4. Microsatellite DNA analyses

A total of 12 microsatellite loci were used in this study; 11 markers were taken from Brown et al. (2011) and Slotman et al. (2007) and a new marker developed by the team (Labiros et al., 2022) (Additional file 1). Each paired microsatellite loci based on non-overlapping size ranges was amplified in a multiplex PCR reaction with a single fluorescent M13 primer (Boutin-Ganache et al., 2001; Oetting et al., 1995; Steffens et al., 1993), two forward primers with M13 at 5' tail and two reverse primers. All PCR reactions (10 µL) contained 1× Type-it multiplex PCR master mix (Qiagen) (6.45 µL nuclease free water [NFW], 1.0 µL buffer, 0.5 µL dNTPs, 0.05 µL taq polymerase), 0.075 µM of each forward primer, 0.250 µM of each reverse primer, 0.5 µM of fluorescently labelled M13 primer and one µL of DNA extract. Cycling conditions included the following: initial denaturation (94 °C) for 10 min; 35 cycles of denaturation (94 °C) for 30 s, annealing (62 °C) for 30 s, and extension (72 °C) for 30 s; and final extension (72 °C) for five minutes. Annealing PCR products were run on an Applied Biosystems 3730xl DNA genetic analyzer (Thermo Fisher Scientific, USA) with a GS 500 Rox internal size standard (Applied Biosystems, CA, USA). Microsatellite alleles were scored using GeneMapper 5 (Applied Biosystems, CA, USA) and microsatellite processing was done at the Philippine Genome Center (PGC), Quezon city (Additional file 2). Microsatellite alleles were scored using GeneMapper 5 (Thermo Fisher Scientific, Singapore). All genotyped data were double checked for allelic drop-outs by running each population using MICROCHECKER 2.2.3 (Van Oosterhout et al., 2004).

2.5. Data analyses

Population genetic diversity was characterized at each locus within populations (i.e., within sites) with the following: allelic richness (AR) and private allelic richness (PAR) by using HP-RARE 1.1 (Kalinowski, 2005), and by the number of alleles and heterozygosities (observed [H_O] and expected [H_E]) by using GENEPOP 4.7.5 (Raymond and Rousset,

1995). Each locus was tested separately for goodness-of-fit for Hardy-Weinberg equilibrium (HWE) by using a probability test, inbreeding coefficient (F_{IS}) (Weir and Cockerham, 1984), and pairwise tests for linkage disequilibrium (LD) between loci in each population (10,000 dememorisations, 1000 batches and 10,000 iterations per batch). The significance level for multiple simultaneous comparisons for HWE and LD was adjusted following the sequential Bonferroni technique. Other GENEPOP 4.7.5 analyses included global estimates (i.e., two-year data, yearly and seasonal) and pairwise between populations of Fisher's statistics (F_{ST}) and gene flow (Nm) between populations. Nm (i.e., effective number of migrants) was calculated by private allele method to provide the corrected multilocus estimate of Nm by population pairs (Slatkin, 1995) and by using the values from the closest regression line (Barton and Slatkin, 1986). GENECLASS 2.0 (Piry et al., 2004) was used to estimate the yearly migration rate of individual *Ae. aegypti* across sites and seasons. Detection of the potential first generation (F0) migrants was done by computing the likelihood of L_{home}/L_{max} using Bayesian method (Rannala and Mountain, 1997) with Monte-Carlo resampling algorithm. Number of simulated individuals was set at 10,000 and 0.05 p -value. Pairwise F_{ST} and geographical distance (km) by year were examined using Mantel's test of correlation with 10,000 permutations through XLSTAT 2021 package of Microsoft Excel 2016. Effective population size (N_e) (i.e., the number of individuals in a theoretically ideal population having the same magnitude of random genetic drift as the actual populations) (Hartl and Clark, 2018) for each population was calculated using LD model of NeEstimator v. 2.1 program (Do et al., 2014). The Bayesian clustering method through STRUCTURE 2.3.4 program (Pritchard et al., 2000) was used to investigate the population structure using the two-year (i.e., one time analysis) and yearly (i.e., separate analyses) multi-locus genotype datasets without prior information on sampling locations considering the admixture model. Genotypes were run with a burn-in of 100,000 iterations and 500,000 replications. The optimal number of K clusters was determined using Delta K (Evanno et al., 2005) using the HARVESTER software (Earl and von Holdt, 2012).

Student's t -tests were performed using Microsoft excel 2016 to determine the differences between some seasonal (i.e., wet versus dry season) genetic analyses. Generalized Linear Model (GLM) statistical tests with the use of Akaike Information Criterion (AIC) were performed using SPSS software (v 21) (IBM Corporation, NY, USA) to explore the relationship between the meteorological data (mean values of the mean monthly of temperature, rainfall and RH) from each site in the following combinations of study period and some genetic analyses (mean values per site of: AR, PAR, number of loci that deviated from HWE, average F_{IS} and N_e). GLM statistical tests were done by: 1) year (i.e., yearly average of the wet and dry seasons) and two-site categories (highland vs. lowland) in each major island, 2) season (i.e., yearly average of wet or dry seasons) and two-site categories, and 3) season and six sites regardless of elevation.

3. Results

Most populations of *Ae. aegypti* across the three Philippine major islands ($n = 450$ in each season, total $n = 1800$) during the two years of study (2017–2019) provided genotypes of the 12 microsatellite loci. A few populations had null alleles in A1, AC1, AG5, CT2, B3 loci, and AC2 loci, and the null allele frequencies ranged from 0.00 to 0.45 in all loci.

3.1. Genetic diversity

Table 2 shows the summary of the genetic diversity of each mosquito population. All 12 microsatellite loci were polymorphic in all populations with a total of 541 and 513 alleles in the wet seasons of 2017–2018 and 2018–2019, respectively; and a total of 444 and 521 alleles in the dry seasons of 2018 and 2019, respectively (Additional file 3). Student's t -tests showed that the number of alleles of these loci

Table 2

Number of alleles, mean allelic richness (AR), mean private allelic richness (PAR), mean observed (H_O) and expected heterozygosities (H_E), number of loci that deviated from HWE and inbreeding coefficient (F_{IS}) for all loci of *Ae. aegypti* collected from all Philippine study sites during the wet and dry seasons (2017–2019) ($n = 450$ each season).

Sites*	Wet Season (2017–2018)						Dry Season (2018)					
	No. of alleles	Mean AR	Mean PAR	Mean H_O (Mean H_E)	Deviations from H-WE	Mean F_{IS}	No. of alleles	Mean AR	Mean PAR	Mean H_O (Mean H_E)	Deviations from H-WE	Mean F_{IS}
BG	91	4.15	0.31	0.67 (0.65)	12	0.54	73	3.52	0.32	0.72 (0.56)	11	0.49
QC	87	3.98	0.24	0.71 (0.63)	11	0.58	69	3.61	0.39	0.67 (0.60)	9	0.44
CC	89	4.28	0.33	0.63 (0.65)	10	0.48	67	3.58	0.30	0.77 (0.60)	11	0.61
LIL	89	4.24	0.36	0.61 (0.66)	12	0.50	80	3.91	0.45	0.74 (0.30)	12	0.59
BUK	95	4.10	0.35	0.71 (0.61)	11	0.58	71	3.53	0.35	0.68 (0.60)	9	0.47
CDO	90	3.90	0.33	0.67 (0.59)	11	0.51	84	3.80	0.51	0.66 (0.61)	9	0.45
Overall mean	90	4.11	0.32	0.67 (0.63)	11.17	0.53	74	3.66	0.39	0.71 (0.60)	10.17	0.51
Total	541						444					
	Wet Season (2018–2019)						Dry Season (2019)					
BG	87	3.82	0.36	0.61 (0.63)	7	0.41	102	4.44	0.48	0.48 (0.70)	9	0.29
QC	83	4.07	0.49	0.60 (0.65)	8	0.40	83	3.67	0.29	0.62 (0.61)	9	0.41
CC	83	3.79	0.34	0.57 (0.62)	9	0.34	90	4.04	0.33	0.51 (0.63)	9	0.29
LIL	87	4.02	0.37	0.53 (0.63)	6	0.21	95	4.01	0.47	0.57 (0.63)	8	0.33
BUK	77	3.84	0.33	0.59 (0.64)	11	0.37	77	6.24	0.32	0.45 (0.60)	8	0.25
CDO	96	4.34	0.51	0.53 (0.68)	10	0.32	74	3.51	0.20	0.50 (0.61)	5	0.25
Overall mean	86	3.98	0.40	0.57 (0.64)	8.50	0.34	87	4.32	0.35	0.52 (0.63)	8	0.30
Total	513						521					

BG: Baguio city, QC: Quezon city, CC: Cebu city mountains, LIL: Liloan, Cebu, BUK: Maramag, Bukidnon, CDO: Cagayan de Oro city.

differed significantly ($p < 0.01$) between wet (2017–2018) and dry seasons (2018), but did not differ significantly ($p > 0.05$) between wet and dry seasons in 2018–2019 across all sites. The mean AR across sites in the wet season of 2017–2018 ranged from 3.9 (CDO) to 4.28 (CC), and in the dry season, 3.52 (BG) to 3.91 (LIL); whereas those in the wet season of 2018–2019 ranged from 3.82 (BG) to 4.34 (CDO), and in the dry season, 3.51 (CDO) to 6.24 (BUK). The mean PAR across sites in 2017–2018 ranged from 0.24 (QC) to 0.36 (LIL) in the wet season, and from 0.30 (CC) to 0.51 (CDO) in the dry season; whereas those in 2018–2019 ranged from 0.33 (BUK) to 0.51 (CDO) in the wet season, and 0.20 (CDO) to 0.48 (BG) in the dry season. Mosquito populations in 2017–2018 from CC mountains and LIL during the wet season, and all populations in 2018–2019 except from QC during the dry season did not conform to HWE expectations ($H_E > H_O$), indicating heterozygote deficits and possible inbreeding within each mosquito population (Carvajal et al., 2020).

Based on the GLM statistical test results by year and 2-site category in each major island (Table 3A), PAR had borderline level of significance ($p = 0.05$) across highland and lowland sites. Temperature, rainfall and RH (Fig. 2) had insignificant ($p > 0.05$) influence on both AR and PAR, which were similar ($p > 0.05$) 2017–2018 to 2018–2019. Moreover, both GLM test results by season and 2-site categories (Table 3B) and by season and six sites regardless of elevation (Table 3B) did not show any significant ($p > 0.05$) results on both AR and PAR.

3.2. Hardy-Weinberg equilibrium (HWE)

Table 2 shows the locus-specific departures from HWE within each *Ae. aegypti* population in each season. More loci deviated from HWE across populations in the wet seasons of 2017–2018 (mean = 11.17) and 2018–2019 (mean = 8.50) than in the dry seasons of 2018 (mean = 10.17) and 2019 (mean = 8) (Additional file 3). GLM test results (Tables 3A–3B) revealed that the yearly number of loci that deviated from HWE differed significantly ($p < 0.001$) (Table 3A) but not seasonally ($p > 0.05$) (Table 3B). This number of loci that deviated from HWE differed ($p < 0.001$) across sites (Table 3B), except those mosquitoes

from BG, having the highest elevation, and CDO (Table 1) as influenced by rainfall and RH (Fig. 2).

3.3. Inbreeding coefficient (F_{IS})

The range of the mean F_{IS} estimates (Table 2) across populations in 2017–2018 (wet season: 0.48–0.58; dry season: 0.44–0.61) were significantly ($p < 0.001$) higher than those of 2018–2019 (wet season: 0.21–0.41; dry season: 0.25–0.41) (Additional file 3). Results indicate the mosquitoes were under intense selection in 2017–2018, and some degree of selection in the 2018–2019. AC4 locus consistently had 0.9 to fixed F_{IS} and deviated from HWE across sites in the entire study period except for the mosquito populations in the Visayas during the wet season of 2018–2019, whereas CT2 locus had 0.96 to fixed F_{IS} and did not conform to HWE among the mosquito populations across the three major islands during the wet season of 2017–2018 only but not in the rest of the two-year study period.

GLM test results by year and two-site categories (Table 3A) showed that the mean F_{IS} (Table 2) differed yearly ($p < 0.001$) as influenced by rainfall ($p < 0.05$) (Fig. 2). Likewise, the mean F_{IS} differed by season ($p < 0.05$) and across sites ($p < 0.05$) (Table 3B) except those from BG and CDO as influenced by RH ($p < 0.001$) (Fig. 2).

3.4. Linkage disequilibrium (LD)

Separate LD analyses showed that all loci comparisons after Bonferroni correction ($p < 0.00015$) for multiple tests within each population did not show LD in both wet (2017–2018) and dry seasons (2018). However, during 2018–2019, there were a pair (1.52%) of loci (AG1 and AG5) from CC highlands, four pairs (6.06%) (AC1 and AC5, AC2 and AC5, AC1 and AG5, and AC5 and CT2) from BUK, and three pairs (4.55%) (AG1 and AG2, AC5 and AG5, and CT2 and B2) from CDO city in the wet season that displayed LD. In the dry season, there were four pairs (6.06%) of loci (A1 and AG2, A1 and CT2, AC2 and CT2, and AG2 and CT2) from QC and a pair (1.52%; AC2 and B2) from CDO city showed LD. LD results in 2018–2019 apparently imply some degree of

Table 3A

Statistical test results of Generalized Linear models by year and two-site categories (highland vs. lowland) in each Philippine big island. Dependent variables of models with their AIC scores are: 1 - AR of *Ae. aegypti*, 2 - PAR, 3 - the number of loci that deviated from HWE, 4 - N_e and 5 - mean F_{IS} .

Source	Wald Chi-Square	df	p-value
Model 1: AR (AIC = 40.02)			
Year	7.54	3	0.06
2-site categories	0.53	1	0.47
Temperature	0.71	1	0.40
Rainfall	0.11	1	0.74
RH	1.38	1	0.24
Model 2: PAR (AIC = -43.23)			
Year	6.38	3	0.10
2-site categories	3.76	1	0.05 ¹
Temperature	0.02	1	0.89
Rainfall	0.00	1	0.95
RH	1.81	1	0.18
Model 3: HWE (AIC = 96.16)			
Year	21.84	3	0.00
2-site categories	1.14	1	0.29
Temperature	0.17	1	0.68
Rainfall	1.55	1	0.21
RH	0.03	1	0.85
Model 4: N_e (AIC = 354.95)			
Year	7.80	5	0.05 ¹
2-site categories	0.48	2	0.49
Temperature	0.58	1	0.45
Rainfall	0.32	1	0.57
RH	0.24	1	0.62
Model 5: F_{IS} (AIC = -56.77)			
Year	89.42	5	0.00
2-site categories	0.91	2	0.34
Temperature	0.70	1	0.40
Rainfall	5.14	1	0.02
RH	0.50	1	0.48

AIC: Akaike Information Criterion, AR: Allelic richness, PAR: Private allelic richness, HWE: Hardy-Weinberg Equilibrium, N_e : Effective population size, F_{IS} : Inbreeding coefficient.

¹ Borderline level of significance.

selection.

3.5. Effective population size (N_e)

N_e estimates based on LD model for *Ae. aegypti* across sites and in the overall Philippine sites were higher in the wet season of 2017–2018 than in the dry season of 2018, but the reverse was true in 2018–2019 (Table 4). Based on GLM statistical test results by year and two-site categories (Table 3A), yearly N_e did not differ but had a borderline level of significance ($p = 0.05$). Neither the elevation nor the season along with meteorological data across study sites regardless of elevation (Table 3B) had significant ($p > 0.05$) roles on N_e (Table 3A).

3.6. Population structure

The global F_{ST} 2017–2019 was estimated at 0.16. The global F_{ST} estimate of 2017–2018 was 0.16 (wet season = 0.04; dry season = 0.09) and that of 2018–2019 was 0.06 (wet season = 0.07; dry season = 0.04). Tables 5A–B show pairwise genetic differentiations (F_{ST}) of *Ae. aegypti* populations. In the two-year study period, population pairs within big islands during the dry season had little ($F_{ST} < 0.05$) genetic differentiations, whereas those during the wet season had little ($F_{ST} = 0.03$) to moderate ($F_{ST} = 0.07–0.09$) genetic differentiations (Wright, 1978). Population pairs across the major islands had little to moderate genetic

differentiations during both the wet ($F_{ST} = 0.02–0.07$ in 2017–2018; $F_{ST} = 0.03–0.13$ in 2018–2019) and dry seasons ($F_{ST} = 0.04–0.16$ in 2017–2018; $F_{ST} = 0.02–0.07$ in 2018–2019). The Mantel tests between the pairwise genetic (F_{ST}) and geographic distances of all mosquito populations did not show yearly significant correlation for each year (year 1: $R^2 = 0.008$, $p = 0.96$; year 2: $R^2 = 0.042$, $p = 0.48$).

3.7. Gene flow (N_m)

Gene flow based on N_m estimates (Table 6A) in 2017–2018 ranged from 2.14 to 6.02 (average = 3.66) effective migrants per generation in the wet season, and from 0.64 to 4.44 (average = 1.59) in the dry season, which were significantly different from one another ($p < 0.001$). Also, gene flow estimates within the major islands (Table 6A) were higher in the wet season than in the dry season. In 2018–2019, N_m values (Table 6B) ranged from 0.87 to 3.63 (average = 2.36) in the wet season, and from 1.44 to 4.85 (average = 3.04) in the dry season, which were also significantly different seasonally ($p < 0.05$). The reverse was true in 2018–2019; N_m estimates within the major islands (Table 6B) were higher in the dry season than in the wet season. GENECLASS analysis identified 172 (19.1%) and 145 (16.1%) out of 900 individuals of *Ae. aegypti* yearly across sites as F0 migrants in 2017–2018 (wet season = 10%); dry season = 9.1%) and 2018–2019 (wet season = 7%; dry season = 9.1%), respectively (Additional file 4). These yearly F0 migrants were observed between lowlands and highlands within and across major islands. The yearly migration rates across all sites ($p = 0.38$) and between seasons (2017–2018: $p = 0.71$; 2018–2019: $p = 0.29$) were similar based on the z-score test for two sample for means.

3.8. Bayesian clustering analyses and marker validation

Overall results of Bayesian analyses showed yearly variation of genetic clusters (K) with overall K = 3 (Fig. 3) in *Ae. aegypti* from Luzon, Visayas and Mindanao using STRUCTURE simulations of the two-year dataset that was run at one time (Table 7). The most probable genetic clusters were K = 2 in 2017–2018 and K = 1 in 2018–2019 (Fig. 3). In 2017–2018, Fig. 3 shows one genetic cluster (depicted green) across all sites during the wet season with a dominant haplotype membership that ranged from 92.7% to 98.3% (Table 7), and another cluster (depicted purple) across all sites during the dry season (membership range = 78.8%–97.2%). In 2018–2019, Fig. 3 shows another cluster (depicted yellow) across all sites regardless of seasons although the range of haplotype membership was broader in the wet season (45.2%–94.2%) than that of the dry season (85.8%–97.6%).

Alternatively, Bayesian analyses using STRUCTURE simulations of the yearly dataset (Fig. 4) showed also yearly variation of genetic clusters, with similar K = 2 in 2017–2018 (Fig. 3) but K = 4 in 2018–2019. Moreover, to assess the robustness of our results, the analyses for both two-year and yearly datasets were repeated using an 11-locus dataset that excluded AC4 locus (Slotman et al., 2007) because it consistently had F_{IS} of 0.9 to fixed, and deviated from HWE across sites in the two-year study except for populations in the Visayas during the wet season of 2018–2019. This smaller dataset produced similar pattern of population clustering (Additional file 5) as the full 12-locus dataset, hence, we report only the latter here. This also validates the novel and good microsatellite marker used in this study (Aaeg 1-3D) developed by the team (Labiros et al., 2022).

4. Discussion

This is the first study, to our knowledge, on the spatio-temporal genetic structure of *Ae. aegypti* from selected highland and lowland sites in the Philippine major islands (Luzon, Visayas and Mindanao) that explored the relationship between meteorological data (i.e., temperature, RH and rainfall) and the genetic structure analyses during the wet (2017–2018 and 2018–2019) and dry seasons (2018 and 2019). Most

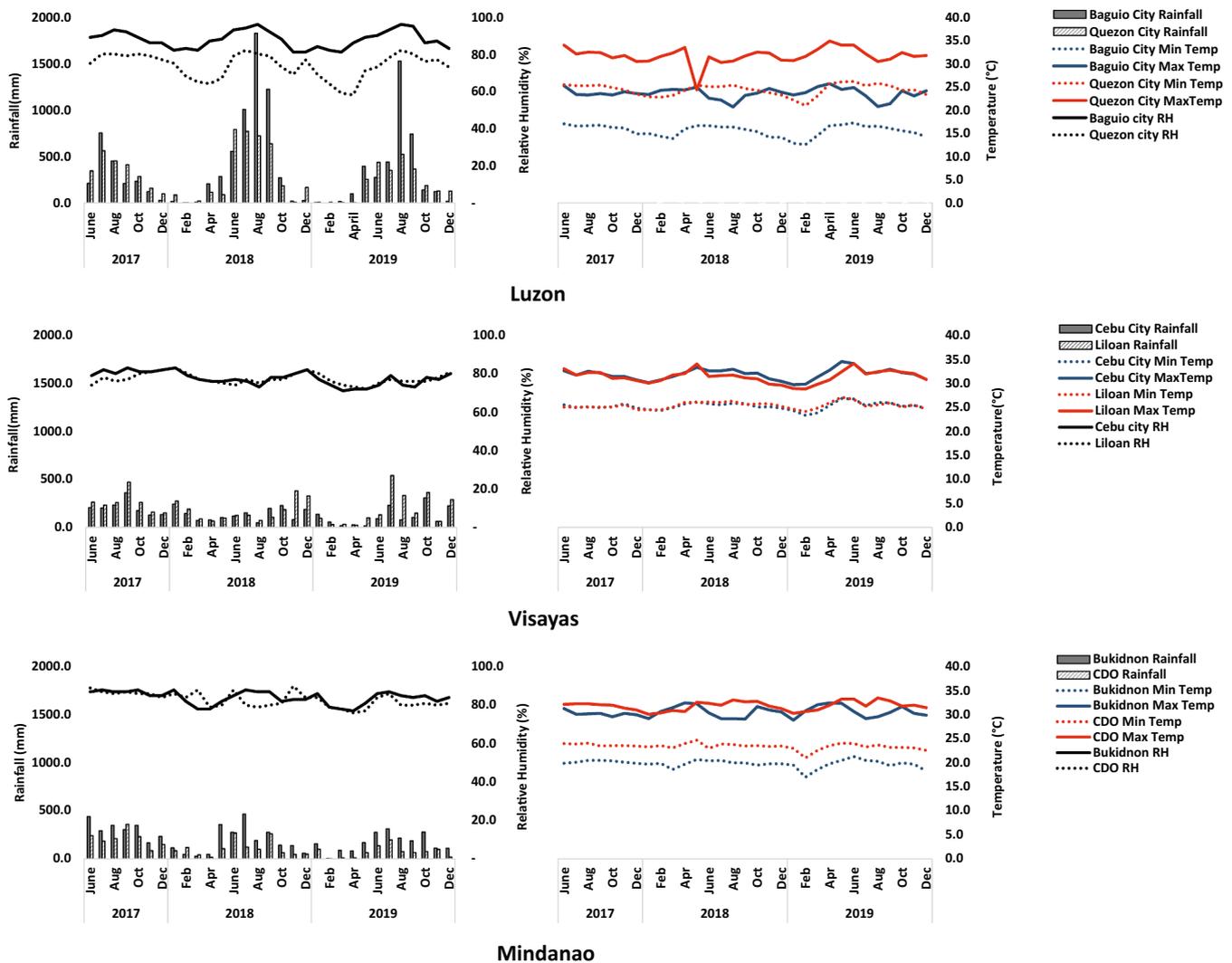


Fig. 2. Monthly temperature, rainfall and relative humidity (RH) in the wet seasons (2017–2018 and 2018–2019) and dry seasons (2018 and 2019) of the nearest PAG-ASA centers to the study sites.

studies report on the genetic structure of *Ae. aegypti* without considering the seasonal and or weather conditions such as in Brazil (Wilke et al., 2017; Maitra et al., 2019; Mendonca et al., 2014), Gabon and Kenya (Xia et al., 2020), Penang, Malaysia (Naim et al., 2020), Peruvian Amazon (Guagliardo et al., 2019), Philippines (Carvajal et al., 2020; Fonzi et al., 2015; Sayson et al., 2015), North America (Pless et al., 2020), Thailand (Olanratmanee et al., 2013), Yogyakarta, Indonesia (Rašić et al., 2015), Yunnan, China (Lv et al., 2020; Shi et al., 2017) among others.

There are six main results of this study. First, GLM statistical test results affirmed the overall Bayesian genetic clustering analyses (Figs. 3–4) that showed yearly variations in both datasets (2017–2019 and yearly) using STRUCTURE. These included significant yearly variations (Table 3A) on F_{IS} estimates as influenced by rainfall (Fig. 2). The mosquitoes were under intense selection in 2017–2018 (overall mean F_{IS} : wet season = 0.53; dry season = 0.51) (Table 2), suggesting more dramatic reduction on the frequency of heterozygotes leading to small refuges of mosquitoes that exhibit random genetic differentiation than those in 2018–2019 (overall mean F_{IS} : wet season = 0.34; dry season = 0.30), when there was some degree of selection only. Huber et al. (2002) associated rainfall and factors related with human activity (e.g., breeding site density and insecticide treatment) with the genetic structure of *Ae. aegypti* populations in Ho Chi Minh city, Vietnam during the rainy season. Moreover, GLM analysis (Table 3B) also showed seasonal

(wet versus dry season) differences on F_{IS} estimates across sites (Table 2), except those from BG and CDO as influenced by RH (Fig. 2). These results might be attributed to the fact that mosquito abundance depends on the presence of water-filled containers and water-storage habits of inhabitants in most study sites, which influence the breeding pattern of *Ae. aegypti* (Moore et al., 1978; Edillo et al., 2012). Sayson et al. (2015) also observed inbreeding of *Ae. aegypti* in the wet season (2011–2012) and in the dry season (2012) in Cebu city, Philippines.

In the case of BG site, mean monthly values of RH and rainfall (Fig. 2) were similar ($p > 0.05$) between seasons in the two-year (2017–2019) period, whereas temperatures (Fig. 2) were similar ($p > 0.05$) between seasons in 2017–2018. Mean monthly RH in BG during the wet seasons were 87.78% (2017–2018) and 87.89% (2018–2019); whereas those during the dry seasons were 85.67% (2018) and 85.33% (2019). Mean monthly rainfall in BG during the wet seasons were higher (223.4 mm [2017–2018] and 545.5 mm [2018–2019]) than those during the dry seasons (164.3 mm [2018] and 167.9 mm [2019]). Mean monthly temperatures in BG were comparable (wet season: 19.80 °C [2017–2018] and 18.94 °C [2018–2019]; dry season: 19.98 °C [2018] and 20.48 °C [2019]). Moreover, the egg resistance of *Ae. aegypti* to desiccation (Perez and Noriega, 2012; Diniz et al., 2017), water availability, and the species tolerance to colder environmental temperature (Kearney et al., 2009) in BG, as the Philippine summer capital with the

Table 3B

Statistical test results of Generalized Linear models by season and two-site categories based on altitudes (left) in each Philippine big island, and by season and six sites (right). Dependent variables of models with their AIC scores are: 1 – AR of *Ae. aegypti*, 2 - PAR, 3 - the number of loci that deviated from HWE, 4 - N_e and 5 - mean F_{IS} .

Source	By season and 2-site categories			By season and six sites		
	Wald chi-square	df	p-value	Wald chi-square	df	p-value
Model 1: AR (AIC = 50.57)						
Season	0.00	1	0.99	0.36	1	0.55
Sites	0.61	1	0.43	4.62	5	0.46
Temperature	0.18	1	0.67	1.22	1	0.27
Rainfall	0.28	1	0.60	0.00	1	0.99
RH	0.00	1	0.98	0.00	1	0.99
Model 2: PAR (AIC = -42.11)						
Season	0.54	1	0.46	1.09	1	0.30
Sites	2.97	1	0.09	4.90	5	0.43
Temperature	0.00	1	0.98	0.80	1	0.37
Rainfall	0.06	1	0.81	0.00	1	0.96
RH	0.87	1	0.35	0.54	1	0.46
Model 3: HWE (AIC = 107.13)						
Season	0.56	1	0.45	0.00	1	0.97
Sites	0.32	1	0.57	20.04	5	0.00
Temperature	0.09	1	0.76	1.95	1	0.16
Rainfall	0.62	1	0.43	6.39	1	0.01
RH	1.06	1	0.30	15.62	1	0.00
Model 4: N_e (AIC = 357.48)						
Season	0.23	1	0.63	0.02	1	0.90
Sites	0.29	1	0.59	6.25	5	0.28
Temperature	0.86	1	0.35	0.01	1	0.91
Rainfall	0.12	1	0.73	0.09	1	0.76
RH	0.99	1	0.32	2.26	1	0.13
Model 5: F_{IS} (AIC = -56.77)						
Season	0.62	1	0.43	3.95	1	0.04
Sites	0.03	1	0.87	14.41	5	0.01
Temperature	1.43	1	0.23	0.47	1	0.49
Rainfall	1.62	1	0.20	0.00	1	0.97
RH	1.39	1	0.24	15.06	1	0.00

AIC: Akaike Information Criterion, RH: Relative humidity, AR: Allelic richness, PAR: Private allelic richness, HWE: Hardy-Weinberg Equilibrium, N_e : Effective population size, F_{IS} : Inbreeding coefficient

highest elevation (Table 1; Fig. 2) across sites, are important driving factors for the mosquitoes to have adapted there. Meanwhile, CDO is an urban lowland site in Mindanao (Table 1), and its nearest airport for domestic flights is 36.9 km away, which has provided the human transportation networks from around its neighboring areas including BUK site for possible dispersal of *Ae. aegypti* (Soper, 1967). BUK is a rural highland site (Table 1), mostly agricultural, and is 135.1 km away from CDO. Results were consistent with Guagliardo et al. (2019) in which human transportation networks by boats explain the high levels of population mixing in the Peruvian Amazon. Cargo shipments are more efficient than passenger ships in the dispersal of *Ae. aegypti* across 15 seaports in central-western Philippines (Fonzi et al., 2015).

Second, GLM analyses showed both yearly differences across highland and lowland sites (Table 3A) and seasonal differences (Table 3B) on the number of loci that deviated from HWE across six sites as influenced by rainfall and RH. There were more loci deviating from HWE in the wet season than in the dry season in both years (Table 2), indicating either natural selection, random genetic drift or non-random mating that occurred more strongly in the wet season as confirmed with F_{IS} results (Edillo et al., 2009; Lanzaro et al., 1998; Lehmann et al., 2003; Midega et al., 2010). These results might have been attributed by higher rainfall and higher overall mean RH in the wet season (2017–2018 = 83.24%;

Table 4

Effective population size of *Ae. aegypti* populations from all study sites during the wet and dry seasons of 2017–2019 based on the linkage disequilibrium model with 95% confidence interval.

Sites	Wet Season (2017–2018)		Dry Season (2018)	
	N_e	95% CI	N_e	95% CI
BG	147.2	82.3–459.4	39.1	26.4–64.0
QC	383.1	123.7 – infinite	71.3	43.6–149.5
CC	234.4	112.2–4732.5	62.9	40.2–117.4
LIL	406.9	136.7 – infinite	59.3	35.2–136.7
BUK	1717.4	184.7 – infinite	35.4	25.2–52.7
CDO	167.4	83.2–1123.4	156.6	75.0–1965.9
All sites	485.4	343.2–767.4	96.3	79.6–117.4
<hr/>				
	Wet Season (2018–2019)		Dry Season (2019)	
BG	41.5	29.8–61.5	76.0	51.9–126.0
QC	58.6	32.1–183.1	31.3	23.6–42.7
CC	30.6	23.3–41.2	31.9	24.5–42.7
LIL	123.1	67.5–404.1	120.5	68.3–341.4
BUK	23.6	18.4–30.6	66.4	44.4–112.3
CDO	49.0	36.8–68.3	39.5	27.8–60.2
All sites	70.9	60.8–82.9	166.4	134.7–209.0

BG: Baguio city, QC: Quezon city, CC: Cebu city mountains, LIL: Liloan, Cebu, BUK: Maramag, Bukidnon, CDO: Cagayan de Oro city, N_e : Effective population size, CI: Confidence interval.

Table 5A

Genetic differentiation using F_{ST} of *Ae. aegypti* populations between study sites and between seasons (wet 2017–2018: below diagonal line; dry 2018: above diagonal line).

Sites	BG	QC	CC	LIL	BUK	CDO
BG		0.05	0.11	0.08	0.16	0.11
QC	0.02		0.08	0.04	0.11	0.06
CC	0.07	0.06		0.05	0.13	0.10
LIL	0.06	0.06	0.01		0.08	0.04
BUK	0.06	0.04	0.03	0.02		0.04
CDO	0.06	0.03	0.03	0.02	0.02	

BG: Baguio city, QC: Quezon city, CC: Cebu city mountains, LIL: Liloan, Cebu, BUK: Maramag, Bukidnon, CDO: Cagayan de Oro city

2018–2019 = 80.70%) than in the dry season (2018 = 77.56%; 2019 = 74.5%) with comparable overall mean temperatures in the wet (2017–2018 = 26.16 °C; 2018–2019 = 25.84 °C) and dry seasons (2018 = 26.44 °C; 2019 = 26.86 °C) in both years of study (Fig. 2). Female mosquitoes' flight from indoor to outdoor or vice versa between 10 °C and 35 °C is tightly linked with their host-seeking behavior (Reinhold et al., 2018) and egg laying (Campos et al., 2012). Because *Ae. aegypti* can fly as fast as 34.1 m/min at 32 °C with 50% RH (Rowley and Graham, 1968), apparently at higher overall mean RH in the wet season with comparable seasonal temperatures in the study sites, females might have flown slower and oviposited in breeding sites closer to resting sites (Campos et al., 2012), and might have seemingly facilitated non-random mating. These current results are interestingly the reverse of the previous findings conducted in Cebu city (Sayson et al., 2015) and in Manaus, Amazonas, Brazil (Mendonca et al., 2014) that reported more deviations from HWE of *Ae. aegypti* populations in the dry season than in the wet season. Differences could be due to the changing seasonal weather patterns of this current study that was done six to seven years after those studies (Sayson et al., 2015; Mendonca et al., 2014), sensitivity or usefulness of the markers (Lanzaro et al., 1995), and local ecology of the vector populations (Sayson et al., 2015; Azil et al., 2010).

Moreover, results of LD analyses in 2017–2018 imply that all loci were randomly associated and were not physically linked validating

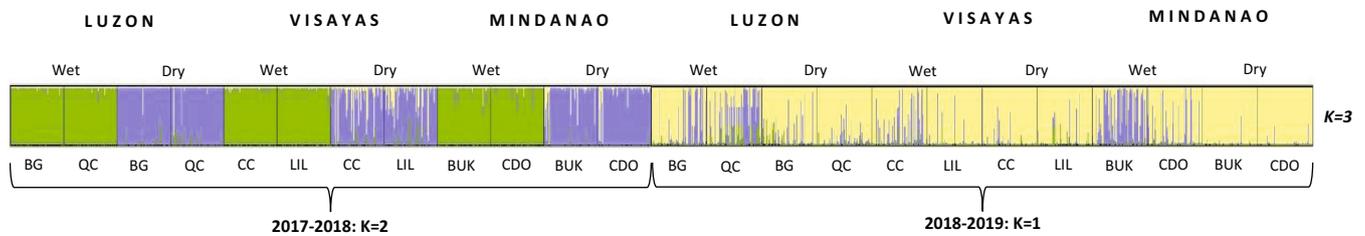


Fig. 3. Year-to-year variation of the genetic structure bar plots of *Ae. aegypti* samples ($n = 1800$) from Luzon, Visayas and Mindanao sites using 12 loci two-year dataset that was run at one time in STRUCTURE. Overall $K = 3$, year 1 (2017–2018): $K = 2$, and year 2 (2018–2019): $K = 1$. The height of each colored line segment represents the probability of assignment to that cluster. Legend: BG - Baguio city; QC - Quezon city; CC - Cebu city mountains; LIL - Liloan, Cebu; CDO - Cagayan de Oro city; BUK - Bukidnon.

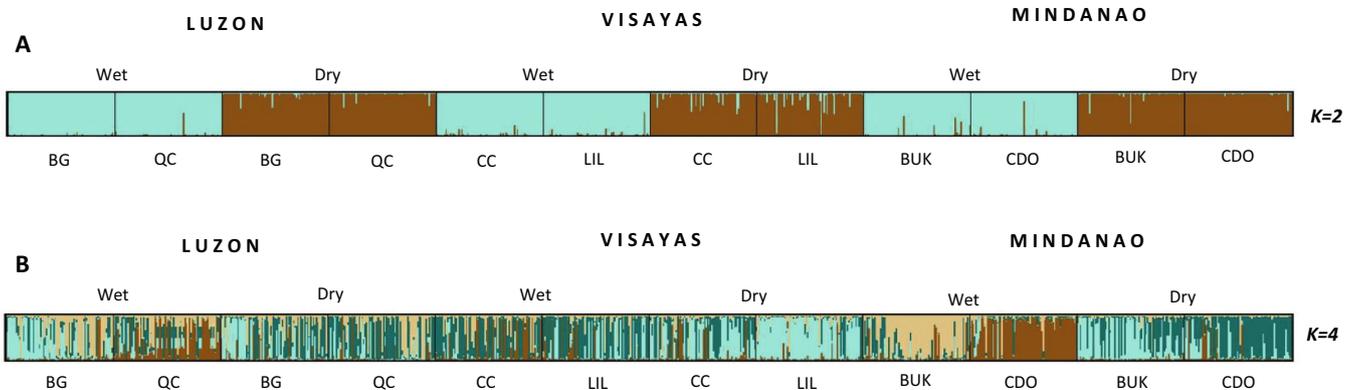


Fig. 4. A–B Year-to-year variation of the genetic structure bar plots of *Ae. aegypti* samples ($n = 1800$) from Luzon, Visayas and Mindanao sites using 12 loci yearly datasets that were run separately in STRUCTURE. Panel A: $K = 2$ in 2017–2018 and panel B: $K = 4$ in 2018–2019. The height of each colored line segment represents the probability of assignment to that cluster. Legend: BG - Baguio city; QC - Quezon city; CC - Cebu city mountains; LIL - Liloan, Cebu; CDO - Cagayan de Oro city; BUK - Bukidnon.

Brown et al. (2011). Based on the chromosomal map of microsatellite markers of *Ae. aegypti* (Labiros et al., 2022) in reference with the genome (Matthews et al., 2018), only AC5 and CT2 loci on the first chromosome used in this study had relatively short distance (i.e., 4,582,215 bp apart), but were not physically linked to cause LD in 2017–2018. However, there were a few pairs of loci that showed LD in 2018–2019, that might have been attributed to non-random mating as confirmed by F_{IS} estimates with some degree of selection (Ridley, 2007; Hartl and Clark, 2018). However, there were a few pairs of loci that showed LD in 2018–2019, that might have been attributed to non-random mating as confirmed by F_{IS} estimates with some degree of selection (Ridley, 2007; Hartl and Clark, 2018). The amplification of dengue viruses (DENVs) within *Ae. aegypti* increases also with humidity (over 60%) and increased environmental temperature ($\sim 24\text{--}31^\circ\text{C}$) (Thu et al., 1998). Edillo et al. (2015) reported the rising minimum infection rate (MIR) of DENV-infected *Ae. aegypti* (i.e., from zero in the wet season of 2011–2012 to 46.22 MIR in mid-dry season of 2012) in Cebu city, Philippines. Although control interventions in the non-outbreak dry season were recommended to suppress the re-emerging pattern of dengue transmission in the following outbreak wet season (Edillo et al., 2015), the enhanced 4S strategy has been commonly practiced during the epidemic wet season. Results of the current study also suggest that the enhanced 4S strategy (DOH, 2020) should be implemented all-year round during this global warming in the Philippines (Edillo et al., 2022), in particular, and related dengue vector control strategies in the tropics, in general.

Third, Bayesian approach showed three genetic clusters with $K = 2$ in 2017–2018 and $K = 1$ in 2018–2019. Clustering with a $K = 2$ (Fig. 3) indicates that all *Ae. aegypti* populations across sites could be grouped by two seasons with consistent seasonal difference on haplotype memberships (Table 7), as influenced significantly ($p < 0.05$) by season and RH

(Fig. 2) on F_{IS} by GLM test results (Table 3B). The high mean F_{IS} estimates (0.51–0.53) in 2017–2018 (Table 2) imply that high non-random mating of mosquitoes as explained above might have acted as an ancillary process for an intense selection to have occurred. AC4 locus consistently had 0.9 to fixed F_{IS} , and deviated from HWE across sites in the entire study period except for the mosquito populations in the Visayas during the wet season of 2018–2019, consistent with that of Slotman et al. (2007). Clustering with a $K = 1$ in 2018–2019 (Fig. 3) indicates genetic homogeneity or population admixture for all mosquito populations across sites in both wet and dry seasons. However, because global genetic differentiation ($F_{ST} = 0.06$) in 2018–2019 was moderate, alternative Bayesian clustering with a $K = 4$ (Fig. 4B; Table 8) using the yearly datasets would imply all samples could be geographically differentiated. Mosquitoes from Luzon and Visayas sites showed population admixture (Fig. 4B) independent of altitude and season, whereas those from Mindanao sites showed an apparent population structure and seasonal genetic clustering as shown also in Table 5B (F_{ST} : wet season =

Table 5B

Genetic differentiation using F_{ST} of *Ae. aegypti* populations between study sites and between seasons (wet 2018–2019: below diagonal line; dry 2019: above diagonal line).

Sites	BG	QC	CC	LIL	BUK	CDO
BG		0.03	0.04	0.04	0.04	0.06
QC	0.07		0.05	0.05	0.06	0.07
CC	0.09	0.07		0.02	0.02	0.03
LIL	0.06	0.07	0.03		0.02	0.06
BUK	0.13	0.08	0.12	0.13		0.02
CDO	0.06	0.06	0.03	0.03	0.09	

BG: Baguio city, QC: Quezon city, CC: Cebu city mountains, LIL: Liloan, Cebu, BUK: Maramag, Bukidnon, CDO: Cagayan de Oro city

0.09; dry season = 0.02). Results imply population admixture in Luzon and Visayas sites (Fig. 4B), which are highly and moderately urbanized areas, respectively, with greater human transportation networks that facilitate population mixing, consistent in other studies (Diaz-Nieto et al., 2016; Guagliardo et al., 2019; Huber et al., 2004; Rasić et al., 2015). Also, passive transport of *Ae. aegypti* eggs or immatures introduces new genetic variants by migration into established populations (Maffey et al., 2020). Moreover, secondary and minor roads in residential areas, pedestrian lane and intersections can act as a route of passive dispersal of *Ae. aegypti* (Regilme et al., 2021). Decreased genetic diversity is facilitated when there is an increased urbanization (Gubler, 2011). Because *Ae. aegypti* can be endophilic (i.e., taking shelter indoor) and endophagic (i.e., blood-feeding indoor), they can flourish in urbanized areas causing dengue epidemic (Gubler, 2011; Brown et al., 2014; Jansen and Beebe, 2010).

Fourth, isolation by distance based on Mantel test results did not influence F_{ST} estimates of *Ae. aegypti* populations in the Philippine major islands. Pairs of mosquito populations within major islands had low F_{ST} estimates in the dry seasons of 2018 and 2019 and wet season of 2017–2018, whereas low to moderate genetic differentiations in the wet seasons of 2017–2019. Results with low genetic differentiation were consistent with studies that had microgeographic-scale setting of *Ae. aegypti* populations in Cebu city (Sayson et al., 2015) and Metro Manila (Carvajal et al., 2020). Landscape features such as highways, rivers and primary roads create also a barrier to dispersal of *Ae. aegypti* on a fine spatial scale (Regilme et al., 2021). Population pairs across sites between major islands had low to moderate F_{ST} estimates indicating that long-distance dispersal might be driven by human-mediated transportation (Brown et al., 2011; Guagliardo et al., 2019; Huber et al., 2008; Mendonca et al., 2014; Paupy et al., 2008; Paupy et al., 2012; Ravel et al., 2001; Scarpassa et al., 2008) and human-mediated interventions such as insecticide use (Scarpassa et al., 2008). There was no evidence of a founder effect in any mosquito populations for there were low F_{ST} estimates among some sites in either of the wet or dry seasons.

Fifth, gene flow (Nm) among populations of *Ae. aegypti* was observed between lowlands and highlands within and across major islands (Tables 6A–6B) as confirmed by similar yearly migration rates (19.1% in 2017–2018 and 16.1% in 2018–2019) across all sites and between seasons by GENECLASS analysis. These results imply that *Ae. aegypti* populations were not completely isolated from each other at any time. Since *Ae. aegypti* is both endophilic and endophagic, their movement from inside to outside shelters or vice versa would allow them to encounter temperature fluctuations (10 °C–32 °C) that can affect their behavior, including host-seeking (Reinhold et al., 2018). Most households in rural highland sites of the current study do not have direct water pipelines, forcing householders to store water in plastic drums for domestic use, thus facilitating the mosquitoes to breed all-year round on their expanded distribution to rural highlands posing a challenge for vector control programs. Moreover, their eggs can undergo quiescence (i.e., non-seasonal irregular dormancy that resist desiccation) that allows the embryo to survive in unfavorable environmental conditions (Diniz et al., 2017), as confirmed in our separate study (Edillo et al.,

Table 6A

Gene flow (Nm) of *Ae. aegypti* populations between Philippine study sites and between seasons (wet 2017–2018: below diagonal line; dry 2018: above diagonal line).

Sites	BG	QC	CC	LIL	BUK	CDO
BG		4.44	1.47	1.73	0.68	0.71
QC	6.02		0.97	1.52	0.64	0.82
CC	2.77	3.35		2.99	0.81	0.84
LIL	2.14	3.05	3.89		1.69	2.06
BUK	2.97	3.74	2.88	4.20		2.46
CDO	3.50	3.79	3.23	3.70	5.70	

BG: Baguio city, QC: Quezon city, CC: Cebu city mountains, LIL: Liloan, Cebu, BUK: Maramag, Bukidnon, CDO: Cagayan de Oro city

Table 6B

Gene flow (Nm) of *Ae. aegypti* populations between study sites and between seasons (wet 2018–2019: below diagonal line; dry 2019: above diagonal line).

Sites	BG	QC	CC	LIL	BUK	CDO
BG		3.56	3.55	4.14	3.34	2.35
QC	0.87		3.52	3.17	2.66	2.16
CC	1.66	3.26		4.85	3.21	1.45
LIL	2.12	3.23	2.56		3.28	1.44
BUK	2.37	1.89	2.07	1.73		2.98
CDO	2.16	2.72	3.63	3.19	1.86	

BG: Baguio city, QC: Quezon city, CC: Cebu city mountains, LIL: Liloan, Cebu, BUK: Maramag, Bukidnon, CDO: Cagayan de Oro city

2022). We observed the highest percentage of pharate larvae of *Ae. aegypti* formed at 18 °C and even a few were produced at 38 °C, facilitating their spread to new areas via transportation of materials (Soper, 1967). Dispersal rate of *Ae. aegypti* at distances greater than their average flight is associated with human activity such as long distance transport (Guagliardo et al., 2019; Bennett et al., 2019; Failloux et al., 1997), however, human-made landscape structures such as primary roads limit also mosquito movement and gene flow (Regilme et al., 2021).

Sixth, N_e had yearly borderline level of significance ($p = 0.05$) from based on GLM test results (Table 3A). Likewise, PAR but not AR had borderline level of significance ($p = 0.05$) across highland and lowland sites in the Philippine major islands (Table 3B). The abundance of *Ae. aegypti* depends on the availability of water-filled containers (Kearney et al., 2009), water-storage habits of humans, and water supply system in localities (Edillo et al., 2012) that might have affected their breeding pattern. The mosquitoes usually reach their maximum after peak rainfall (Huber et al., 2002). The mean rainfall (Fig. 2) in the current study during wet seasons of both years were higher than those in the dry

Table 7

Percentage haplotype memberships of *Ae. aegypti* in the wet and dry seasons of 2017–2018 ($K = 2$) and 2018–2019 ($K = 1$) across Philippine study sites with overall $K = 3$ based on the one-time STRUCTURE analysis of the two-year 12-loci genotype dataset.

Season	Site	Cluster 1 (%)	Cluster 2 (%)	Cluster 3 (%)
Wet Season (2017–2018)	BG	1.1	0.6	98.3
	QC	1.2	0.8	98.0
	CC	1.4	95.6	3.0
	LIL	3.1	92.7	4.3
	BUK	1.2	1.2	97.6
	CDO	1.2	1.1	97.7
Dry Season (2018)	BG	17.8	78.8	3.4
	QC	14.5	80.0	5.6
	CC	1.4	2.0	96.6
	LIL	0.9	1.9	97.2
	BUK	9.6	88.1	2.3
	CDO	7.5	91.6	0.9
Wet Season (2018–2019)	BG	74.5	24.1	1.4
	QC	50.0	40.9	9.1
	CC	85.8	9.9	4.3
	LIL	91.2	7.5	1.4
	BUK	75.5	22.8	1.8
	CDO	94.2	4.7	1.1
Dry Season (2019)	BG	93.7	5.1	1.1
	QC	91.3	4.9	3.9
	CC	45.2	53.0	1.8
	LIL	78.5	19.2	2.3
	BUK	97.6	1.6	0.8
	CDO	97.3	2.1	0.6

BG: Baguio city; QC: Quezon city; CC: Cebu city mountains; LIL: Liloan, Cebu; BUK: Maramag, Bukidnon; CDO: Cagayan de Oro city.

Table 8

Percentage haplotype memberships of *Ae. aegypti* in the wet and dry seasons of 2017–2018 ($K = 2$) and 2018–2019 ($K = 4$) across the Philippine study sites based on separate STRUCTURE analyses of yearly 12-loci genotype datasets.

Season	Site	Cluster 1 (%)	Cluster 2 (%)	Cluster 3 (%)	Cluster 4 (%)
Wet Season (2017–2018)	BG	0.6	99.4	0.0	0.0
	QC	0.9	99.1	0.0	0.0
	CC	1.1	98.9	0.0	0.0
	LIL	1.3	98.7	0.0	0.0
	BUK	2.4	97.6	0.0	0.0
	CDO	2.1	97.9	0.0	0.0
Dry Season (2018)	BG	97.5	2.5	0.0	0.0
	QC	95.9	4.1	0.0	0.0
	CC	96.2	3.8	0.0	0.0
	LIL	92.5	7.5	0.0	0.0
	BUK	97.1	2.9	0.0	0.0
	CDO	99.2	0.8	0.0	0.0
Wet Season (2018–2019)	BG	5.9	40.7	17.9	35.4
	QC	42.0	12.5	24.8	20.7
	CC	24.2	8.2	52.1	15.5
	LIL	12.9	23.8	54.0	9.3
	BUK	8.0	5.2	19.2	67.6
	CDO	78.9	7.3	5.5	8.3
Dry Season (2019)	BG	8.2	33.1	38.6	20.1
	QC	7.2	15.0	49.5	28.3
	CC	14.9	42.8	34.8	7.5
	LIL	4.8	74.7	11.8	8.7
	BUK	2.5	53.1	38.4	6.0
	CDO	9.3	20.4	66.7	3.6

BG: Baguio city; QC: Quezon city; CC: Cebu city mountains; LIL: Liloan, Cebu; BUK: Maramag, Bukidnon; CDO: Cagayan de Oro city.

seasons. This might have contributed to the greater N_e estimates in the wet season than in the dry season of 2017–2018 (Table 4), suggesting reconstitution of the genetic composition of *Ae. aegypti* population during the wet season by the expansion of refuges that survived the preceding dry season, consistent with Sayson et al. (2015) for *Ae. aegypti* subpopulations in Cebu city, Philippines and in Ho Chi Minh city, Vietnam (Huber et al., 2002). Moreover, the effect of N_e estimates on genes differs under varying levels of selection pressure, and is more pronounced in genes under high purifying selection (Subramanian, 2018). However, perhaps because N_e fluctuates as a result of environmental stochasticity, N_e estimates were lower in the wet season than in the dry season of 2018–2019 (Table 4), that might have contributed also to the striking difference in the population structure between 2017 and 2018 and 2018–2019. Because of similar ($p > 0.05$) seasonal mean temperatures across sites in 2017–2019, except BG and BUK in 2018–2019 (Fig. 2), the increase in the variance in allele frequency of the LD model (Whitlock and Barton, 1997) might have influenced the similar breeding pattern of *Ae. aegypti* in the current study. In response to this environmental context of global warming (IPCC, 2014), *Ae. aegypti* might have developed several methods of adaptation to protect themselves from environmental stress (Reinhold et al., 2018). Moreover, residents in rural highlands have utilized hose delivery system of spring water to their houses, and have stored water in artificial containers for domestic use. These water-filled containers served as potential breeding sites of *Ae. aegypti*.

5. Conclusions

In conclusion, *Ae. aegypti* populations in the Philippines underwent striking yearly differences in genetic structure; seasonal population clustering in 2017–2018 and population admixture in more urbanized sites (Luzon and Visayas) in 2018–2019. These yearly variations

indicated selection and random genetic drift as influenced by rainfall and RH, and a more dramatic reduction of heterozygotes due to non-random mating leading to a more intense selection in 2017–2018 than in 2018–2019 as influenced by rainfall, and differences of N_e estimates although with borderline level of significance. In both years (2017–2019), human-mediated activities and not isolation by distance influenced genetic differentiations within and across big islands. *Ae. aegypti* expanded their distribution to the Philippine highlands. Findings of this work are extremely relevant to better respond to the effects of global warming on integrated vector management that includes vector control strategies and education of the public not just in the country but also in the tropics. Vector control strategies during the epidemic wet season are recommended to be changed into a whole year-round approach to optimize the economic cost and burden of dengue and other arboviral diseases (chikungunya and Zika) transmitted by *Ae. aegypti*. Installation of water pipelines to residents in rural mountains nation-wide is an important practical recommendation in preventing the spread of *Aedes*-borne arboviral diseases there.

Abbreviations

HWE	Hardy-Weinberg equilibrium
RH	relative humidity
CFR	case fatality rate
DENV	dengue virus
BG	Baguio city
QC	Quezon city
CC	Cebu city
LIL	Liloan, Cebu
BUK	Maramag, Bukidnon
CDO	Cagayan de Oro city
O/L traps	Ovicidal/ Larvicidal traps
DOST	Department of Science and Technology
AR	allelic richness
PAR	private allelic richness
H_o	observed heterozygosity
H_E	expected heterozygosity
F_{IS}	inbreeding coefficient
LD	linkage disequilibrium
F_{ST}	genetic differentiation
N_m	gene flow
N_e	effective population size
GLM	generalized linear model
AIC	Akaike information criterion

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CRedit authorship contribution statement

Frances Edillo: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Software, Supervision, Writing – original draft, Writing – review & editing. **Rhoniel Ryan Ymbong:** Data curation, Formal analysis, Investigation, Methodology, Project administration, Software, Supervision, Visualization, Writing – original draft, Writing – review & editing. **Maureen Mathilde Cabahug:** Data curation, Formal analysis, Investigation, Software, Visualization, Writing – original draft. **Dinesse Labiros:** Data curation, Investigation. **Mark Windy Suycano:** Data curation, Investigation, Software, Visualization. **Louis Lambrechts:** Conceptualization, Writing – review & editing. **Anavaj Sakuntabhai:** Conceptualization, Methodology, Writing – review & editing.

Declaration of Competing Interest

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Appendix A. Supplementary data

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References

- Azil, A.H., Long, S.A., Ritchie, S.A., Williams, C.R., 2010. The development of predictive tools for pre-emptive dengue vector control: a study of *Aedes aegypti* abundance and meteorological variables in North Queensland, Australia. *Trop. Med. Int. Health* 15, 1190–1197.
- Barton, N.H., Slatkin, M., 1986. A quasi-equilibrium theory of the distribution of rare alleles in a subdivided population. *Heredity* 56 (Pt 3), 409–415. <https://doi.org/10.1038/hdy.1986.63>.
- Belkin, J.N., 1962. *The Mosquitoes of the South Pacific (Diptera, Culicidae)*. University of California Press, California.
- Bennett, K.L., Gómez Martínez, C., Almanza, A., Rovira, J.R., McMillan, W.O., Enriquez, V., Barraza, E., Díaz, M., Sanchez-Galan, J.E., Whiteman, A., Gittens, R.A., Loaiza, J.R., 2019. High infestation of invasive *Aedes* mosquitoes in used tires along the local transport network of Panama. *Parasit. Vectors* 12 (1), 264. <https://doi.org/10.1186/s13071-019-3522-8>.
- Bhatt, S., Gething, P.W., Brady, O.J., Messina, J.P., Farlow, A.W., Moyes, C.L., Drake, J. M., Brownstein, J.S., Hoen, A.G., Sankoh, O., Myers, M.F., George, D.B., Jaenisch, T., Wint, G.R.W., Simmons, C.P., Scott, T.W., Farrar, J.J., Hay, S.I., 2013. The global distribution and burden of dengue. *Nature* 496 (7446), 504–507. <https://doi.org/10.1038/nature12060>.
- Boutin-Ganache, I., Raposo, M., Raymond, M., Deschepper, C.F., 2001. M13-tailed primers improve the readability and usability of microsatellite analyses performed with two different allele-sizing methods. *Biotechniques* 31 (1), 24–28. <https://doi.org/10.2144/01311bm02>.
- Brady, O.J., Gething, P.W., Bhatt, S., Messina, J.P., Brownstein, J.S., Hoen, A.G., Moyes, C.L., Farlow, A.W., Scott, T.W., Hay, S.I., 2012. Refining the global spatial limits of dengue virus transmission by evidence-based consensus. *PLoS Negl. Trop. Dis.* 6 (8), e1760 <https://doi.org/10.1371/journal.pntd.0001760>.
- Brown, J.E., McBride, C.S., Johnson, P., Ritchie, S., Paupy, C., Bossin, H., Lutomiah, J., Fernandez-Salas, I., Ponlawat, A., Cornet, A.J., Black 4th, W.C., Gorrochotegui-Escalante, N., Urdaneta-Marquez, L., Sylla, M., Slotman, M., Murray, K.O., Walker, C., Powell, J.R., 2011. Worldwide patterns of genetic differentiation imply multiple 'domestications' of *Aedes aegypti*, a major vector of human diseases. *Proc. Biol. Sci.* 278 (1717), 2446–2454. <https://doi.org/10.1098/rspb.2010.2469>.
- Brown, J.E., Evans, B.R., Zheng, W., Obas, V., Barrera-Martinez, L., Egizi, A., Zhao, H., Caccone, A., Powell, J.R., 2014. Human impacts have shaped historical and recent evolution in *Aedes aegypti*, the dengue and yellow fever mosquito. *Evolution* 68 (2), 514–525. <https://doi.org/10.1111/evo.12281>.
- Campos, M., Spenassatto, C., da Graça Macoris, M.L., dos Santos Paduan, K., Pinto, J., Ribolla, P.E.M., 2012. Seasonal population dynamics and the genetic structure of the mosquito vector *Aedes aegypti* in São Paulo, Brazil. *Ecol. Evol.* 2 (11), 2794–2802. <https://doi.org/10.1002/ece3.392>.
- Capinha, C., Rocha, J., Sousa, C.A., 2014. Macroclimate determines the global range limit of *Aedes aegypti*. *Ecohealth* 11 (3), 420–428. <https://doi.org/10.1007/s10393-014-0918-y>.
- Carvajal, T.M., Ogishi, K., Yaegashi, S., Hernandez, L., Viacrusis, K.M., Ho, H.T., Amalin, D.M., Watanabe, K., 2020. Fine-scale population genetic structure of dengue mosquito vector, *Aedes aegypti*, in Metropolitan Manila, Philippines. *PLoS Negl. Trop. Dis.* 14 (5), e0008279 <https://doi.org/10.1371/journal.pntd.0008279>.
- Cheng, K.J.G., Lam, H.Y., Rivera, A.S., Tumanan-Mendoza, B.A., Alejandra, M.M., Wu, D. B.C., 2018. Estimating the burden of dengue in the Philippines using a dynamic transmission model. *Acta Medica Philippina*. <https://doi.org/10.47895/amp.v5i2i.427>.
- Corporal-Lodangco, I.L., Leslie, L.M., 2017. Defining Philippine climate zones using surface and high-resolution satellite data. *Procedia Comput. Sci.* 114, 324–332. <https://doi.org/10.1016/j.procs.2017.09.068>.
- Dhimal, M., Gautam, I., Joshi, H.D., O'Hara, R.B., Ahrens, B., Kuch, U., 2015. Risk factors for the presence of chikungunya and dengue vectors (*Aedes aegypti* and *Aedes albopictus*), their altitudinal distribution and climatic determinants of their abundance in Central Nepal. *PLoS Negl. Trop. Dis.* 9 (3), e0003545 <https://doi.org/10.1371/journal.pntd.0003545>.
- Díaz-Nieto, L.M., Chiappero, M.B., Díaz de Astarloa, C., Maciá, A., Gardenal, C.N., Berón, C.M., 2016. Genetic evidence of expansion by passive transport of *Aedes* (*Stegomyia*) *aegypti* in eastern Argentina. *PLoS Negl. Trop. Dis.* 10 (9), e0004839 <https://doi.org/10.1371/journal.pntd.0004839>.
- Diniz, D., de Albuquerque, C., Oliva, L.O., de Melo-Santos, M., Ayres, C., 2017. Diapause and quiescence: dormancy mechanisms that contribute to the geographical expansion of mosquitoes and their evolutionary success. *Parasit. Vectors* 10 (1), 310. <https://doi.org/10.1186/s13071-017-2235-0>.
- Do, C., Waples, R.S., Peel, D., Macbeth, G.M., Tillett, B.J., Ovenden, J.R., 2014. NeEstimator v2: re-implementation of software for the estimation of contemporary effective population size (Ne) from genetic data. *Mol. Ecol. Resour.* 14 (1), 209–214. <https://doi.org/10.1111/1755-0998.12157>.
- DOH, 2017. Dengue Diseases Surveillance Report. https://www.doh.gov.ph/sites/default/files/statistics/2017_Dengue_MW1-MW48.pdf (accessed 18 January 2021).
- DOH, 2020. Dengue Prevention and Control Program. <https://doh.gov.ph/national-dengue-prevention-and-control-program> (accessed 28 February 2020).
- Earl, D.A., von Holdt, B.M., 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conserv. Genet. Resour.* 4 (2), 359–361. <https://doi.org/10.1007/s12686-011-9548-7>.
- Edillo, F.E., Kiszewski, A., Manjourides, J., Pagano, M., Hutchinson, M., Kyle, A., Arias, J., Gaines, D., Lampman, R., Novak, R., Foppa, I., Lubelczyk, C., Smith, R., Moncayo, A., Spielman, A., Culex pipiens Working Group, 2009. Effects of latitude and longitude on the population structure of *Culex pipiens* s.l., vectors of West Nile virus in North America. *Am. J. Trop. Med. Hyg.* 81 (5), 842–848. <https://doi.org/10.4269/ajtmh.2009.08-0605>.
- Edillo, F.E., Roble, N.D., Otero 2nd, N.D., 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 (6), 1365–1374.
- Edillo, F.E., Sarcos, J., Sayson, S., 2015. Natural vertical transmission of dengue viruses in *Aedes aegypti* in selected sites in Cebu city, Philippines. *J. Vector Ecol.* 40 (2), 282–291. <https://doi.org/10.1111/jvec.12166>.
- Edillo, F.E., Ymbong, R.R., Bolneo, A.A., Hernandez, R.J., Fuentes, B.L., Cortes, G., Cabrera, J., Lazaro, J.E., Sakuntabhai, A., 2022. Temperature, season and latitude influence development-related phenotypes of Philippine *Aedes aegypti* (Linnaeus): implications for dengue control amidst global warming. *Parasites & Vectors* 15 (74). <https://doi.org/10.1186/s13071-022-05186-x>.
- Evanno, G., Regnaut, S., Goudet, J., 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Mol. Ecol.* 14 (8), 2611–2620. <https://doi.org/10.1111/j.1365-294X.2005.02553.x>.
- Failloux, A.B., Raymond, M., Ung, A., Chevillon, C., Pasteur, N., 1997. Genetic differentiation associated with commercial traffic in the Polynesian mosquito, *Aedes polynesiensis* Marks 1951. *Biol. J. Linn. Soc.* 60 (1), 107–118. <https://doi.org/10.1111/j.1095-8312.1997.tb01486.x>.
- Fonzi, E., Higa, Y., Bertuso, A.G., Futami, K., Minakawa, N., 2015. Human-mediated marine dispersal influences the population structure of *Aedes aegypti* in the Philippine archipelago. *PLoS Negl. Trop. Dis.* 9 (6), e0003829 <https://doi.org/10.1371/journal.pntd.0003829>.
- Guagliardo, S., Lee, Y., Pierce, A.A., Wong, J., Chu, Y.Y., Morrison, A.C., Astete, H., Brosi, B., Vazquez-Prokopec, G., Scott, T.W., Kitron, U., Stoddard, S.T., 2019. The genetic structure of *Aedes aegypti* populations is driven by boat traffic in the Peruvian Amazon. *PLoS Negl. Trop. Dis.* 13 (9), e0007552 <https://doi.org/10.1371/journal.pntd.0007552>.
- Gubler, D.J., 2011. Dengue, urbanization and globalization: the unholy trinity of the 21 (st) century. *Trop. Med. Health.* 39 (4 Suppl), 3–11. <https://doi.org/10.2149/tmh.2011-S05>.
- Hartl, D.L., Clark, A.G., 2018. *Principles of Population Genetics*, 4th ed. Sinauer Associates, Inc., Massachusetts.
- Huber, K., Loan, L.L., Hoang, T.H., Tien, T.K., Rodhain, F., Failloux, A.B., 2002. Temporal genetic variation in *Aedes aegypti* populations in Ho Chi Minh City (Vietnam). *Heredity* 89 (1), 7–14. <https://doi.org/10.1038/sj.hdy.6800086>.
- Huber, K., Loan, L.L., Chantha, N., Failloux, A.B., 2004. Human transportation influences *Aedes aegypti* gene flow in Southeast Asia. *Acta Trop.* 90 (1), 23–29. <https://doi.org/10.1016/j.actatropica.2003.09.012>.
- Huber, K., Ba, Y., Dia, I., Mathiot, C., Sall, A.A., Diallo, M., 2008. *Aedes aegypti* in Senegal: genetic diversity and genetic structure of domestic and sylvatic populations. *Am. J. Trop. Med. Hyg.* 79 (2), 218–229. <https://doi.org/10.4269/ajtmh.2008.79.218>.
- IPCC, 2014. *Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* [Core Writing Team, R.K. Pachauri and L.A. Meyer (Eds.)]. IPCC, Geneva, Switzerland (151 pp.).
- Jansen, C.C., Beebe, N.W., 2010. The dengue vector *Aedes aegypti*: what comes next. *Microbes Infect.* 12 (4), 272–279. <https://doi.org/10.1016/j.micinf.2009.12.011>.
- Kalinowski, S.T., 2005. HP-RARE 1.0: a computer program for performing rarefaction on measures of allelic richness. *Mol. Ecol. Notes* 5 (1), 187–189. <https://doi.org/10.1111/j.1471-8286.2004.00845.x>.
- Kearney, M., Porter, W.P., Williams, C., Ritchie, S., Hoffmann, A.A., 2009. Integrating biophysical models and evolutionary theory to predict climatic impacts on species' ranges: the dengue mosquito *Aedes aegypti* in Australia. *Funct. Ecol.* 23, 528–538.
- Kutsuna, S., Kato, Y., Moi, M.L., Kotaki, A., Ota, M., Shinohara, K., Kobayashi, T., Yamamoto, K., Fujiya, Y., Mawatari, M., Sato, T., Kunimatsu, J., Takeshita, N., Hayakawa, K., Kanagawa, S., Takasaki, T., Ohmagari, N., 2015. Autochthonous

- dengue fever, Tokyo, Japan, 2014. *Emerg. Infect. Dis.* 21 (3), 517–520. <https://doi.org/10.3201/eid2103/141662>.
- Labrios, D.A., Catalig, A.M.P., Ymbong, R.R.J., Sakuntabhai, A., Luisma, A.O., Edillo, F. E., 2022. Novel and broadly applicable microsatellite markers in identified chromosomes of the Philippine dengue mosquitoes, *Aedes aegypti* (L.) (Diptera: Culicidae). *J. Med. Entomol.* <https://doi.org/10.1093/jme/tjab194>.
- Lanzaro, G.C., Zheng, L., Toure, Y.T., Traore, S.F., Kafatos, F.C., Vernick, K.D., 1995. Microsatellite DNA and isozyme variability in a west African population of *Anopheles gambiae*. *Insect Mol. Biol.* 4, 105–112.
- Lanzaro, G.C., Touré, Y.T., Camahan, J., Zheng, L., Dolo, G., Traoré, S., Petrarca, V., Vernick, K.D., Taylor, C.E., 1998. Complexities in the genetic structure of *Anopheles gambiae* populations in West Africa as revealed by microsatellite DNA analysis. *Proc. Natl. Acad. Sci. U. S. A.* 95 (24), 14260–14265. <https://doi.org/10.1073/pnas.95.24.14260>.
- Lehmann, T., Licht, M., Elissa, N., Maega, B.T., Chimubwa, J.M., Watsenga, F.T., Wondji, C.S., Simard, F., Hawley, W.A., 2003. Population structure of *Anopheles gambiae* in Africa. *J. Hered.* 94 (2), 133–147. <https://doi.org/10.1093/jhered/esg024>.
- Lourenço, J., Recker, M., 2014. The 2012 Madeira dengue outbreak: epidemiological determinants and future epidemic potential. *PLoS Negl. Trop. Dis.* 8 (8), e3083. <https://doi.org/10.1371/journal.pntd.0003083>.
- Lv, R.-C., Zhu, C.-Q., Wang, C.-H., Ai, L.-L., Lv, H., Zhang, B., Li, C.-M., An, J., Wang, P.-G., Hu, D., Tan, X.-Z., Yang, L., Zhou, H.-N., Tan, W.-L., 2020. Genetic diversity and population structure of *Aedes aegypti* after massive vector control for dengue fever prevention in Yunnan border areas. *Sci. Rep.* 19 (1), 12731. <https://doi.org/10.1038/s41598-020-69668-7>.
- Maffey, L., Garzón, M.J., Confalonieri, V., Chanampa, M.M., Hasson, E., Schweiggmann, N., 2020. Genome-wide screening of *Aedes aegypti* (Culicidae: Diptera) populations from northwestern Argentina: active and passive dispersal shape genetic structure. *J. Med. Entomol.* 57 (6), 1930–1941. <https://doi.org/10.1093/jme/tjaa125>.
- Maitra, A., Cunha-Machado, A.S., Leandro, A.S., da Costa, F.M., Scarpassa, V.M., 2019. Exploring deeper genetic structures: *Aedes aegypti* in Brazil. *Acta Trop.* 195, 68–77.
- Matthews, B.J., Dudchenko, O., Kingan, S.B., et al., 2018. Improved reference genome of *Aedes aegypti* informs arbovirus vector control. *Nature.* 563, 501–507.
- McKenzie, B.A., Wilson, A.E., Zohdy, S., 2019. *Aedes albopictus* is a competent vector of Zika virus: a meta-analysis. *PLoS One* 14 (5), e0216794. <https://doi.org/10.1371/journal.pone.0216794>.
- Mendonça, B.A., de Sousa, A.C., de Souza, A.P., Scarpassa, V.M., 2014. Temporal genetic structure of major dengue vector *Aedes aegypti* from Manaus, Amazonas, Brazil. *Acta Trop.* 134, 80–88. <https://doi.org/10.1016/j.actatropica.2014.02.014>.
- Midega, J.T., Muturi, E.J., Baliraine, F.N., Mbogo, C.M., Githure, J., Beier, J.C., Yan, G., 2010. Population structure of *Anopheles gambiae* along the Kenyan coast. *Acta Trop.* 114 (2), 103–108. <https://doi.org/10.1016/j.actatropica.2010.01.011>.
- Moore, C.G., Cline, B.L., Ruiz-Tiben, E., Lee, D., Romney-Joseph, H., Rivera-Correa, E., 1978. *Aedes aegypti* in Puerto Rico: environmental determinants of larval abundance and relation to dengue virus transmission. *Am. J. Trop. Med. Hyg.* 27, 1225–1231.
- Naim, D.M., Kamal, N.Z.M., Mahboob, S., 2020. Population structure and genetic diversity of *Aedes aegypti* and *Aedes albopictus* in Penang as revealed by mitochondrial DNA cytochrome oxidase I. *Saudi. J. Bio. Sci.* 27 (3), 953–967. <https://doi.org/10.1016/j.sjbs.2020.01.021>.
- Oetting, W.S., Lee, H.K., Flanders, D.J., Wiesner, G.L., Sellers, T.A., King, R.A., 1995. Linkage analysis with multiplexed short tandem repeat polymorphisms using infrared fluorescence and M13 tailed primers. *Genomics.* 30 (3), 450–458. <https://doi.org/10.1006/geno.1995.1264>.
- Olanratnane, P., Kittayapong, P., Chansang, C., Hoffmann, A.A., Weeks, A.R., Endersby, N.M., 2013. Population genetic structure of *Aedes (Stegomyia) aegypti* (L.) at a micro-spatial scale in Thailand: implications for a dengue suppression strategy. *PLoS Negl. Trop. Dis.* 7 (1), e1913. <https://doi.org/10.1371/journal.pntd.0001913>.
- Paupy, C., Brengues, C., Kamgang, B., Hervé, J.P., Fontenille, D., Simard, F., 2008. Gene flow between domestic and sylvan populations of *Aedes aegypti* (Diptera: Culicidae) in North Cameroon. *J. Med. Entomol.* 45 (3), 391–400. [https://doi.org/10.1603/0022-2585\(2008\)45\[391:gfbdas\]2.0.co;2](https://doi.org/10.1603/0022-2585(2008)45[391:gfbdas]2.0.co;2).
- Paupy, C., Le Goff, G., Brengues, C., Guerra, M., Revollo, J., Barja Simon, Z., Hervé, J.P., Fontenille, D., 2012. Genetic structure and phylogeography of *Aedes aegypti*, the dengue and yellow-fever mosquito vector in Bolivia. *Infect. Genet. Evol.* 12 (6), 1260–1269. <https://doi.org/10.1016/j.meegid.2012.04.012>.
- Perez, M.H., Noriega, F.G., 2012. *Aedes aegypti* paratubercle 1st instar quiescence affects larval fitness and metal tolerance. *J. Insect. Physiol.* 58 (6), 824–829. <https://doi.org/10.1016/j.jinsphys.2012.03.005>.
- Piry, S., Alapetite, A., Cornuet, J.M., Paetkau, D., Baudouin, L., Estoup, A., 2004. GENECLASS 2: a software for genetic assignment and first-generation migrant detection. *J. Hered.* 95 (6), 536–539. <https://doi.org/10.1093/jhered/esh074>.
- Pless, E., Hopperstad, K.A., Ledesma, N., Dixon, D., Henke, J.A., Powell, J.R., 2020. Sunshine versus gold: the effect of population age on genetic structure of an invasive mosquito. *Ecol. Evol.* 10 (18), 9588–9599.
- Pritchard, J.K., Stephens, M., Donnelly, P., 2000. Inference of population structure using multilocus genotype data. *Genetics.* 155 (2), 945–959. <https://doi.org/10.1093/genetics/155.2.945>.
- Rannala, B., Mountain, J.L., 1997. Detecting immigration by using multilocus genotypes. *Proc. Natl. Acad. Sci. U. S. A.* 94, 9197–9221.
- Rasić, G., Endersby-Harshman, N., Tantowijoyo, W., Goundar, A., White, V., Yang, Q., Filipović, I., Johnson, P., Hoffmann, A.A., Arguni, E., 2015. *Aedes aegypti* has spatially structured and seasonally stable population in Yogyakarta, Indonesia. *Parasit. Vectors.* 8, 610. <https://doi.org/10.1186/s13071-015-1230-6>.
- Ravel, S., Monteny N., Velasco Olmos, D., Escalante Verdugo, J., Cuny, G., 2001. A preliminary study of the population genetics of *Aedes aegypti* (Diptera: Culicidae) from Mexico using microsatellite and AFLP markers. *Acta. Trop.* 78 (3), 241–250. [https://doi.org/10.1016/s0001-706x\(01\)00083-3](https://doi.org/10.1016/s0001-706x(01)00083-3). PMID: 11311187.
- Raymond, M., Rousset, F., 1995. GENEPOP, population genetics software for exact tests and ecumenicism, version 3.4. *J. Hered.* 86, 248–249. <https://doi.org/10.1093/oxfordjournals.jhered.a111573>.
- Regilme, M.A.F., Carvajal, T.M., Honnen, A., Amalin, D.M., Watanabe, K., 2021. The influence of roads on the fine-scale population genetic structure of the dengue vector *Aedes aegypti* (Linnaeus). *PLoS Negl. Trop. Dis.* 15 (2), e0009139. <https://doi.org/10.1371/journal.pntd.0009139>.
- Reinhold, J.M., Lazzari, C.R., Lahondère, C., 2018. Effects of the environmental temperature on *Aedes aegypti* and *Aedes albopictus* mosquitoes: a review. *Insects.* 9 (4), 158. <https://doi.org/10.3390/insects9040158>.
- Ridley, M., 2007. *Evolution*, 2nd Ed. Oxford University Press, USA.
- Rowley, W.A., Graham, C.L., 1968. The effect of temperature and relative humidity on the flight performance of female *Aedes aegypti*. *J. Insect Physiol.* 14 (9), 1251–1257. [https://doi.org/10.1016/0022-1910\(68\)90018-8](https://doi.org/10.1016/0022-1910(68)90018-8).
- Ryan, S.J., Carlson, C.J., Mordecai, E.A., Johnson, L.R., 2019. Global expansion and redistribution of *Aedes*-borne virus transmission risk with climate change. *PLoS Negl. Trop. Dis.* 13 (3), e0007213. <https://doi.org/10.1371/journal.pntd.0007213>.
- Sayson, S.L., Gloria-Soria, A., Powell, J.R., Edillo, F.E., 2015. Seasonal genetic changes of *Aedes aegypti* (Diptera: Culicidae) populations in selected sites of Cebu City, Philippines. *J. Med. Entomol.* 52 (4), 638–646. <https://doi.org/10.1093/jme/tjv056>.
- Scarpassa, V.M., Conn, J.E., 2007. Population genetic structure of the major malaria vector *Anopheles darlingi* (Diptera: Culicidae) from the Brazilian Amazon, using microsatellite markers. *Mem. Inst. Oswaldo Cruz* 102 (3), 319–327. <https://doi.org/10.1590/s0074-02762007005000045>.
- Scarpassa, V.M., Cardoza, T.B., Cardoso Junior, R.P., 2008. Population genetics and phylogeography of *Aedes aegypti* (Diptera: Culicidae) from Brazil. *Am. J. Trop. Med. Hyg.* 78 (6), 895–903. <https://doi.org/10.4269/ajtmh.2008.78.895>.
- Shi, Q.-M., Zhang, H.-D., Wang, G., Guo, X.-X., Xing, D., Dong, Y.-D., Xiao, L., Gao, J., Liu, Q.-M., Sun, A.-J., Li, C.-X., Zhao, T.-Y., 2017. The genetic diversity and population structure of domestic *Aedes aegypti* (Diptera: Culicidae) in Yunnan Province, southwestern China. *Parasit. Vectors* 10 (1), 292. <https://doi.org/10.1186/s13071-017-2213-6>.
- Slatkin, M., 1995. A measure of population subdivision based on microsatellite allele frequencies. *Genetics.* 139 (1), 457–462. <https://doi.org/10.1093/genetics/139.1.457>.
- Slotman, M.A., Kelly, N.B., Harrington, L.C., Kitthawee, S., Jones, J.W., Scott, T.W., Caccione, A., Powell, J.R., 2007. Polymorphic microsatellite markers for studies of *Aedes aegypti* (Diptera: Culicidae), the vector of dengue and yellow fever. *Mol. Ecol. Notes* 7 (1), 168–171. <https://doi.org/10.1111/j.1471-8286.2006.01533.x>.
- Soper, F.L., 1967. Dynamics of *Aedes aegypti* distribution and density. Seasonal fluctuations in the Americas. *Bull. World Health Organ.* 36 (4), 536–538.
- Souza-Neto, J.A., Powell, J.R., Bonizzoni, M., 2019. *Aedes aegypti* vector competence studies: a review. *Infect. Genet. Evol.* 67, 191–209. <https://doi.org/10.1016/j.meegid.2018.11.009>.
- Steffens, D.L., Sutter, S.L., Roemer, S.C., 1993. An alternate universal forward primer for improved automated DNA sequencing of M13. *Biotechniques.* 15 (4), 580–582.
- Subramanian, S., 2018. Influence of effective population size on genes under varying levels of selection pressure. *Genome Biol. Evol.* 10 (3), 756–762.
- The City Government of Baguio, 2020. Baguio Dengue Cases in Increase by 400 percent. <https://www.baguio.gov.ph/content/baguio-dengue-cases-increase-400-percent#:~:text=The%20City%20Health%20services%20office,the%20same%20period%20this%20year> (accessed 14 February 2021).
- Thu, H.M., Aye, K.M., Thein, S., 1998. The effect of temperature and humidity on dengue virus propagation in *Aedes aegypti* mosquitoes. *Southeast Asian J. Trop. Med. Public Health.* 29 (2), 280–284.
- Van Oosterhout, C., Hutchinson, W.F., Wills, D.P.M., Shipley, P., 2004. MICROCHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Mol. Ecol. Notes* 4, 535–538. <https://doi.org/10.1111/j.1471-8286.2004.00684.x>.
- Weir, B.S., Cockerham, C.C., 1984. Estimating F-statistics for the analysis of population structure. *Evol.: Int. J. Org. Evol.* 38 (6), 1358–1370. <https://doi.org/10.1111/j.1558-5646.1984.tb05657.x>.
- Whitlock, M.C., Barton, N.H., 1997. The effective size of a subdivided population. *Genetics.* 146 (1), 427–441. <https://doi.org/10.1093/genetics/146.1.427>.
- WHO, 2012. *Handbook for Integrated Vector Management*. WHO, Geneva, Switzerland.
- WHO, 2019a. WHO Scales Up Response to Worldwide Surge in Dengue. <https://www.who.int/news-room/feature-stories/detail/who-scales-up-response-to-worldwide-surge-in-dengue> (accessed 18 January 2021).
- WHO, 2019b. Dengue and Severe Dengue: Who Spread Dengue and Severe Dengue? <https://www.who.int/news-room/q-a-detail/dengue-and-severe-dengue> (accessed 18 January 2021).
- WHO, 2021. Dengue Situation Update Number 610. https://www.who.int/docs/default-source/wpro---documents/emergency/surveillance/dengue/dengue-20201217.pdf?sfvrsn=fc80101d_45 (accessed 18 January 2021).
- Wilke, A.B.B., Wilk-da-Silva, R., Marelli, M.T., 2017. Microgeographic population structuring of *Aedes aegypti* (Diptera: Culicidae). *PLoS One* 12 (9), e0185150. <https://doi.org/10.1371/journal.pone.0185150>.

World Weather Online, 2022. Liloan Climate Weather Averages. <https://www.worldweatheronline.com/liloan-weather-averages/cebu/ph.aspx> (accessed 10 February 2022).

Wright, S., 1978. Evolution and the genetics of populations, 4. University of Chicago Press, Chicago.

Xia, S., Cosme, L.V., Lutomiah, J., Sang, R., Ngangue, M.F., Rahola, N., Ayala, D., Powell, J.R., 2020. Genetic structure of the mosquito *Aedes aegypti* in local forest and domestic habitats in Gabon and Kenya. *Parasit. Vectors* 13 (1), 417. <https://doi.org/10.1186/s13071-020-04278-w>.