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Effects of Plant-Community Composition on the Vectorial Capacity and Fitness of the Malaria Mosquito *Anopheles gambiae*

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Abstract. Dynamics of *Anopheles gambiae* abundance and malaria transmission potential rely strongly on environmental conditions. Female and male *An. gambiae* use sugar and are affected by its absence, but how the presence or absence of nectariferous plants affects *An. gambiae* abundance and vectorial capacity has not been studied. We report on four replicates of a cohort study performed in mesocosms with sugar-poor and sugar-rich plants, in which we measured mosquito survival, biting rates, and fecundity. Survivorship was greater with access to sugar-rich plant species, and mortality patterns were age-dependent. Sugar-poor populations experienced Weibull mortality patterns, and of four populations in the sugar-rich environment, two female and three male subpopulations were better fitted by Gompertz functions. A tendency toward higher biting rates in sugar-poor mesocosms, particularly for young females, was found. Therefore, vectorial capacity was pulled in opposing directions by nectar availability, resulting in highly variable vectorial capacity values.

INTRODUCTION

Spatial and temporal heterogeneity in malaria transmission depends on climatic and environmental factors. Perhaps most well-studied are the effects of rainfall and availability of mosquito breeding sites and of temperature fluctuations. In addition, certain models have included vegetation as a predictive factor, as an indicator either of rainfall, and thus suitable conditions for *Anopheles* larval development,[1] or of tree canopy cover that lowers temperature, thereby affecting adult survival or larval development.[2,3] However, vegetation also provides shelter and nectar meals for vectors, and provides forage for potential blood hosts (in the case of zoophilic or generalist species), and as such can be an effective predictor of vector dispersion and disease transmission.[4] In this study, we focused on the question whether the species composition of the plant community, and in particular the presence of nectariferous plant species, should be considered as a component of the landscape that influences mosquito abundance and malaria transmission. If so, this is a factor that not only changes geographically and seasonally, but also with land development and agricultural practices.

The effects of environmental factors, or of vector control methods, on malaria transmission are best investigated through their effects on vectorial capacity,[5,6] a measure that encompasses the entomologic aspects of the basic reproductive rate of malaria (R_0).[7] Vectorial capacity describes the number of secondary infections caused by a population of mosquitoes per daily exposure to an infected host, and is, at its most basic, a function of mosquito density relative to humans, biting frequency, survival rate, and duration of the extrinsic cycle of the pathogen. Survival and biting rate are particularly important components because they affect vectorial capacity exponentially.

One aspect where concern about the accuracy of vectorial capacity has been expressed relates to the assumption of a constant mortality factor because analyses show that hazard functions of mosquito populations in nature and in laboratory settings are typically better described by age-dependent mortality functions.[8–10] These concerns would be minor if vectorial capacity in two environments were to be overestimated or underestimated to the same degree (i.e., a quantitative difference),[5] but they could be particularly serious if use of an exponential mortality function results in qualitatively different outcomes when environments or success of control measures are compared. One theoretical investigation so far suggests that this may be the case.[11]

Presence of sugar in the environment of the malaria vector *Anopheles gambiae* s.s. affects most of the components of vectorial capacity (Stone CM, Foster WA, unpublished data). For instance, biting rates are reported to be higher when sugar is absent,[12,13] whereas for survivorship the opposite is true.[12] Mosquito density or cohort size is affected by female fecundity[14] and by the proportion of females that are inseminated. In the absence of sugar, male reproductive performance may be affected to the extent that too few females can become inseminated to sustain a viable population.[15,16] Thus, sugar may affect vectorial capacity of *An. gambiae* in opposing directions, by simultaneously decreasing biting rates and increasing survival and density. However, although in laboratory cages these opposing factors tilt toward a greater vectorial capacity in the absence of sugar,[12] this will not necessarily be the case in nature. In the field, mosquitoes likely experience higher levels of mortality because of increased energetic expenditures incurred in host seeking and locating oviposition sites and mates, and to a higher background mortality caused by predation, host-defensive behavior, and physical factors. Furthermore, in nature, sugar will have to be obtained from a variety of sources. That these sources differ in quality is clear from experiments where access to different plants resulted in widely varying survival times of mosquitoes.[14,17,18] In addition, in one field study, populations of *An. sergentii* Theobald in two oases were reported to differ in vectorial capacity by a factor of 250, in favor of the oasis with two prominent sugar-bearing plants.[19] In a second study at the same site and in an adjacent month, vectorial capacities differed by a factor of 7,[20] nonetheless a large difference.

The questions we specifically wanted to answer, by using three-week cohort studies in mesocosms, were whether productive plant hosts enhance or depress vectorial capacity of

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An. gambiae, and by what means. To assess these questions, we investigated the effects of two simulated plant-species communities, differing in their nectar availability, on three components of this measure: biting rate, survivorship, and adult density. In the case of survivorship, we wished to determine whether mortality is best described by an exponential (i.e., constant) model or one of several, age-dependent functions, and whether mortalities in sugar-poor and sugar-rich environments are best described by the same or different functions. For biting rates, we wished to determine not only whether, but also how, they are affected. For instance, when sugar is readily available, young female *An. gambiae* may prefer to feed on sugar,[21] whereas when sugar is restricted and/or blood hosts are readily available even young females may seek a blood meal.[22,23] Thus, the age at which mosquitoes obtain their first blood meal may depend on environmental access to sugar. Consequently, vectorial capacity may differ qualitatively between environments with sugar-rich plants and sugar-poor plants. In the case of mosquito density, we investigated the effects of plant community on fecundity and rates of population increase. Thus, we measured the following female fitness parameters: daily fecundity, net replacement rate (R_o), and intrinsic rate of increase (r) in nectar-rich and nectar-poor environments. If mortality, biting rates, and reproductive fitness of mosquitoes differ between such environments, how do their combined effects determine vectorial capacity, and in which direction? The answer has implications for potential methods of malaria control that reduce vector population biting rate and age structure and limit or suppress population density.

MATERIALS AND METHODS

Mosquitoes (*An. gambiae* s.s., Mbita strain) were reared according to standard methods, as described,[16] with human blood to support egg production and a human host to study biting behavior (Institutional Review Board permit 2004H0193, International Building Code permit 2005R0020). Experiments were performed during March–June 2011 in mesocosms set up for this purpose in the clear-glass-enclosed portion of The Ohio State University Biological Sciences Greenhouse. An advantage over field mark-release-recapture studies is a lack of confounding emigration or immigration of mosquitoes, and better control over experimental factors such as blood host presence. A disadvantage is that certain mortality factors (e.g., predation, extreme weather) are absent, and the energetic costs associated with foraging and mating are probably still underestimated, although less so than in small laboratory cages.

The mesocosms (Figure 1) were customized insect cages manufactured by Megaview LLC (Salem, OR). A full description is available from the authors. In brief, the sides of the mesocosm, ceiling, and sleeves were made of white polyester netting (42 × 12 mesh/per cm^2), and the floor material was made of white vinyl. The dimensions of the cage were 5.66 × 4.87 × 3.00 meters (length × weight × height) for a total of 82.69 $meters^3$. Nine cylindrical sleeves were located in the ceiling to allow 500-W growing lights to be suspended inside of the cage. These lights were on between 9:00 AM and 5:00 PM to add to the natural ambient daylight entering the greenhouse and their mesocosms, but not to interfere with crepuscular light conditions. At 39°57′40″N during March–June, sunrise occurred between approximately 6:00 AM and 7:45 AM, and sunset occurred between approximately 6:30 PM and 9:00 PM. Temperature was controlled through the greenhouse heating and cooling system, and humidity was maintained with an ultrasonic humidifier. Recordings of temperature and humidity were taken with data loggers placed at the entrance to a resting pot.

To simulate environments with plant communities that comprised species that were either rich or poor nectar sources for mosquitoes, we selected plant species endemic to western Kenya of which survival and sugar intake by *An. gambiae* exposed to them had been studied.[14,17,18,24] Plants showing a high level of sugar output and extended mosquito survival in those studies were used in the sugar-rich environment. These plants were *Senna didymobotrya* (Fabaceae), *Ricinus communis* (Euphorbiaceae), and *Tecoma stans* (Bignoniaceae); *Senna occidentalis* (Fabaceae) was also included in this category because of prior observations of *An. gambiae* in the laboratory and the field feeding on its abundant and visible droplets of nectar (Jackson BT, Foster WA, Njiru B, unpublished data). Plants used in the sugar-poor environment were *Tithonia diversifolia* (Asteraceae), *Parthenium hysterophorus* (Asteraceae), *Lantana camara* (Verbenaceae), and *Datura stramonium* (Solanaceae). Although *P. hysterophorus* was reported to give a high level of fructose positivity in one study,[24] survival was

FIGURE 1. Interior of one mesocosm, showing resting pots, oviposition sites, lights, temperature and humidity sensors, a chair for the blood host, and nectariferous plants.

comparable to that of negative (water only) controls,[14] matching our own preliminary observations.

Seven plants were present in each mesocosm and watered daily throughout the experiments. Plant size was limited by the size of pots used; larger pots would have been difficult to move. In replicates 1 and 2, the sugar-rich environment consisted of three *S. didymobotrya*, two *R. communis*, and two *T. stans*. In replicates 3 and 4, one *R. communis* was replaced by one *S. occidentalis*. The sugar-poor environment consisted of two plants each of *T. diversifolia, P. hysterophorus,* and *L. camara,* and one *D. stramonium*, during replicates 1 and 2. During replicate 2, copious amounts of nectar were observed on the new growth of *T. diversifolia*, indicating that it is occasionally not a nectar-poor plant. For that reason, for replicates 3 and 4, two *T. diversifolia* were replaced with one extra *P. hysterophorus* and one extra *D. stramonium*. Biological control agents (i.e., *Neoseiulus californicus*, *Neoseiulus cucumeris*, and *Phytoseiulis persimilis*) were released in our plant stock room to help control pest populations.

Four empty terracotta pots (diameter = 36 cm) served as mosquito resting sites, the openings of which were covered with a thin sheet of plywood with a circular hole (diameter = 12.75 cm) in the middle. To prevent mosquitoes from resting and dying on the soil of potted plants, where their bodies were more likely to go unnoticed, the soil was covered from plant-pot rim to plant stem by white nylon fabric. Two clear plastic pans (59 × 39 × 17 cm [length × width × depth] of aged tap water served as oviposition sites and were always present on the floor of each mesocosm. A few leaves were strewn on the surface of the water to break its surface tension, and brown paper was placed under the oviposition sites to add contrast between the mesocosm floor and the containers.

At the start of each replicate, approximately 1,000 mixed-sex pupae were placed in each mesocosm. Nearly all emerged as adults by the morning of the next day, designated day 0. Any pupae remaining on day 0 (generally < 2%) were allowed an additional 24 hours to emerge. Survival of adult males and females was estimated by removing and counting dead bodies each morning from resting sites and from the white vinyl floor, rather than by aspirating and counting the survivors every day, to minimize their disturbance. A human blood host (C.M.S.) with feet and legs exposed was available for 30 minutes per mesocosm during the hour after sunrise, before the overhead lights came on, from day 1 onwards. The order in which the blood host was exposed in the two mesocosms was alternated each day. Biting rate was assessed by counting engorging females, and relating this number to the estimate of surviving females present on that day. Each morning the oviposition sites were inspected for eggs. Eggs were transferred to a round white filter paper, photographed with a Sony (Tokyo, Japan) digital camera with 50-mm macro lens mounted on a copy stand, and their number was estimated in ImageJ.[25] Each replicate was run for 21 days, after which all survivors were collected by backpack aspirator and counted.

To account for differences between the final number of surviving mosquitoes collected and the number released at the outset (minus the dead bodies collected and counted throughout the experiment), we assumed that a constant proportion of dead bodies went unnoticed (e.g., around the base of the plants) and multiplied the bodies counted by this factor. If subtracting the body counts over the duration of the experiment from the number of males or females released resulted in a negative number still living in the mesocosm, we assumed a slight deviation from a 1:1 sex ratio and adjusted the numbers of males and females released accordingly. Subsamples of surviving females of replicates 3 and 4 were dissected and their spermathecae were inspected by using a compound microscope for sperm to determine insemination status after cohabitation with males for 21 days in sugar-poor and sugar-rich mesocosms.

Survivorship of mosquitoes in sugar-poor and sugar-rich environments were analyzed by constructing Kaplan-Meier survivorship curves per replicate for males and females and testing for differences in survivorship using a Cox proportional-hazards analysis in R.[26,27] Mortality functions describing the distribution of ages at death[28,29] were fitted to the data and their parameters estimated by using the Survomatic package for R.[30] Differences in biting rates between environments were analyzed for each replicate separately by using generalized least squares in R, using treatment (sugar-rich and sugar-poor) and order (blood fed first or second on a given day because the timing affected light conditions and possibly biting response of females) as explanatory variables. A temporal auto-correlation structure was included in the regression model, which enabled one to differentiate a rich and poor mesocosm. The auto-correlation structure takes into account the fact that a measurement on a given day will be more closely correlated (positively or negatively) to a measurement on the next day than to a later or earlier measurement.[31] Biting rates were log-transformed and the models were validated by inspecting the residuals for homogeneity and normality.

Reproductive fitness of females in sugar-poor and sugar-rich environments was assessed by creating life tables for each replicate, which enabled calculation of net reproductive rate, R_0.[32] The intrinsic rate of increase, r, was calculated by taking the natural log of the dominant eigenvalue (i.e., λ) of corresponding Leslie matrices,[33] using Mathematica 7 (Wolfram, Champaign, IL).

To calculate the vectorial capacity, allowing for age-dependent mortality and biting rates, we used a formula similar to that for total population vectorial capacity.[10,34] We calculated in Mathematica the expected number of potentially infective bites by cohorts in either environment according to the equation

$$C = m\sum_{x=1}^{T-n}\left(\varepsilon_x\left(\prod_{i=1}^{x-1}\mu_i\varepsilon'_i\right)\left(\prod_{i=x}^{n+x-2}\mu_i\right)\sum_{i=n+x}^{T}\varepsilon_i\mu_{i-1}\right)$$

where m is the cohort size; T is the terminal time, i.e., the end point under consideration (day 21 in this study); n is the extrinsic incubation period of malaria; ε_x is the probability of biting at time x; ε' is $1-\varepsilon$; and μ_i is the probability of survival on a given day. Thus, we calculated and summed, over day x = 1 through $x = T - n$, the probability of not biting but surviving until day x, the probability of biting on day x and then surviving through the extrinsic incubation period, and the expected number of infective bites from a female taking her first infectious, i.e., gametocytemic, meal on day x.

The main assumptions are that all blood meals taken will infect females and that all bites after the extrinsic incubation period will be infective. Both assumptions are fundamental to vectorial capacity in its simplest form[6] and are violated in nature. Thus, the formula overestimates the number of infective bites arising directly from one infective human. However,

TABLE 1

Female mean age at death in plant sugar–rich and plant sugar–poor mesocosms, according to replicate, including a test to detect a difference in survival between treatments according to Cox proportional-hazards, and showing the mortality function that best describes the distribution of ages at death and the estimated parameter values of that function*

			Cox proportional hazard			Parameters		
Replicate	Sugar treatment	Mean age at death, days	z	*P*	Mortality function	λ	B or γ	c or s
1	Poor	10.8	–9.326	< 0.0001	Weibull	0.095	1.91	
	Rich	14.5			Weibull	0.0676	2.79	
2	Poor	13.6	11.7	0.00089	Weibull	0.074	1.858	
	Rich	11.7			Weibull	0.084	2.02	
3	Poor	9.46	–18.14	< 0.0001	Weibull	0.11	1.2	
	Rich	33.8			Gompertz-Makeham	5.5×10^6	0.50	0.015
4	Poor	10.6	–4.255	< 0.0001	Logistic	0.123	0.15	1.7
	Rich	14.0			Gompertz	0.026	0.11	

this should not affect a qualitative comparison between environments unless vector competence of mosquitoes in sugar-poor and sugar-rich environments differs. It has been suggested that sugar feeding by *An. gambiae* does not play a major role in the immune response to infection with *Plasmodium falciparum*,[35] but for other mosquito-parasite systems this may differ. Wilcoxon and *t*-tests were used to detect significant differences in fitness parameters and vectorial capacities between sugar-rich and sugar-poor environments.

RESULTS

Survival of female *An. gambiae* in three of four replicates was significantly greater in mesocosms with sugar-rich plants than in mesocosms with sugar-poor plants (Table 1), despite females having similar access to a blood host each day. The same pattern was found for male mosquitoes (Table 2), although here the difference in survival was greater between treatments than for females (Figures 2 and 3). A series of semi-hierarchical mortality functions (exponential, Weibull, Gompertz, Gompertz-Makeham, Logistic, and Logistic-Makeham) was fitted against observed mortality patterns. In none of the replicates for either sex, was an exponential mortality function the best model (i.e., the model with the lowest Akaike Information Criterion value).

Best models and the estimated parameter values are shown in Tables 1 and 2, and the corresponding survivorship functions[29] are plotted against the survivorship values in Figures 2 and 3. Females in poor environments displayed mortality patterns best described by Weibull distributions in three of four replicates. In the fourth replicate, a logistic function gave the best fit, but the Gompertz and Weibull models had Akaike Information Criterion differences (δ_i) of only 1, suggesting that all three models should be considered.[36] In the sugar-rich environments of replicates 1 and 2, mortality was best described by a Weibull function. Replicates 3 and 4 were better described by Gompertz-Makeham and Gompertz functions, respectively. Male survival patterns were comparable to those of females, to the extent that in sugar-poor environments Weibull distributions gave the best fits, whereas in the sugar-rich mesocosms, versions of the Gompertz function gave the best fit to male data from three of four replicates in the sugar-rich mesocosms.

The mean ± SD biting rate over all days, all four replicates combined, for sugar-poor mesocosms was 0.198 ± 0.08 bites per day per female mosquito; in sugar-rich mesocosms this value was 0.142 ± 0.07 bites per female per day (Figure 4). A comparison of Figure 4A and Figure 4B indicated a more pronounced difference in biting rates between treatments when sugar-poor mesocosms did not include *T. diversifolia* (Figure 4B), in particular during the first two days of the experiment. Treatment had a significant effect on biting rate in replicate 3 ($t = 5.3$, $P < 0.001$) and replicate 4 ($t = 3.6$, $P = 0.001$), but not in the replicates that included *T. diversifolia*. Blood feeding a mesocosm either first or second had a significant effect on biting rate in all replicates except replicate 4 ($t = 1.8$, $P = 0.08$).

To test for differences between the slopes of regression lines fitted to sugar-poor and sugar-rich mesocosms, an analysis of covariance was performed, which took a significant interaction factor between days and treatment as an indication of differing slopes. In replicates 1 and 2, in which the sugar-poor treatment included *T. diversifolia*, there was no difference between the slopes of the two treatments ($t = 0.39$,

TABLE 2

Male mean age at death in plant sugar–rich and plant sugar–poor mesocosms, according to replicate, including a test to detect a difference in survival between treatments according to Cox proportional-hazards, and showing the mortality function that best describes the distribution of ages at death and the estimated parameter values of that function

			Cox proportional hazard			Parameters		
Replicate	Sugar treatment	Mean age at death, days	z	*P*	Mortality function	λ	B or γ	c or s
1	Poor	6.07	–23.83	< 0.0001	Weibull	0.16	1.87	
	Rich	20.8			Gompertz-Makeham	0.00065	0.27	0.02
2	Poor	21.4	6.222	< 0.0001	Weibull	0.045	1.566	
	Rich	15.8			Weibull	0.063	1.96	
3	Poor	3.88	–26.02	< 0.0001	Weibull	0.26	1.7	
	Rich	36.7			Gompertz	8.3×10^3	0.068	
4	Poor	4.07	–17.55	< 0.0001	Weibull	0.24	1.282	
	Rich	12.14			Gompertz-Makeham	1.1×10^5	0.5	0.074

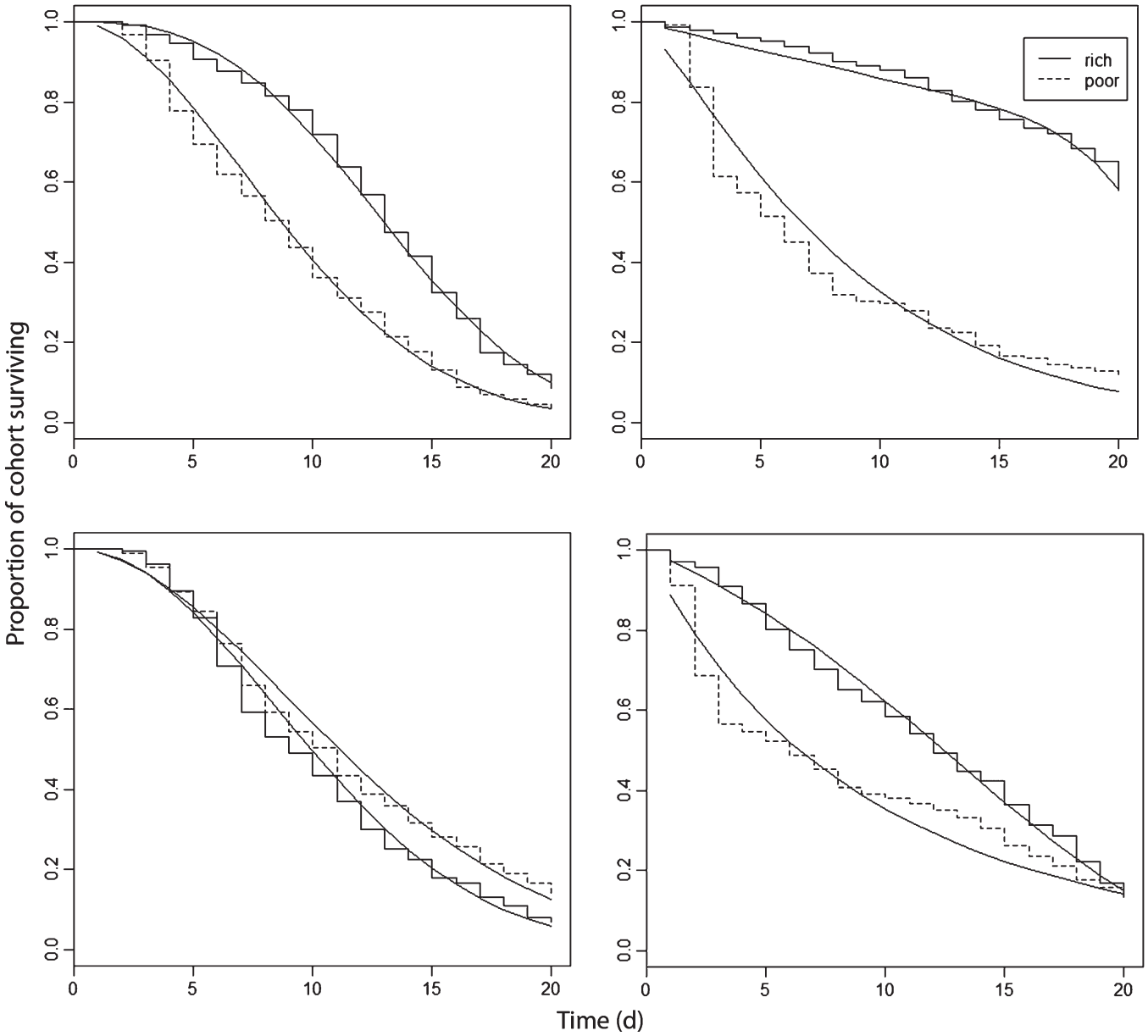

FIGURE 2. Female Kaplan-Meier survivorship curves in plant sugar–poor and plant sugar–rich mesocosms, per replicate (left, upper: 1; left, lower: 2, right, upper: 3; right, lower: 4), and fitted lines based on associated estimated survivorship functions (see Table 1). d = days.

$P = 0.69$). In both treatments of those replicates, the biting rates increased slightly, but not significantly, with time. In replicates 3 and 4, the biting rate of females in the sugar-poor treatment decreased, but not significantly ($t = 0.89$, $P = 0.38$), whereas the biting rate in the sugar-rich room tended to increase with time ($t = 2.01$, $P = 0.051$). Among those two replicates there was a marginally significant difference in slopes ($t = 1.86$, $P = 0.066$).

Values of fitness parameters for females in both environments are shown in Table 3. There were no significant differences in mean daily fecundity between treatments over four replicates. In replicate 3, mean daily fecundity was remarkably low, particularly in the sugar-poor environment. The mean net reproductive rate and the intrinsic rate of increase were higher (not significant) in the sugar-rich mesocosms. In samples of surviving females from replicate 3, 56 (98.2%) of 57 from the sugar-rich mesocosm had been inseminated, but only 2 (3.6%) of 55 from the sugar-poor treatment had been inseminated. In replicate 4, these figures were 53 (98.1%) of 54 and 51 (86.4%) of 59, respectively.

Mean ± SD daily temperatures over all replicates at floor level were 25.42 ± 1.57°C, and 25.3 ± 1.63°C in the sugar-poor and sugar-rich mesocosms, respectively ($t = 0.46$, $P = 0.64$). Mean ± SD daily minimum and daily maximum temperatures were 22.6 ± 1.1°C and 30.6 ± 2.6°C, and 22.5 ± 1.4°C and 30.2 ± 2.5°C in the sugar-poor and sugar-rich mesocosms, respectively. Mean ± SD daily relative humidities at floor level were 59.5 ± 5.33% and 60.63 ± 4.61% in the sugar-poor and sugar-rich mesocosms, respectively ($t = 1.46$, $P = 0.15$). Mean ± SD daily minimum and daily maximum relative humidities were 38.9 ± 9.1% and 68.9 ± 6.9%, and 41.7 ± 10.4% and 70.7 ± 3.8%, respectively.

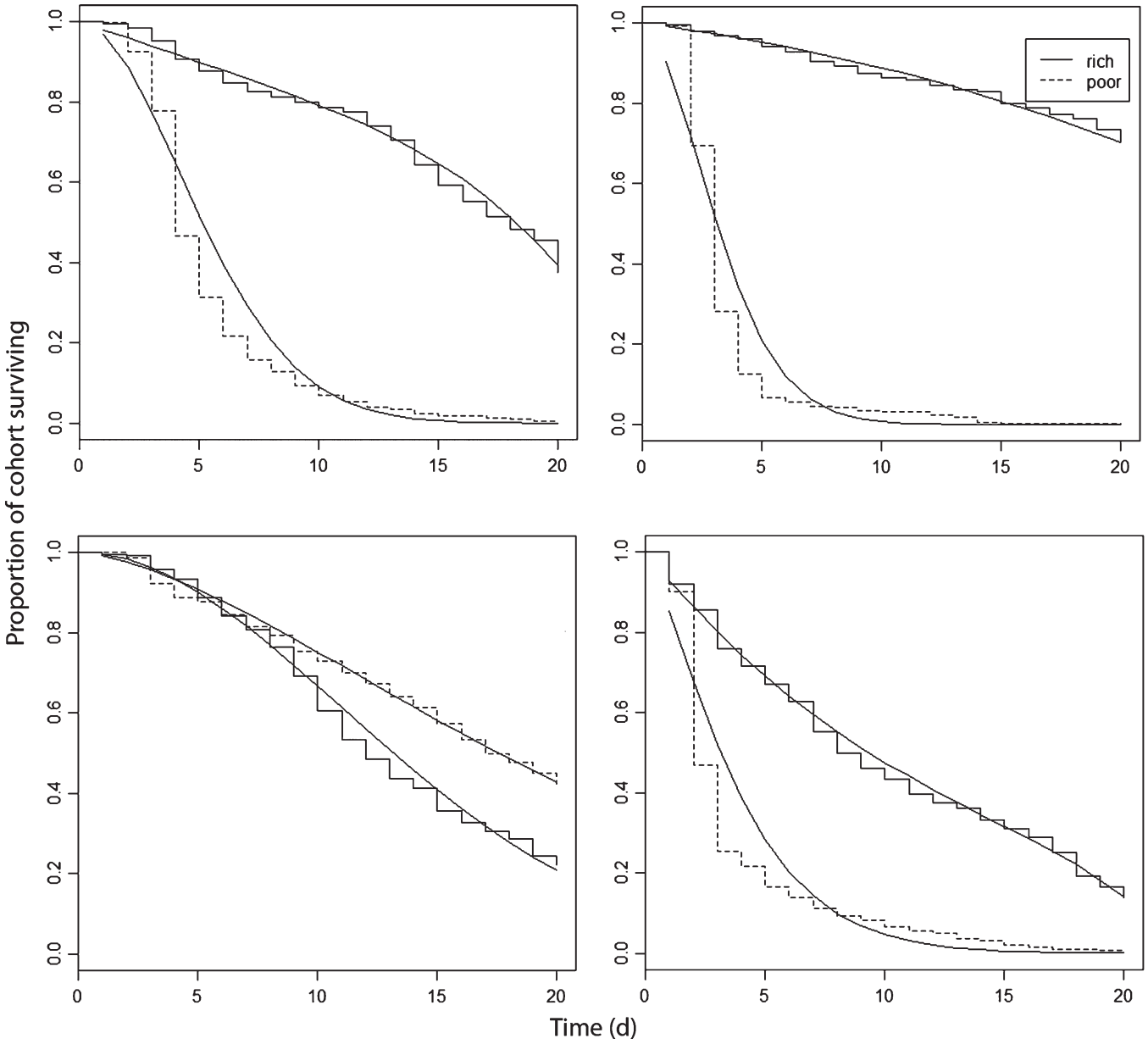

FIGURE 3. Male Kaplan-Meier survivorship curves in plant sugar–poor and plant sugar–rich mesocosms, per replicate (left, upper: 1; left, lower: 2, right, upper: 3; right, lower: 4), and fitted lines based on associated estimated survivorship functions (see Table 2). d = days.

For vectorial capacity calculations, we used the mean temperature (25.36°C) recorded during the experiments, which led to an extrinsic incubation period for *P. falciparum* of 11.96 days,[37] which was rounded up to 12 days, The outcomes representing the potential number of infectious bites stemming from one cohort of 500 females are shown in Table 4. The calculations of age-dependent vectorial capacity for each replicate used the daily survival probabilities (p_x) from the life tables of that cohort and the daily biting rates (i.e., the actual number of bites counted on a given day, divided by the estimated number of female mosquitoes present). Vectorial capacity also was calculated with constant values (i.e., the mean p_x and biting rate). On average, the age-dependent vectorial capacity of cohorts in sugar-poor environments was higher by 25% than those in sugar-rich environments, although because of wide variation in outcomes among replicates, the difference was not statistically significant (t = 0.6, P = 0.56). By either method (constant or age-dependent), in two of four replicates (2 and 4) the treatment giving a substantially higher vectorial capacity was the same; in replicate 3, the difference between treatments was small. Only in replicate 1 was vectorial capacity much higher in the sugar-rich treatment (Table 4).

Use of constant measures of survival and biting gave a vectorial capacity for sugar-poor mesocosms roughly similar to that with age-dependent measures, but underestimated the vectorial capacity in the sugar-rich treatments. Therefore, as a comparative measure, the constant values disagreed with the age-dependent values because the cohorts in

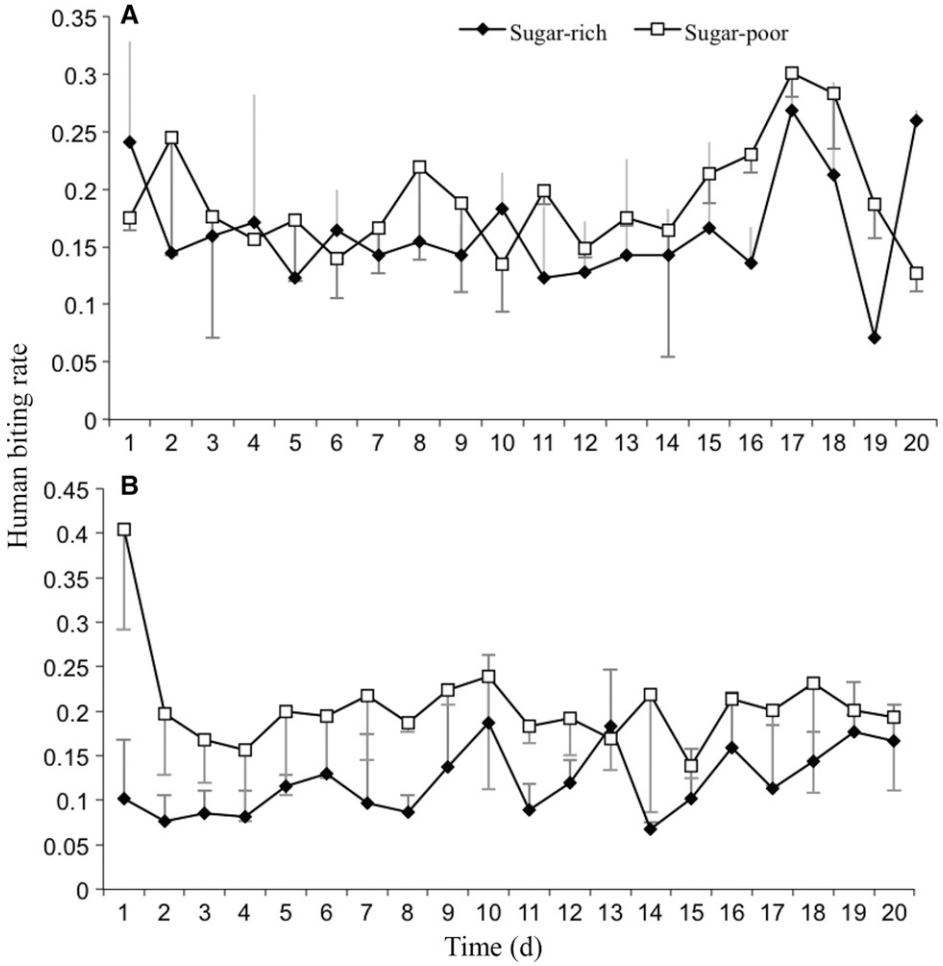

FIGURE 4. **A**, Mean biting rate per female per day for replicates 1 and 2, when the sugar-poor mesocosm included *Tithonia diversifolia*. **B**, Mean biting rate per female per day for replicates 3 and 4, when *T. diversifolia* was not used as a sugar-poor plant. d = days.

sugar-poor mesocosms were estimated by constant values to have vectorial capacities 46% greater than in sugar-rich mesocosms. Perhaps most notable of these results is the variation in vectorial capacity outcomes. The constant survival and biting rates Figure 5 suggest that this variation is particularly high in the sugar-poor environments, possibly the result of vulnerability of biting rates to minor differences in sugar supply when it is scarce.

TABLE 3

Measures of reproductive success of *Anopheles gambiae* in plant sugar–poor and plant sugar–rich mesocosms

Treatment	Replicate	Mean daily fecundity (M_x)	Net reproductive rate (R_o)	Rate of increase (r)
Sugar poor	1	25.50	118.42	0.57
	2	18.86	151.15	0.64
	3	3.84	21.93	0.41
	4	26.6	150.89	0.55
	Mean	18.7	110.6	0.54
Sugar rich	1	18.12	167.87	0.56
	2	22.08	135.44	0.71
	3	8.38	131.84	0.5
	4	18.4	139.32	0.56
	Mean	16.75	143.62	0.58
	z	0.72	0.43	0.14
	P	0.47	0.66	0.88

DISCUSSION

Plant community composition clearly affected survival and biting rate of *An. gambiae*, which are two major components of vectorial capacity. Increased accessibility to plant sugar increased survivorship of male and female mosquitoes, whereas it depressed the biting rate of females. These opposing effects of sugar on vectorial capacity have been documented in cage studies.[12] These effects resulted in a potential number of infectious bites that was on average, but not significantly, 25% higher in environments with poor sugar-hosts, despite the reduced survivorship. The variation in vectorial capacity highlights that the effects of plants on

TABLE 4

Vectorial capacity of cohorts in sugar-poor and sugar-rich environments, calculated either with age-dependent or age-constant (i.e., mean) biting rates and mortalities

	Age-dependent			Constant		
Replicate	Poor	Rich	Poor:Rich	Poor	Rich	Poor:Rich
1	72.6	129.1	0.56	55.3	86.8	0.64
2	138.8	88.1	1.57	135.9	66.9	2.02
3	68.5	77.4	0.88	97.5	86.7	1.12
4	215.9	109.9	1.96	200.6	97.6	2.06
Mean	123.9	101.1	1.25	122.3	84.5	1.46

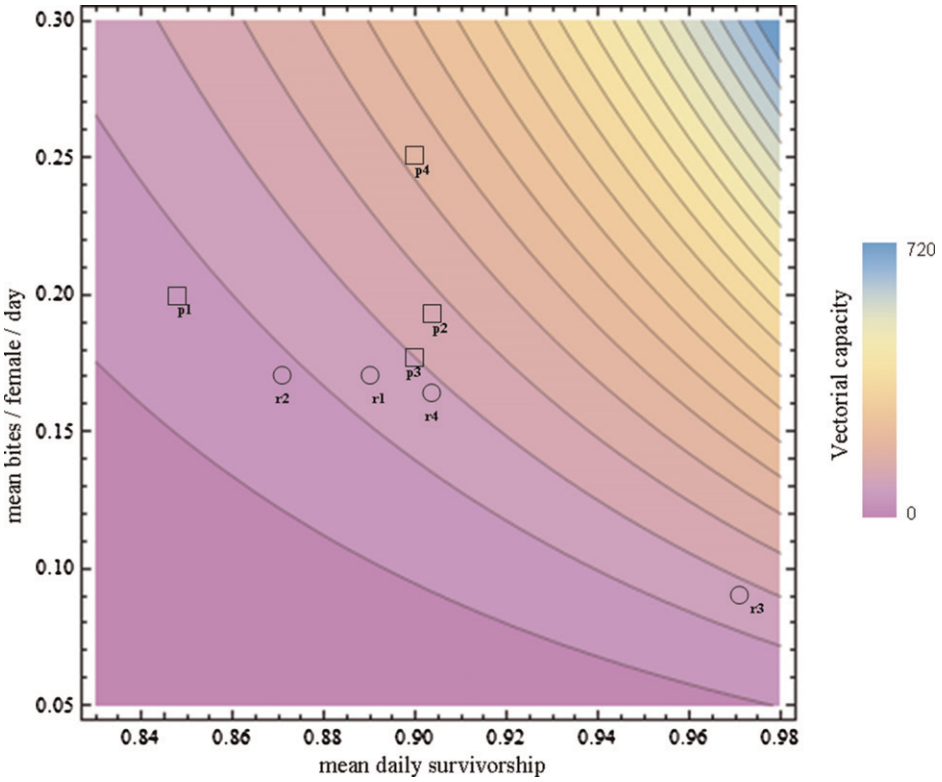

FIGURE 5. Values for mean, i.e., constant, daily survival rates (p_x) and biting rates (bites per female per day), in the four replicates of sugar-poor and sugar-rich treatments, plotted onto vectorial-capacity isolines with increasing values toward the upper right. r1-4 and p1-4 represent the values of replicates 1-4 in rich (r) or poor (p) mesocosms, respectively.

biting, survival, and the insemination rate of females, and the interactions between them are not straightforward. These issues are discussed further below.

Although it is evident that the plant community can affect the age distribution of a mosquito population, less clear from these results is whether this finding will have any effect on the growth rate of a mosquito population. If it does, it appears that it would not be caused by the reliance of the female mosquito on sugar, but rather to a severe hampering of male reproductive performance when sugar is rare or absent, as suggested by prior experiments.[15,16] Such a reduction in male mating ability occurred in the sugar-poor treatment only in replicate 3, the probable cause of its low egg production (Table 3), because uninseminated females rarely lay eggs. In that instance, only 3.6% of surviving females were inseminated and their fitness measures were correspondingly depressed.

It is surprising that minute differences in mean age of males at death (e.g., 3.88 days and 4.07 days in replicates 3 and 4, respectively) could result in such dramatic differences in female insemination rates. It raises the question whether male mating performance responds in a binary manner to a threshold of environmental sugar, perhaps by sustaining a few highly fit males, instead of a linear function of male mortality. Given our lack of knowledge of mosquito plant-foraging behavior, it is difficult to extrapolate these results to a natural situation. However, on the basis of similarities in reproductive rates in sugar-rich and sugar-poor environments in three of the replicates, we may assume that mosquito populations can be sustained even if only sugar-poor plant hosts are present. This assumption is valid only if they nevertheless provide sufficient nectar to fuel male mating activity, not compromising the efficiency with which males and females find each other in the field.[38] Further work may show how male-mating performance depends on the sugar concentration and accessibility of different plant host species. For example, if the tissues of some species can be pierced,[39,40] will their phloem sap be sufficient to sustain male mating activity?

With the exception of replicate 3, in which insemination and egg production were correspondingly quite low in the sugar-poor environment, fitness parameters were equivalent or slightly higher when nectar was readily available. In this regard, *An. gambiae* differs from *Aedes aegypti* L., another anthropophilic mosquito also reported to achieve higher reproductive success when it does not feed on sugar sources.[41–43] For *Ae. aegypti* L., results are not unequivocal, and spatial constraints may matter. For *An. gambiae*, the environment does matter because in laboratory cages, fitness (R_0 and r) was reported to be slightly[12] or substantially[44] higher for sugar-deprived females. One can conclude that in more realistic settings the reproductive value of sugar to females is greater. Whether this can be extrapolated to even more energetically demanding field environments remains to be investigated. It may explain why sugar feeding is retained by this mosquito species.

Given comparable climatic conditions, the differences in male survival between replicates are a reasonable indication of variation in nectar production or state of the plants, leading to, for instance, the higher survival rate in the third replicate in the sugar-rich mesocosms. *A priori*, we applied sugar-poor and sugar-rich labels to the different plant species used in these experiments on the basis of the results of Manda and others.[14,24] However, variation in survival between replicates

suggests that this division is too simplistic because sugar-poor and sugar-rich plants apparently provide at times sufficient nutrients for *An. gambiae* males and females. This variability likely depends on plant age and condition. For instance, *T. diversifolia*, a putative nectar-poor plant, produced copious amounts of nectar during replicate 2, necessitating its removal from the sugar-poor environment in subsequent replicates. To come to a better understanding of the nutritive value of certain plant species for *An. gambiae*, it will be necessary to assess their range of nectar production.

When we compared the final two sugar-poor replicates, we noticed that despite comparable mortality, vectorial capacity was drastically different, resulting from differences in biting rates. Although in both replicates biting peaked on day 1, a phenomenon we have observed in sugar-deficient habitats[22] caused by opportunistic blood feeding after emergence if acceptable sugar sources are absent, the biting rate remained stable after that in replicate 4, but showed a decrease in replicate 3. This finding can be ascribed to the marked difference in insemination rates between the two replicates. In the third replicate, 86.8% of surviving unmated females collected at the end of the experiment retained Christopher's stage V eggs, i.e., were gravid and unable to make more eggs, likely causing the decrease in biting rate with age by limiting the capacity of the gut for blood and the need for additional protein. Conversely, the outcome of replicate 4, in which sugar had opposite effects on biting rate and survival, is consistent with results of two laboratory cage studies, which compared a diet of blood only with a diet of blood plus a 10% sucrose solution.[12,13] The results are in stark contrast to those of two field studies conducted with *An. sergentii*, in which vectorial capacity was reported to be many times higher in a sugar-rich oasis than in a sugar-deficient oasis,[19,20] a result of higher biting rate and greater survival in the sugar-rich oasis. Whether this finding was caused by differences in the biology of the two species, or a reflection of the difference between the field and confined environments, is not known.

The utility of vectorial capacity when mortality rates are inconstant, as in nature, has been questioned. The main contribution of this study is to show that different environments, if they differ in plant species composition and abundance, may also differ in the pattern of age-dependent mortality of the mosquitoes. A consequence of this contribution is that one can no longer assume that constant mortality values will provide qualitatively sensible values when comparing the vectorial capacity in two regions. Complicating matters further is that the age at which mosquitoes first bite matters when mortality is age dependent.[9,10] The biting peak we observed on day 1 in sugar-poor, but not in sugar-rich mesocosms, suggests that availability of sugar sources also may influence the age at which females first obtain blood. Biting rates found in this study resulted in gonotrophic cycles considerably longer than the 2–3-day cycle traditionally associated with *An. gambiae*.[45] The most likely reason is the limited window of time per day in which a blood host was present. Therefore, extrapolation of results to the field should be undertaken with great care, and these results may be most applicable to situations where host accessibility is similarly restricted, e.g., areas with high bed net coverage. Further studies on the occurrence and timing of a decrease or increase in biting rates as mosquitoes age would justify use of the more elaborate vectorial-capacity formula we used in this report. Some studies support this suggestion. In these situations, the oldest age cohort of sugar-deprived females, given the opportunity to mate before withdrawal of sugar, had increased biting rates.[9,10] An increase in biting activity also has been observed in *Plasmodium*-infected mosquitoes,[46] which typically coincides with increased age.[47]

Further studies clarifying the impact of environmental sugar on mosquito behavior are warranted. Ideally, these would incorporate wild-type anophelines, use various blood hosts at different levels of accessibility, and be performed under semi-field conditions. At this point, we conclude that environments differing in composition of nectar-producing plants will have vector populations with different biting behaviors and age distributions, and therefore different vectorial capacities. In consequence, this aspect may be worth incorporating in epidemiologic models.

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