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Parameterization and Sensitivity Analysis of a Complex Simulation Model for Mosquito Population Dynamics, Dengue Transmission, and Their Control

Alicia M. Ellis,* Andres J. Garcia, Dana A. Focks, Amy C. Morrison, and Thomas W. Scott

Department of Entomology, University of California Davis, Davis, California; Fogarty International Center, National Institutes of Health, Bethesda, Maryland; Department of Environmental and Global Health, Emerging Pathogens Institute, University of Florida, Gainesville, Florida; Department of Geography, Emerging Pathogens Institute, University of Florida, Gainesville, Florida

Abstract. Models can be useful tools for understanding the dynamics and control of mosquito-borne disease. More detailed models may be more realistic and better suited for understanding local disease dynamics; however, evaluating model suitability, accuracy, and performance becomes increasingly difficult with greater model complexity. Sensitivity analysis is a technique that permits exploration of complex models by evaluating the sensitivity of the model to changes in parameters. Here, we present results of sensitivity analyses of two interrelated complex simulation models of mosquito population dynamics and dengue transmission. We found that dengue transmission may be influenced most by survival in each life stage of the mosquito, mosquito biting behavior, and duration of the infectious period in humans. The importance of these biological processes for vector-borne disease models and the overwhelming lack of knowledge about them make acquisition of relevant field data on these biological processes a top research priority.

INTRODUCTION

Worldwide, 2.5 billion people are at risk of contracting dengue. Approximately 50 million dengue fever cases, 500,000 cases of dengue hemorrhagic fever, and 22,000 deaths occur annually.[1] Local and regional control is complicated by the facts that no vaccine has yet been licensed for broad-scale application and that dengue epidemiology is complex. Not only are there four different virus serotypes with potentially different ecologies, but transmission dynamics are also influenced by variation in the behavior and population dynamics of mosquito vectors and human hosts and the nature of their interactions with the environment and each other.[2]

Mathematical and computer simulation models provide a systematic way to explore and analyze the complexity of dengue transmission and can be useful tools for devising more effective disease surveillance and control strategies. Models can integrate data from different sources and at different spatiotemporal scales, identify and prioritize gaps in data or knowledge, and address questions that are too complex, expensive, or dangerous to address in other ways.[3,4] Models, therefore, provide a basic theoretical framework so that the intricate transmission processes of dengue can be represented in a simplified form that can be analyzed in ways that are not possible in the natural system itself.

To explore the complexity of infectious disease systems, both simple[5] and complex[6–8] models have been developed. More complex disease models tend to be more realistic and can be used to develop location-specific control strategies. However, the behavior of complex models can be difficult to explore analytically,[9] and as the number of model parameters increases, the likelihood also increases that parameters for which little empirical data exist are included. Evaluating the suitability, accuracy, and performance of complex disease models can be difficult.

Sensitivity analysis provides a way to explore the behavior of complex disease models by identifying how variation in model parameters affects model output.[9] Model outputs obtained with different parameter values are evaluated to identify the parameters to which the model is most sensitive. In this way, sensitivity analysis can be useful not only for understanding model behavior, but also for identifying the biological processes that may be most important in determining pathogen transmission. Obtaining accurate empirical estimates of the parameters capturing the most important biological processes may then improve the accuracy of disease models and the ability of models to inform surveillance and control strategies.

In this study, we conducted a sensitivity analysis of the complex dengue simulation models originally developed by Focks and others.[7,8] The Container-Inhabiting Mosquito Simulation Model (CIMSiM) simulates mosquito population dynamics, and the Dengue Transmission Model (DENSiM) simulates dengue transmission in a human population. Both models can be parameterized for a specific location using local weather and entomological and epidemiological data, and both can be used to test the effects of various prevention measures on local dengue transmission. Our objectives were to (1) parameterize the models for Iquitos, Peru, using data collected during longitudinal studies in the city (i.e., set-up the models with required data from location to be modeled), (2) identify parameters to which the models are most sensitive using sensitivity analysis, and (3) determine parameters for which more empirical data are needed. Our overall goal was to evaluate the behavior of the model and set an empirical research agenda for improving the understanding and modeling of dengue and its control.

OVERVIEW OF MODELS

The dengue model is comprised of two interacting models. CIMSiM is an age- and stage-structured model that tracks the dynamics of mosquito eggs, larvae, pupae, and adults over time.[8] The model uses local weather information and information obtained from a survey of breeding sites in the focal location. For the survey, users collect information about the number of water-filled containers in a defined area, container dimensions, container characteristics, and number of pupae in the containers. These raw data are used to define container categories for use in the model. Egg, larval, and water dynamics within each container category are simulated with survival and development based on predation (for eggs), temperature,

*Address correspondence to Alicia M. Ellis, Department of Entomology, University of California Davis, One Shields Ave., Davis, CA 95618. E-mail: alicia.m.ellis@gmail.com

saturation deficit, and food dynamics. Adult population dynamics are determined by age-dependent survival with two age classes (young and old), the effects of temperature and saturation deficit on survival, and host biting.

DENSiM takes information from CIMSiM and local information about the human age structure, age-specific birth and death rates, age- and serotype-specific serostatus, and population size to simulate infection dynamics in human and mosquito populations. It is individual-based and age-structured for the human population and tracks infection dynamics for all user-defined dengue serotypes circulating in the system.[7] The dengue transmission model uses CIMSiM results to model the mosquito population and its interaction with the human population. Both models have stochastic and discrete elements.

METHODS

Data. Long-term entomological and epidemiological surveys have been conducted simultaneously in Iquitos, Peru, since 1999,[10] and data obtained from these surveys were used to parameterize the models. Iquitos is located in the northeastern part of Peru (73.2°W, 3.7°S; 120 m above sea level) and has a tropical climate with an average daily temperature of 25.8°C and an average annual precipitation of 3.4 m during the study. The study was restricted to an ~16 km^2 area in the districts of Mayanas, Punchana, Belen, and San Juan.

For the entomological component of the monitoring study, a team of researchers visited houses on a daily, rotational basis since 1999 and recorded information about each water-filled container in and around each home. Each container was defined as a certain type (e.g., bucket, tire, or vase), and several measurements related to container size, water use, location, and number of pupae were obtained. Details of the study and results from the first 3 years of the study are presented by Morrison and others.[11]

The epidemiological study monitored the serostatus of ~2,400 individuals once every 6 months beginning in 1999. The sampled population represented a wide range of ages and individuals of both sexes. A team of phlebotomists obtained blood samples from study participants once every 6 months, and the plaque reduction neutralization test was used to determine serotype-specific serostatus of individuals when blood was drawn. Differences in test results between subsequent blood draws were used to identify infections for each of the dengue serotypes, although the exact timing of the infection between negative and positive blood draws (~6-month intervals) could not be determined. This technique did, however, allow the detection of all infections, including those that were asymptomatic.[10]

Model parameterization. *Containers.* CIMSiM simulates the dynamics of immature *Ae. aegypti* and water dynamics within user-specified container categories. Each container category should represent the average characteristics and density of that container category in the focal location. We used containers sampled in the long-term entomological study in Iquitos, Peru, just before the transmission season in 2000 to parameterize the model.[11] Container measurements used for the parameterization included shape (circular or rectangular), dimensions, presence or absence of lid, fill method (manual or rain), fill frequency (daily, weekly, or monthly), drawdown frequency (daily, weekly, or monthly), if it was located on the edge of a roof or similar device to capture rain water, if it was in shade or sun, and total number of pupae present. Drawdown percent was estimated by expert opinion (Morrison AC, personal communication). For each container, we calculated volume and watershed ratio where the watershed ratio was the surface area of the container opening divided by the surface area of the opening at its' widest location in the container (i.e., the surface area of a horizonal plane in the widest part of the container). If the container was partially under a roof or rain gutter, the watershed ratio was multiplied by 5, and if the container was completely under a roof or similar device, the ratio was multiplied by 10.

Container categories for CIMSiM were then defined by the local container type (e.g., bucket or tire) and fill method (rain- or manually filled), and averages for all container characteristics required in the model were calculated for the resulting 68 container categories (Supplemental Appendix A, Tables 1 and 2). A few changes to these averages were necessary for the model to produce sustained transmission of dengue in DENSiM. Mean drawdown was reduced from 50% to 0% for rain-filled boxes, drawdown frequency was changed from daily to weekly for large rain-filled tanks, and height was changed from 100 to 1.1 cm for large rain-filled and elevated tanks.

Weather. Weather data used for parameterization were obtained from the National Climatic Data Center for a weather station in Iquitos for 2000–2004 (station: 84377099999–IQUITOS; information available at http://www.ncdc.noaa.gov/oa/ncdc.html). Weather inputs included daily minimum, average, and maximum temperature, precipitation, relative humidity, and saturation deficit.

Food. After entering the appropriate container and weather data into the model, the food fitter described in Focks and others[8] was used to determine food inputs for each container category in CIMSiM using default values for biological parameters (Table 1). Food values were modified within the food fitter until the difference between the predicted number of pupae and the observed number of pupae per hectare was less than or equal to one for each container category (calculated from the Iquitos entomological study described above) for each container category. In one case (rain-filled tires), changing food did not alter the difference between observed and predicted numbers of pupae. In this case, an arbitrary value of 60 for food was used. The food values obtained when using default values for biological parameters were used for all model runs in the sensitivity analysis described below (i.e., the food fitter was not used for each of the 5,000 parameter combinations described below).

Human demographics. Data used to calculate the human population size of Iquitos were obtained for 1999 (simulations began January of 2000) from El Instituto Nacional de Estadística e Informática (INEI; information available at http://www1.inei.gob.pe/biblioineipub/bancopub/Est/Lib0004/Loreto.htm). Estimated population sizes for the districts of Iquitos and Punchana (the areas sampled in the longitudinal studies conducted in Iquitos) were summed to give a population size of 400,000 for DENSiM. Host density was then calculated as 400,000 divided by the estimated area of the region surveyed (16 km^2).[11]

Age-structure and age-specific fertility rates were obtained by the US Census Bureau international database online for Peru in 1999 (information available at http://www.census.gov/ipc/www/idb/country.php). Age-specific death rates were calculated from several sources. Numbers of age-specific deaths and population size for Peru in 2003 were obtained from the

TABLE 1
Parameter defaults and ranges for CIMSiM sensitivity analysis

Biology tab	Parameters	Distribution	Default	Minimum value	Maximum value
Adults	Young mosquito cut-off age	Uniform	10	8	12
Adults	Old mosquito survival	Uniform	0.89	0.8	0.9
Adults	Young mosquito survival	Uniform	0.91	0.9	1
Adults	Double blood mean high weight threshold	Uniform	3.5	2.8	4.2
Adults	Double blood meal high weight ratio	Uniform	0.01	0.05	0.12
Adults	Double blood meal low weight threshold	Uniform	0.5	0	1.2
Adults	Double blood meal low weight ratio	Uniform	1	0.8	1
Adults	Dry to wet weight factor	Uniform	1.655	1.324	1.986
Adults	Fecundity factor	Uniform	45.9	36.72	55.08
Adults	Interrupted feeds per meal	Uniform	3	0	5
Adults	Minimum oviposition temperature	Uniform	18	14.4	21.6
Adults	Proportion of mosquitoes feeding on humans	Uniform	0.09	0.72	1
Adults	Proportion of interrupted feeds on different host	Uniform	0.35	0.2	0.6
Adults	Survival at or above high saturation-deficit threshold	Uniform	0.6	0.48	0.72
Adults	High saturation-deficit threshold	Uniform	30	24	36
Adults	Low saturation-deficit threshold	Uniform	10	4	16
Adults	Second developmental threshold	Uniform	0.58	0.464	0.696
Adults	High-temperature threshold	Uniform	40	30	45
Adults	Survival at or above high lethal temperature threshold	Uniform	0.05	0.04	0.06
Adults	High lethal temperature threshold	Uniform	50	45	60
Adults	Low-temperature threshold	Uniform	4	2	14
Adults	Survival at or below low lethal temperature threshold	Uniform	0.05	0.04	0.06
Adults	Low lethal temperature threshold	Uniform	0	–10	2
Eggs	Flood hatch ratio	Uniform	0.596	0.4768	0.7152
Eggs	Minimum hatch temperature	Uniform	22	13.5	26.4
Eggs	Nominal survival	Uniform	0.99	0.792	1
Eggs	Predation survival at high-temperature threshold	Uniform	0.7	0.56	0.84
Eggs	High-temperature predation threshold	Uniform	30	25	36
Eggs	Low-temperature predation threshold	Uniform	20	14	25
Eggs	Survival at or above high saturation-deficit threshold	Uniform	0.95	0.76	1
Eggs	High saturation-deficit threshold	Uniform	40	32	48
Eggs	Survival at low saturation-deficit threshold	Uniform	0.99	0.792	1
Eggs	Low saturation-deficit threshold	Uniform	10	2	18
Eggs	High sun-exposure threshold	Uniform	0.85	0.68	1
Eggs	Survival at or above high sun-exposure threshold	Uniform	0.95	0.76	1
Eggs	Spontaneous hatch ratio	Uniform	0.197	0.1576	0.2364
Eggs	High-temperature threshold	Uniform	30	20.6	38.5
Eggs	Survival at or above high lethal temperature threshold	Uniform	0.05	0.04	0.06
Eggs	High lethal temperature threshold	Uniform	47	38.5	56.4
Eggs	Low-temperature threshold	Uniform	–6	–10	3.4
Eggs	Survival at or below low lethal temperature threshold	Uniform	0.05	0.04	0.06
Eggs	Low lethal temperature threshold	Uniform	–14	–23.4	–10
Larvae	Cadaver weight to food ratio	Uniform	0.4	0.32	0.48
Larvae	Dry container survival	Uniform	0.05	0.04	0.06
Larvae	Assimilation rate	Uniform	0.3	0.24	0.36
Larvae	Exploitation rate	Uniform	0.8	0.64	0.96
Larvae	Exploitation rate independence	Uniform	0.1	0.08	0.12
Larvae	Metabolic weight-loss rate	Uniform	0.016	0.0128	0.0192
Larvae	Metabolic weight-loss exponent	Uniform	0.667	0.5336	0.8004
Larvae	Fasting survival with lipid reserve	Uniform	0.95	0.76	1
Larvae	Fasting survival with no lipid reserve	Uniform	0.5	0.4	0.6
Larvae	Non-depletable lipid reserves	Uniform	0.15	0.12	0.18
Larvae	Minimum weight for survival	Uniform	0.0009	0.00072	0.00108
Larvae	Nominal survival	Uniform	0.99	0.792	1
Larvae	Minimum weight for pupation	Uniform	0.1	0.08	0.19
Larvae	Pupation survival	Uniform	0.95	0.76	1
Larvae	High-temperature threshold	Uniform	39	30.2	41.5
Larvae	Survival at or above high lethal temperature threshold	Uniform	0.05	0.04	0.06
Larvae	High lethal temperature threshold	Uniform	44	41.5	52.8
Larvae	Low-temperature threshold	Uniform	10	7.5	18.8
Larvae	Survival at or below low lethal temperature threshold	Uniform	0.05	0.04	0.06
Larvae	Low lethal temperature threshold	Uniform	5	–3.8	7.5
Larvae	Weight at hatch	Uniform	0.001	0.0008	0.0012
Pupae	Emergence survival	Uniform	0.83	0.664	0.996
Pupae	Female emergence ratio	Uniform	0.5	0.4	0.6
Pupae	Nominal survival	Uniform	0.99	0.792	1
Pupae	High-temperature threshold	Uniform	39	30.2	41.5
Pupae	Survival at or above high lethal temperature threshold	Uniform	0.05	0.04	0.06
Pupae	High lethal temperature threshold	Uniform	44	41.5	52.8
Pupae	Low-temperature threshold	Uniform	10	7.5	18.8
Pupae	Survival at or below low lethal temperature threshold	Uniform	0.05	0.04	0.06
Pupae	Low lethal temperature threshold	Uniform	5	–3.8	7.5

TABLE 2
Parameter defaults and ranges for DENSiM sensitivity analysis

Parameter	Distribution	Default	Minimum value	Maximum value
Duration of maternally acquired neutralizing titers of antibody (days)	Uniform	90	30	180
Duration of maternally acquired enhancing titers of antibody (days)	Uniform	270	216	324
Duration of heterologous immunity	Uniform	60	30	180
DEN-1 viremia (MID-50)	Uniform	1,000,000	4	8
DEN-1 incubation duration (days)	Uniform	4	2	8
DEN-1 viremic duration (days)	Uniform	5	3	8
DEN-2 viremia (MID-50)	Uniform	1,000,000	4	8
DEN-2 incubation duration (days)	Uniform	4	2	8
DEN-2 viremic duration (days)	Uniform	5	3	8
DEN-3 viremia (MID-50)	Uniform	1,000,000	4	8
DEN-3 incubation duration (days)	Uniform	4	2	8
DEN-3 viremic duration (days)	Uniform	5	3	8
Probability of infection in biters at or below low titer set point (4 logs)	Uniform	0.1	0	0.3
Probability of infection in biters at or above high titer set point (8 logs)	Uniform	1	0.8	1
Nominal probability of mosquito to human infection	Uniform	0.9	0.72	1
EIP factor at or below low set point (3 logs)	Uniform	1.25	1.1	1.4
EIP factor at or above high set point (8 logs)	Uniform	0.75	0.6	0.9
Double blood meal low weight ratio	Uniform	1	0.8	1
Double blood meal low weight limit	Uniform	0.5	0	1.2
Double blood meal high weight ratio	Uniform	0.1	0.05	0.12
Double blood meal high weight limit	Uniform	3.5	2.8	4.2
Proportion of mosquitoes feeding on humans	Uniform	0.9	0.72	1
Interrupted feeds per meal	Uniform	3	0	5
Proportion of interrupted feeds on a different host	Uniform	0.35	0.2	0.6
Young mosquito survival	Uniform	0.91	0.9	1
Young mosquito cutoff age	Uniform	10	8	12
Old mosquito survival	Uniform	0.89	0.8	0.9

MID-50 is the 50% monkey infectious dose

United Nations (UN) Statistics Division online (information available at http://unstats.un.org/unsd/demographic/sconcerns/mortality/mort2.htm, table 19). Estimated age-specific death rates were calculated from these data. The crude death rate for Peru in 2003 was one-half the crude death rate estimated by the US Census Bureau for the same year. It has been suggested that only 50% of deaths are reported in Peru.[12] Doubling age-specific death rates calculated from the UN Statistics Division generated a crude death rate that matched the crude death rate estimated by US Census Bureau. We used these doubled mortality rates in the parameterization of DENSiM.

Age- and serotype-specific serostatus. Data used for estimating age- and serotype-specific serostatus for the human population were obtained from the epidemiological survey conducted in Iquitos, Peru.[10] Because simulations began in 2000, we used results from the last blood sample collected from individuals in 1999 (N = 852). Date of birth was not recorded during the study, and only age at first blood draw in 1999 was known. Age- and serotype-specific serostatus was calculated from the data assuming that all individuals remained the same age throughout the entire year (i.e., January 1 to December 31). Sample sizes differed across age groups, with high sample sizes for younger individuals and relatively small sample sizes for the older age groups. To obtain better estimates of age-specific serostatus for the older age groups, a logistic curve was fit to the proportion of individuals immune to each serotype in each age class for dengue 1 (DEN-1) and 2 (DEN-2), the only serotypes present in 1999. The equation was fit to the first 8, 9, 10, and 11 age classes (which had relatively large sampling sizes) with and without forcing through the origin. The fit obtained using the first nine age classes and forcing through the origin produced immunity rates of 84% and 78% for DEN-1 and DEN-2, respectively, by age 25–29, which best matched expected age-specific seroprevalence for 1999.[10] Predicted values for rates of immunity by serotype and age class from this logistic equation were used in the simulations.

Infection introduction. In Iquitos, DEN-1 was first detected in 1990, DEN-2 was first detected in 1995, and DEN-3 was first detected in 2001. For our simulations, we introduced DEN-1 and DEN-2 on January 1 and January 3, 2000, respectively, and DEN-3 on January 1, 2001. Virus was introduced monthly for the first year for each serotype, with one infected human to produce sustained transmission of the three serotypes.

Sensitivity analysis. *Parameter ranges.* Because most of the parameter defaults for the models were determined from empirical studies and because parameter ranges were rarely presented in those studies, we set parameter ranges for parameters included in the sensitivity analysis as ±20% of the default value, which produced reasonable parameter ranges based on expert opinion (Tables 1 and 2). The ±20% rule was modified in the following ways. (1) If the 20% range gave values that were not possible (e.g., negative values when minimum value should be zero or values above one for survival), the range was truncated to match data requirements. (2) If an empirical range existed and if this range was larger than the 20% range, the empirically measured range was used. (3) If an empirical range existed and if the 20% range was wider than this range on one side of the default but smaller than the empirical range on the other side, the range of the parameter was expanded on the one side where empirical range is wider, but the 20% range was maintained on the other side. (4) For egg, larvae, pupae, and adult survival versus temperature and egg predation survival versus temperature, the numbers of degrees within the 20% range calculated for the highest lethal temperature threshold were used to define the ranges for the lower survival temperature thresholds. In many cases, temperature threshold ranges overlapped. The sensitivity analysis was originally run with the overlapping

ranges, and runs were omitted when parameter values selected for lower thresholds were higher than the parameter values selected for the higher thresholds. Because far more runs were omitted than maintained, the midpoint of the overlapping section of temperature threshold ranges was calculated, and the ranges were shorted to that midpoint so that they did not overlap. In CIMSiM, weight at hatch and minimum weight for survival were rank-correlated to reduce the probability of obtaining impossible parameter values during the Latin Hypercube sampling (LHS) of parameter values (discussed in next section), and because they are likely correlated in natural systems. Because we were only interested in the relative effect of different parameters on model output and because parameter distributions are unknown for most parameters, we assumed a uniform distribution for all parameter values.

LHS and model runs. Sensitivity analyses were conducted separately for CIMSiM and DENSiM using the Iquitos parameterization. DENSiM uses output and parameter values for adult mosquitoes from CIMSiM to resimulate the mosquito population and track infection dynamics in mosquitoes. Thus, for DENSiM sensitivity analysis, parameter defaults for CIMSiM were used in every run except the parameters associated with biting and adult survival, which were varied in both CIMSiM and DENSiM tests. LHS was performed on each set of parameters using Sandia's LHS program (Version 0.5 Beta, 1997; Sandia National Laboratories, Risk Assessment and Systems Modeling Department, Albuquerque, NM) with 5,000 samples for each parameter (Sandia's LHS program was modified to allow 5,000 as opposed to the original maximum of 1,000 samples). A module was created to read the output of the LHS program and automatically run CIMSiM or DENSiM using parameter values selected for each of the 5,000 runs. All simulations used the established population, random food, and food values obtained by using the food fitter with default parameter values.

Analysis of output. For each run of the model, daily measures of several outputs over the 5-year period (2000–2004) of the simulation were obtained. For CIMSiM, we focused on the total daily number of eggs, larvae, pupae, females, new females, host-seeking females, ovipositing females, and new eggs laid summed over all containers in Iquitos and the average weight of female mosquitoes in the city. For DENSiM, we evaluated the total daily number of infective mosquitoes, persons incubating, persons viremic, and persons with virus for each of three serotypes. Because the outputs are time series, we conducted several analyses to facilitate the evaluation of the relative effects of each parameter on model output. First, we calculated several time series descriptors for each output listed: mean, median, minimum, maximum, variance, period, amplitude, first, second, and third autocorrelation coefficients, and 25% and 75% quantiles.[13] Principal components analysis was then conducted to reduce the time series descriptors to three new independent variables (i.e., principal components) for each output and for DENSiM, each output by serotype. Finally, step-wise regressions of the first three principal components against parameter values was conducted for each output. *P* value to enter and remove was set at 0.15, because the goal was to be more inclusive than exclusive. Thus, for each of the nine outputs in CIMSiM, three regressions were performed, one for each of the three principal components (27 regressions total). For DENSiM, 36 regressions were performed—three for each of four outputs for each of the three serotypes. The order in which the parameters were added (i.e., rank) reflects the relative amount of variation in the principal component explained by the parameters and therefore, can be interpreted as their relative importance in determining model output. To assess overall sensitivity of the models to changes in parameters, regressions that had similar principal component loadings on time-series descriptors were grouped and compared. For each regression group, the number of times that the parameter was selected in the regression (output variable versus principal component) and its average rank were calculated, and relative importance was assigned based on count and rank across regression groups.

RESULTS

CIMSiM sensitivity analysis. For CIMSiM, principal component (PC) loadings for the mean, quantiles, minimum, maximum, amplitude, and to a lesser degree, variance were high and positive for PC1 across all output variables. Loadings on the first three autocorrelation coefficients and amplitude were high and positive for PC2 across all output variables and loadings for PC3 varied across output variables but were generally very high and positive for period (Supplemental Appendix A, Table 3A). Loadings for PC3 were similar for eggs, larvae, and host-seeking females and similar for pupae and total females.

For each of these groupings (defined by similarities in PC loadings), the number of times each parameter was included and its average rank (order of inclusion) were calculated (Supplemental Appendix A, Table 4A). Results showed that model outputs for CIMSiM (with total numbers of each life stage summed across containers) were sensitive to (1) nominal survival for eggs, larvae, and pupae and young and old adult survival (a partial surrogate for adult nominal survival present in earlier model versions that allows the potential for age-dependent mosquito mortality), (2) parameters associated with larval feeding (i.e., exploitation rate, assimilation rate, and exploitation rate independence), (3) parameters associated with egg predation (i.e., high and low predation temperature thresholds and predation survival at the high-temperature threshold), (4) parameters associated with pupation (i.e., survival while metamorphosing into pupae, survival while emerging from the pupal stage, and female emergence ratio), (5) high-temperature thresholds for eggs and adults, (6) adult fecundity factor, dry to wet weight ratio, second developmental threshold, and low saturation-deficit threshold, and (7) minimum egg-hatch temperature. Particularly important variables were the larval exploitation rate, high-temperature thresholds for eggs, adults, and egg predation, and nominal survival for larvae and young adults. Overall, the parameters identified as important in determining dynamics of the mosquito population were related to survival within each life stage.

DENSiM sensitivity analysis. For DEN-1, DEN-2, and the three output variables for human infection (i.e., persons incubating, persons viremic, and persons with virus), PC1 loadings were high and positive for mean, variance, the first three autocorrelation coefficients, and amplitude. PC2 loadings were high for quantiles (all negative), the first three autocorrelation coefficients (all positive), and period (positive), and PC3 loadings were high for variance (negative), median (positive), maximum (negative), and amplitude (negative). For DEN-3 and the three human infection outputs, loadings for PC1 were

high and positive for mean variance, maximum, the first three autocorrelation coefficients, and amplitude. PC2 loadings for DEN-3 and the three human infection outputs were high for the first quantile (positive), median (positive), autocorrelation coefficients (negative), and period (negative), and for PC3, they were high for variance (negative), all quantiles (positive), maximum (negative), and autocorrelation coefficients (negative). Loadings for infective mosquitoes were generally different from loadings for the other output variables but were similar across each serotype for each principal component. PC1 loadings for infective mosquitoes were high and positive for mean, variance, median, third quantile, maximum, and amplitude. PC2 loadings were high and positive for the three autocorrelation coefficients, and PC3 loadings were high for first quantile (positive), median (positive), maximum (negative), and amplitude (negative). Counts and average ranks were obtained for each of these groupings (Supplemental Appendix A, Table 3B).

Results showed that infection of humans and mosquitoes were most sensitive to the number of interrupted mosquito feeds per blood meal and parameters associated with age-dependent adult mosquito survival, particularly survival of the older-aged individuals (i.e., young and old survival and cut-off age between young and old adults) (Supplemental Appendix A, Table 4B). For each serotype, the duration of the viremic period was important. The duration of incubation in the humans was occasionally important for each serotype, although viremia was not important. Proportion of mosquito feeds on different hosts, proportion of feeds on humans, duration of heterologous immunity, and double blood meal low weight limit for mosquitoes were also important.

DISCUSSION

Sensitivity analysis results suggest that patterns of mosquito population dynamics and dengue transmission may be primarily determined by factors influencing the survival of each life stage of the mosquito, larval food competition, adult mosquito biting behavior, and duration of the infectious period in humans. These results are consistent with other modeling studies reporting the importance of mosquito survival or mortality,[14–21] biting rate,[6,14,15,17,18,22] and length of the infectious period in the host[6,14,21,23–27] in determining the dynamics of mosquito-borne diseases. However, our results go one step further by identifying specific causes of mosquito mortality and the aspects of biting behaviors that may be most influential. Age-dependent adult mortality, exploitation and assimilation of larval food, egg predation and hatching, survival due to temperature for all life stages, pupation survival, adult fecundity, number of feeds per complete blood meal, and proportion of interrupted or incomplete blood meals that resume on different hosts may all be important in determining mosquito population and virus transmission dynamics.

Unfortunately, little detailed empirical information exists on mosquito survival and biting behavior, and accurate estimates of the length of the host infectious period for mosquito-borne diseases are often difficult to obtain. A growing number of studies have shown age-dependent mortality in mosquitoes,[28–32] but the form of the relationship between age and mortality and how it changes in space and time in natural systems are not known for most mosquito vector species. Accurate estimates of age-dependent mortality are important, because adult survival determines the probability that a mosquito will live long enough to become infectious and transmit a pathogen during its lifetime. Studies have identified potential causes of mortality throughout the mosquito life cycle,[33–35] but the rates at which mortality occurs for each mortality factor are not known. Larval food competition is known to occur in the laboratory and field,[36–39] but the extent to which it occurs in nature is unclear. In most cases, the resources that larvae consume in natural systems have not even been identified. Finally, many investigators have estimated mosquito biting rates in the field,[40–46] but few have defined details of biting behavior that we identified as important in our sensitivity analysis.

CIMSiM and DENSiM are most sensitive to parameters for which little empirical information exists, and although this information can be extremely valuable for setting research priorities, it also places constraints on the use and interpretation of model outputs. Uncertainty in estimates of important parameters limits confidence that the models accurately capture mosquito population and transmission dynamics in a specific location.[47] Outputs should not, therefore, be used to make explicit predications about transmission dynamics or the effect of intervention strategies on them. However, even with limitations in the current state of understanding of dengue transmission, models can be extremely useful tools.[3,4] CIMSiM and DENSiM are based on currently available information, and they can be useful for improving understanding about the complex processes that interact to determine transmission dynamics and allowing users to visualize how slight changes in biological parameters or certain components of intervention strategies (i.e., percent coverage of indoor residual spraying) can have dramatic effects on mosquito populations and dengue transmission. The models are useful for encouraging users to think critically about ecological and transmission processes occurring in their locations, providing the relative effects of different perturbations to mosquito population dynamics and patterns of virus transmission to aid in developing more informed local control strategies that specifically target key components of the virus transmission process.

In addition to restrictions in model use, the limitations and constraints of our analysis and results should be considered. First, we did not test model sensitivity to differences in container classification, weather, human demographics, and serostatus. Model output may be sensitive to a slightly different set of parameters across locations. Second, we focused on the linear effects of parameters and sensitivity of model outputs to individual parameters and not on interactive effects. Because of the large numbers of parameters and because our goal was to conduct an initial analysis of the model, we chose to use linear step-wise regression to evaluate parameter importance. Although non-linear effects may occur, this method permits a simplified first analysis. Interactive parameter effects were also not examined, although results did identify related groups of parameters as being important (e.g., interrupted feeds per meal and proportion resuming on different hosts). More complete analyses of parameter interactions are needed. Finally, the structure of the models may restrict the type of information that can be extracted from them. For example, the models do not include movement of vectors or hosts or heterogeneous distribution of mosquito bites among humans, which prevent an evaluation of the relative importance of these factors in determining transmission dynamics.

Unlike some other studies,[14,19,48] we did not identify parameters associated with the extrinsic incubation period (EIP) in the mosquito as being important. This may be a function of how the processes determining the EIP are incorporated into the model and not because it is unimportant. Mosquito survival interacts with the EIP to determine the number of mosquitoes that become infectious, but the interaction may operate primarily through the parameters determining adult mosquito survival in the model and only secondarily through parameters associated directly with EIP. Overall, the importance of mosquito survival, biting behavior, and length of the incubation period in the host are likely to have important effects across all systems, although the details of those processes that are most important may vary.

Because of their importance in determining transmission dynamics and the scant empirical information about them, mosquito mortality, biting behavior, and duration of the infectious period should be top priorities for empirical research on mosquito-borne diseases. Specifically, studies are needed to better understand (1) age-dependent adult mosquito mortality, (2) mortality factors and rates for all mosquito life stages, (3) the strength, nature, and occurrence of larval density dependence, (4) the number of times a mosquito feeds per replete blood meal, and (5) the number of different hosts that are fed on per meal in natural systems. Focusing empirical research on parameters that have been identified as potentially important in models of virus transmission dynamics will not only improve understanding of transmission patterns but will also improve the accuracy of models and their use as tools for developing more effective surveillance and control strategies.

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Authors' addresses: Alicia M. Ellis and Thomas W. Scott, Department of Entomology, University of California Davis, Davis, CA, and the Fogarty International Center, National Institutes of Health, Bethesda, MD, E-mails: alicia.m.ellis@gmail.com and twscott@caes.ucdavis.edu. Andres J. Garcia, Department of Geography, Emerging Pathogens Institute, University of Florida, Gainesville, FL, E-mail: andygarcia@gmail.com. Dana A. Focks, Department of Environmental and Global Health, Emerging Pathogens Institute, University of Florida, Gainesville, FL, E-mail: DAFocks@id-analysis.com. Amy C. Morrison, Department of Entomology, University of California Davis, Davis, CA, and Naval Medical Research Center Detachment, Washington, DC, E-mail: amy.aegypti@gmail.com.

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