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Patterns of sugar feeding and host plant preferences in adult males of *An. gambiae* (Diptera: Culicidae)

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ABSTRACT: Sugar feeding by male mosquitoes is critical for their success in mating competition. However, the facets of sugar source finding under natural conditions remain unknown. Here, evidence obtained in Western Burkina Faso indicated that the distribution of *An. gambiae* s.s. (M and S molecular forms) males across different peri-domestic habitats is dependent on the availability of potential sugar sources from which they obtain more favorable sites for feeding or resting. Among field-collected anophelines, a higher proportion of specimens containing fructose were found on flowering *Mangifera indica* (Anacardiaceae), *Dolonix regia* (Fabaceae), *Thevetia neriifolia* (Apocynaceae), *Senna siamea*, and *Cassia sieberiana* (both Fabaceae) compared to that recorded on other nearby plants, suggesting that some plants are favored for use as a sugar source over others. Y-tube olfactometer assays with newly-emerged *An. gambiae* s.s. exposed to odors from individual plants and some combinations thereof showed that males use odor cues to guide their preference. The number of sugar-positive males was variable in a no-choice cage assay, consistent with the olfactory response patterns towards corresponding odor stimuli. These experiments provide the first evidence both in field and laboratory conditions for previously unstudied interactions between males of *An. gambiae* and natural sugar sources. **Journal of Vector Ecology 35 (2): 267-276. 2010.**

Keyword Index: *Anopheles gambiae*, males, sugar-feeding, flowering plants, olfactometer, Burkina Faso.

INTRODUCTION

An insect's chances for survival and reproduction depend on its nutrition. Evolutionary fitness depends significantly upon an optimal diet in both quantity and quality (House 1961, Nayar and Sauerman 1975a, 1975b). During the first days after emergence, mosquitoes of both sexes almost exclusively feed on plant-derived sugary fluids including sap, nectar, and honeydew in order to supplement their energy reserves and to sustain life (Foster 1995, Foster and Takken 2004, Clements 1992, Nielsen and Greve 1950, Hocking 1953, Haeger 1955). Their feeding behavior is influenced by several factors. These include the physiological state of the individual dictating what is needed at a particular time and their ability to respond directly to a multiplicity of signals indicating resource availability. These factors strongly regulate how insects feed and how they are distributed (Ma and Roitberg 2008, Takken 2005, Becerra and Venable 1999, Costantini et al. 1996). Attractive diets for female *An. gambiae* and related species are amino acid- and protein-rich foods, typically the blood of vertebrates, which are necessary for egg development (Uchida et al. 2003, Harrington et al. 2001). They may also take plant-derived sugar meals, but both blood and sugar meals are used to fulfill different requirements for females. Males, on the other hand, strictly prefer sugar-rich floral nectars. These non-blood-feeding males play an often ignored, but essential, role in female reproduction. Indeed, energy gain

from sugar intake in the field is necessary to maintain male swarming and therefore female insemination (Clements 1955, Nayar and Van Handel 1971). Although confirmed to be of plant origin (Foster 1995, Gary and Foster 2004), the actual sources of sugary fluids that *An. gambiae* feed on are still unknown.

It is questionable to conclude that male *Anopheles* spp. feed on plant-derived sugar while only limited experimental evidence is available to connect this basic behavior to specific plant species and their phenology. It is standard practice to provide colonies of *Anopheles* spp. with a variety of sugar solutions including sucrose or glucose from the day of emergence onwards in an attempt to mimic natural sugars and to make mosquitoes thrive in insectaries (Benedict 1997, Briegel and Kaiser 1973, Gillett et al. 1962). However, the range of sugary substances that mosquitoes utilize under natural conditions is likely different than those used in husbandry protocols. The habitats where *An. gambiae* thrive are characterized by an immense diversity of flowering plants that *a priori* can serve as sugar sources. It is not likely that they acquire sugar meals on all flowering plants that exist in their environment, although this seems plausible (Müller and Schlein 2005). Reports on sugar feeding by males in nature are scanty. Except for a few field observations of sugar feeding in female *Anopheles gambiae* (Impoinvil et al. 2004, Manda et al. 2007), surprisingly, no work has been carried out for the last 30 years to document feeding conditions (feeding habits and requirements) and

food preference of anopheline males.

Finding a suitable sugar meal is likely one of the most critical challenges that males face under natural conditions. Plants display a multitude of different properties that characterize them, including not only hundreds of different chemicals (Raguso 2004), but also other factors such as their form, color, and texture (Dethier 1982). Sugar source finding in *An. gambiae* depends not only on these characters but may also rely on behavioral traits. Here, we aimed to investigate the sugar feeding behavior of wild adult males of *An. gambiae* in the Kou Valley, a rice growing area situated in Western Burkina Faso, where marked heterogeneity exists in the distribution of flowering plants in the landscape (Robert et al. 1986). A further aim was to evaluate the roles of plant-related odor stimuli for *An. gambiae* adult males in discriminating between different potential sugar sources in classical Y-shaped olfactometer bioassays (Lefèvre et al. 2009). In addition, the number of fructose-positive mosquitoes after simple exposure of males to candidate plants were measured and their association with direct observation of the response to the plants examined. Identification of suitable natural sources of sugar for *An. gambiae* males is of special importance towards an understanding of how important the ecology of males is in mosquito populations (Ferguson et al. 2005) prior to implementing any future control strategy aiming to replace or suppress wild malaria vectors.

MATERIALS AND METHODS

Field surveys were carried out in the Kou Valley (11°24'N; 04°24'W), a rice growing area situated about 30 km northwest of Bobo-Dioulasso, a Sudanian ecozone in Western Burkina Faso. The Kou Valley site contains seven discrete villages covering 1,200 ha surrounded by wooded savannah. Within each village, a marked heterogeneity exists in the distribution patterns and composition of flowering and non-flowering plants both close to, and hundreds of meters from, human habitations. The entomological indices and the malaria situation in the study area have been described previously (Baldet et al. 2003, Diabaté et al. 2005). Both molecular forms of *An. gambiae* s.s. often occur together but show temporal separation, with the M form being relatively abundant throughout the whole year, while the S form is most prevalent in the rainy season.

Field observational surveys of plant-sugar feeding in male *An. gambiae*

The aim of the preliminary field surveys was to determine the local distribution of male *Anopheles* species based on sampling from different trees that bore flowers around human dwellings. For collection of data on male sugar feeding in general, samples of indoor resting male mosquitoes and those found in various outdoor resting sites (abandoned houses, beneath the grass) were collected by manual aspirators. In parallel, adult male mosquitoes were also collected directly from within clay pots suspended on flowering plants amongst the most common plants found

within peridomestic areas. A total of 15 to 20 clay pots (15 cm in diameter by 30 cm depth) at a time, were suspended at 1-2 m above ground level in a shaded area on the plants. A lid was placed at the top of each pot leaving a hole approximately 5 cm in diameter to keep the pot partially closed to avoid over-exposure to the sun. No bait was used to lure mosquitoes into the pots. Setting up these clay pots and measuring the density of males on available host trees was found to be the best compromise between a laboratory assay of randomly selected plants (Impoinvil et al. 2004, Manda et al. 2007) and impractical field observations to assess events of sugar feeding behavior in wild populations of anopheline males.

Our investigations occurred through February to May 2008, in the off-season of the predominant species of flowering plants. Furthermore, the number of flowering plants available was limited and when they flowered, they only did so for a short period. Thus, at three-day intervals for four months, usually soon after sunrise, all mosquitoes found within each clay pot suspended on individual flowering plants and in those placed on the ground beneath the grass were removed with a manual aspirator, counted, and immediately killed to halt sugar digestion. These were transferred to an ice box for transportation to the laboratory where they were sorted to species using morphological identification criteria. Species and molecular forms within the *An. gambiae* complex were determined by PCR-RFLP (Scott et al. 1993, Favia et al. 2001). The presence of nectar in the crop was demonstrated qualitatively by its reaction with the anthrone reagent (Van Handel 1972). Procedures for processing and detecting sugar fed mosquitoes were similar to those described previously (Manda et al. 2007). In brief, field-collected mosquitoes were crushed individually in tubes containing 0.5 ml of anthrone reagent and incubated at room temperature for 30 min. A change of the yellowish color to green or dark blue after incubation was indicative of the presence of undigested plant sugars. The amount of fructose present was not quantified for practical reasons, mainly because the amount of sugar in field mosquitoes is generally so little that treating an individual mosquito would underestimate nectar content (Van Handel 1972). The average number of *An. gambiae* s.l. collected on different flowering trees or resting sites as well as proportion of sugar fed individuals were estimated. Although this analysis could provide evidence that the males found within clay pots have fed on plant sugar, it did not allow a determination of whether they had taken sugar directly from the plants on which the clay pots were suspended. Therefore, subsequent laboratory bioassays were set up to make the distinction between the use of plants as a place to rest or a place to feed.

Selection of plant candidates for laboratory bioassays

Based on the number of males directly collected within clay pots suspended on flowering plants that tested positive in cold anthrone tests, plant species that clearly attracted more male *An. gambiae* than other common trees were selected. These were subsequently tested in the experimental assays to determine if *An. gambiae* is selective

in its feeding behavior and whether it uses odor to regulate its preference for sugar source. Cuttings with inflorescence were taken to the Department of Botany, *Université Polytechnique de Bobo-Dioulasso*, for identification. Other plant samples were used as odor sources in an attempt to monitor the behavioral response of male *An. gambiae* in the olfactometer bioassays. We used live plant tissues (stem with inflorescences), instead of pure extracts or single chemicals, to provide evidence that male *An. gambiae* are attracted to odors of natural sugar sources.

Experimental mosquitoes

Two-day-old male mosquitoes collected as larvae from productive anopheline breeding sites in the study area were used. When required, mosquito larvae were sampled from known *An. gambiae* aquatic habitats using a standard dipping technique. These were transported to the laboratory at the *Institut de Recherche en Science de la Santé* and reared to adulthood under ambient conditions. Developing larvae were fed with Tetramin[®] until all the larvae pupated. Pupae were transferred to holding cages (30x30x30 cm) for emergence. Males were sorted and placed in individual holding cups under ambient conditions at the insectary. They were maintained only on cotton pads soaked with distilled water (i.e., they had no access to sugar) and then assayed when they were two days old. Access to water was discontinued six hours prior to olfactometer assays. Using reared insects offers the advantage that physiological factors such as age and hunger state can be controlled, and starving them prior to the bioassay improved the likelihood of obtaining a successful response.

Y-shaped olfactometer

As a corollary to field observations, we developed a dual-choice olfactometer bioassay, as described by Lefèvre et al. (2009), to establish the degree of attractiveness for *An. gambiae* males towards semiochemicals from potential plant sugar sources. The device consisted of a glass construction (1.2 m in width, 2 m in length, and 45 cm in height) with one releasing cage (30 x 30 x 30 cm) connected centrally to the odor plume downwind from the odor sources, at 50 cm from the fork joint. Both arms (10 x 10 cm) were connected to left and right trapping /collecting boxes (30 x 30 x 30 cm) in its top half. These two trapping boxes were also connected via polythene tubing to two odor chambers (each 90 x 90 x 90 cm) located outside of the experimental room. Each external upwind odor chamber, containing plant materials, was equipped with a small fan producing airflow at 24 cm/s. Plant odors were commonly applied by cutting the branches and placing them inside the upwind boxes. For the control, the odor chamber was left empty, so that outside air could be drawn into the olfactometer. The symmetry of the apparatus has been tested in previous trials without any odor source in either port of the olfactometer (Lefèvre et al. 2009).

Olfactometer bioassays

First, precise grading of male responses to each of the

selected plants was compared to an ambient air stream (no odor). The behavioral response tested for was an increase in upwind flight towards individual plants in the wind tunnel compared with the level of upwind flight in their absence. Each plant was tested three times per day and on four different experimental days (12 replicates per plant) using different batches of newly-emerged males and freshly cut plant parts with flowers from the field. Second, different pairs of selected plants were successively evaluated in a two-choice assay using newly-emerged males. Each plant combination was assayed at two different positions in the olfactometer on every experimental day, and on three different days (six replicates per pair of plants) using different mosquito samples and new plant parts.

Just before the start of scotophase, two-day-old *An. gambiae* males that had not received a sugar meal were randomly drawn from the same larval cohort and put in paper cups and transferred to the olfactometer room. Test mosquitoes (20 males per trial) were released into the downwind box and allowed to acclimate for 30 min. They were prevented from flying towards the odor source before the beginning of the assay with the escape slide covered with a screen. The tests were carried out in a room at ambient temperatures (ranging from 27.4° C to 32.6° C) and humidity (61% to 70%) over the experimental period (from February to May 2008). On each experiment day, fresh plant materials (with flowers still attached to the stem) collected from the field were placed individually in the upwind odor chamber, care being taken by the operator to wear surgical gloves during the process. The observation time of upwind flight choices of males was 30 min after the introduction of the plant materials. This was the time chosen for observations in all assays because the response of mosquitoes in a preliminary trial did not increase after a 30 min exposure. Both in the single plant situation and with two competing stimuli, two different behavioral responses were scored:

Activation, which corresponds to the initiation of flight from the releasing cage and orientation in flight toward the Y-shaped tunnel.

Attraction, which corresponds to the proportion of those attracted and/or that follow the upwind plume to the odor source in one of the two arms of the olfactometer.

These were estimated using the following formula:

N = Number of male mosquitoes released

X_A = Number of male mosquitoes recaptured in cage A (stimulus A)

X_B = Number of male mosquitoes recaptured in cage B (stimulus B)

Thus, Activation = $(X_A + X_B) / N$ and Attraction = $X_A / (X_A + X_B)$.

Males that responded (activation) or that were attracted to either odor plume flowing from respective arms with plants were counted at the end of the observation time. Those not activated within 10 min were also counted but were excluded from the analysis. To remove any odor from previous trials and to minimize experimental artifacts, all parts of the olfactometer were cleaned with 75% alcohol

between test series. In addition, to counteract the effect of any biased directional response of unknown origin, the positions of the plants and controls were alternated between ports in each replicate trial. After every test, mosquitoes were collected from the olfactometer using a mouth aspirator. They were put into cups, killed with chloroform, and identified to species and molecular form by PCR-RFLP (Scott et al. 1993, Favia et al. 2001).

No-choice feeding assay

Based on the field observations, it was assumed that wild male mosquitoes would at least show a predisposition to ingest sugar meals from each plant species on which they were caught. Correspondingly, a no-choice assay was used to provide correlative evidence of a link between the behavioral preference observed in the olfactometer choice assay and the sugar feeding patterns of males on the selected candidate plants. Thus, a single cutting from each plant was placed in a 90x90x90 cm cage with 200 starved two-day-old male *An. gambiae* from the same larval cohort. As starved males within the cage could feed on a plant that otherwise they would not respond to, 50 ml of distilled water in a small cup containing a piece of filter paper was simultaneously placed at one corner of the cage so that male that were less able to feed on plants could eventually imbibe water. The cages were placed on a laboratory bench in a darkened room under ambient conditions similar to those described above. On every experimental day, the beginning of the assay occurred around 18:00 and the evaluation of feeding response was carried out simultaneously with all selected plants using random mosquito samples. Each test series lasted three h, after which mosquitoes were aspirated into paper cups and killed with chloroform. The percentage that had fed on plants was assessed by testing them individually for the presence of fructose using the cold anthrone test (Van Handel 1972) while legs of each mosquito were used for identification of the species or molecular form by PCR (Scott et al. 1993, Favia et al. 2001). A minimum of three replicates were conducted for each plant, using different batches of mosquitoes and fresh plant parts with inflorescences. Data were pooled for each plant and the proportion of sugar-fed males were compared between plants to further determine whether plant species that are more attractive also incite satisfactory feeding responses.

Statistical analysis

Data collected in field observational surveys and by the cold anthrone test (i.e., the proportions of sugar-fed males) were square root transformed to meet normality assumptions and subjected to a univariate Generalized Linear Model (GLM). To determine whether there was a significant difference between treatments for each series of wind-tunnel bioassays, the number of upwind approaches (activation) and number of source orientations toward the plumes of plant odor and/or air (attraction) were subjected to chi-square analysis in a 2x2 contingency table with Yates correction. All data analyses used the Statistical Package for Social Scientists (SPSS 11.0.0, 1998). All probabilities

reported here are two-tailed and statistical significance was accepted at a 0.05 level.

RESULTS

Field observational survey and selection of plant candidates

A total of 5,879 adult *Anopheles gambiae s.l.* comprised of 3,663 females and 2,216 males was collected in different habitat sub-units and within the clay pots that were set up on the sugar sources in the field. The majority of males were collected in outdoor habitats and within clay pots while females predominated in catches inside houses. Only male data are presented here in an attempt to provide a good appreciation for their feeding behavior. Data for females will be presented separately.

An. gambiae s.l. males comprised 59.1% (n = 1,309) of all mosquitoes collected within clay pots suspended on trees. Of these 96.5% and 3.2% were M and S molecular forms of *An. gambiae s.s.*, respectively, and the remaining were *An. arabiensis* (0.22%). As might be expected, the density of mosquitoes found in each clay pot at a given sampling occasion was highly variable and ranged from one to approximately 30 males per pot. In addition, there was significant variation in the number of males of *An. gambiae s.s.* caught on different trees and at outdoor shelters (Figure 1). The higher number of male *An. gambiae* caught on flowering *Mangifera indica* L. (Anacardiaceae), *Delonix regia* (Fabaceae), *Thevetia neriifolia* Juss (Apocynaceae), and two other trees of the Fabaceae family, the Cassod tree *Senna siamea* Lam. and the West African laburnum *Cassia sieberiana* DC, contrasted generally with the rarity of mosquitoes on other nearby plants, with fewer or no males in corresponding clay pots ($p < 0.05$ for a *F*-test applied to the mean number of males per tree). This suggests that in the Kou Valley where a large variety of flowering plants are available, some are favored for use as a sugar source (or resting sites) by males of *An. gambiae* over others. Other common or scattered flowering plants in the locality included *Lantana camara* (Verbenaceae), *Bougainvillea glabra* 'Variegata' (Nyctaginaceae), *Adenium obesum* (Apocynaceae), *Carica papaya*, (Caricaceae), *Allamanda violacea* (Apocynaceae), *Banana musa* (Musaceae), acacia *Annonidium mannii* (Mimosaceae), *Azadirachta indica* (Meliaceae), and *Khaya grandifolia* (Meliaceae).

All wild-caught males of *An. gambiae s.s.* were tested for fructose, a main constituent of plant-derived sugar meals. There was significant variation in the proportion of sugar-fed males among micro-habitats ($F_{7,32} = 208.8$, $p < 0.001$). On average, the proportion of sugar-fed mosquitoes in the samples of resting males collected in peri-domestic habitats (both inhabited and abandoned houses) varied from 69-74% (Figure 1) at the time of collection. The majority of males caught on *M. indica* ($75.3 \pm 16\%$) and half of those caught on *D. regia* ($57.2 \pm 1.7\%$) and *T. neriifolia* ($53.1 \pm 2.5\%$) had ingested a sugar meal (Figure 1). Compared to other plants, significantly lower numbers of males found on *S. siamea* ($35.2 \pm 4.4\%$) and *C. sieberiana* ($48.3 \pm 3.7\%$)

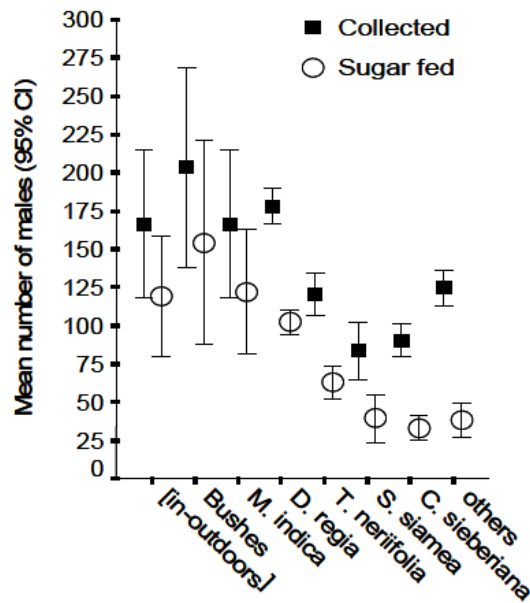


Figure 1. Variation in the density of adult *An. gambiae* males and sugar feeding rates for different flowering plants and resting habitats (Kou Valley, Western Burkina Faso). The “in-outdoors” category in the figure corresponds to the pool of males collected indoors and in outdoor shelters; “others” refer only to peridomestic plant species at which clay pots were also set to sample male mosquitoes. In its broad sense, “bushes” refers to peri-domestic areas covered by weeds or grass families (gramineae). The y-axis indicates the mean number of mosquitoes per individual plant or habitat sub-unit.

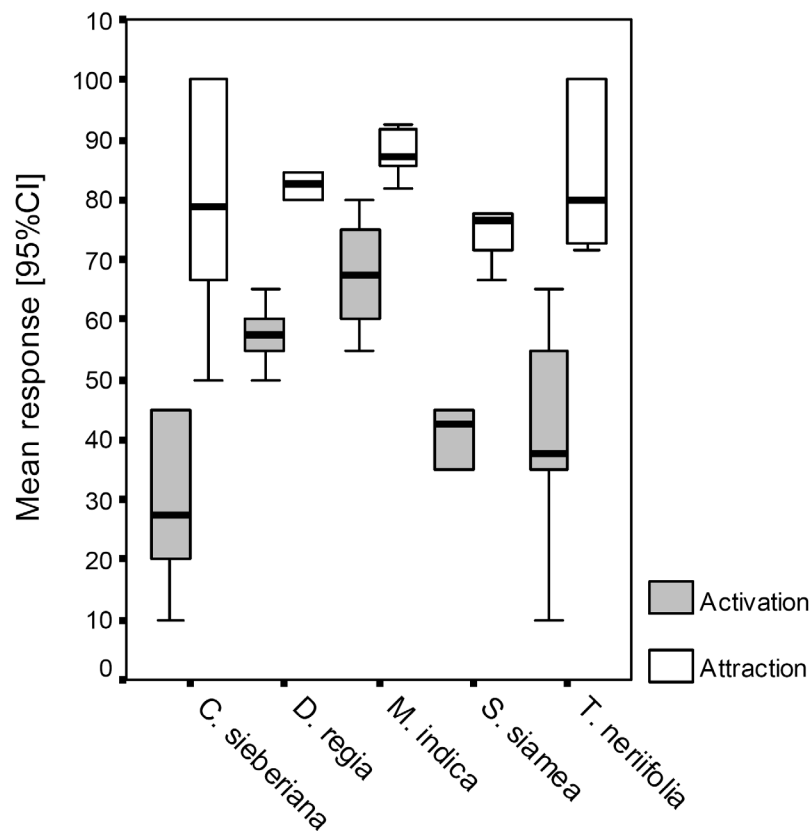


Figure 2. Activation and orientation flights of *An. gambiae* s.s. males to the odors of individual flowering plant species in a Y-shaped olfactometer.

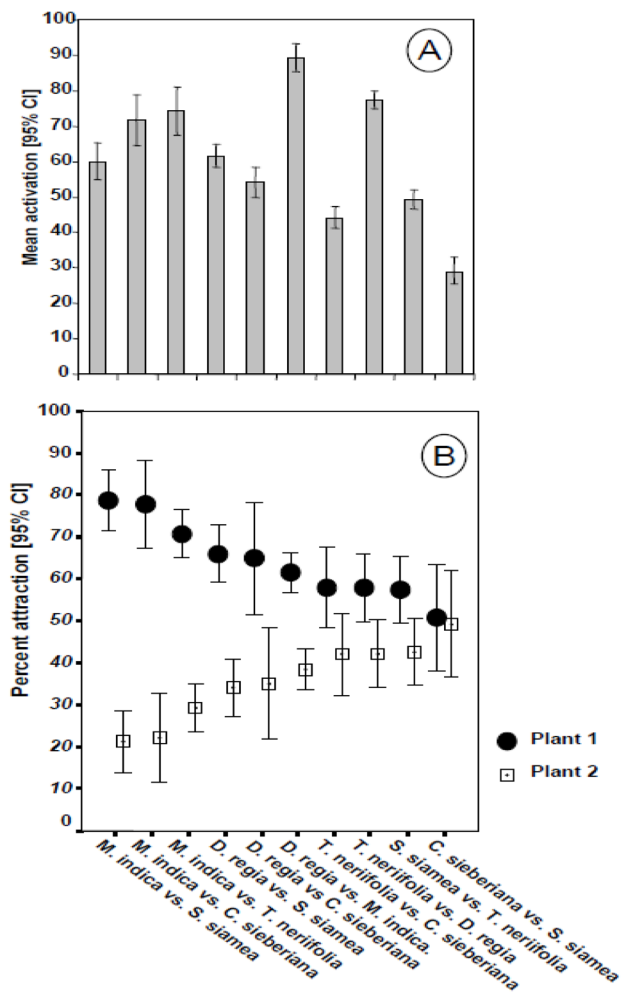


Figure 3. Behavioral responses of newly-emerged (two-three days old) *Anopheles gambiae* males in an olfactometer to competing odors of flowering plants collected from the Kou Valley (Western Burkina Faso). Bars represent the mean proportions ($n =$ six replicates, with 20 specimens each) of *An. gambiae* males that initiated upwind anemotaxic flights (A), and the proportion of males caught on each odor source (B).

had fed on sugar (independent GLM Univariate Pairwise comparisons, $p < 0.001$).

Given the immense heterogeneity of native flowering plants occurring in the Kou Valley, these field observational surveys resulted in a narrowing down of the range of potential sugar sources for laboratory bioassays. A series of experiments comprising the analysis of the olfactory response of the males of *An. gambiae* to the best candidate flowering plants using dual-choice olfactometer and short-term observations of male feeding response under a no-choice situation were performed. Mosquitoes used in these experiments were collected from *An. gambiae* breeding sites when the relative density of the M form was at its highest peak. Indeed the PCR analysis indicated that the mosquitoes tested in behavioral assays were all of the M molecular forms throughout the sampling period.

Relative attractiveness of plant odor vs air

Figure 2 shows the results of the upwind flight responses of male *An. gambiae* to odors of individual plants relative to the ambient air stream. When no odor source was applied at the upwind end, the response was neutral as less than 5% of the male mosquitoes moved away from the releasing cage (data not shown). Only in the presence of plant material at the upwind end was activation noted, and there was significant variation in the relative proportions of mosquitoes responding to individual plants ($F_{4,25} = 9.2$, $p = 0.001$). Responses were highly directional with few mosquitoes flying back to the releasing cage, suggesting true localization to the odor source. On any occasion, about $46 \pm 18\%$ of *An. gambiae* males were activated when plant cuttings were presented in the upwind section of the olfactometer and the response to individual plants was repeatable and significantly greater than the corresponding air controls (Pairwise F -test, $p > 0.05$; see Figure 3). *Mangifera indica* (Activation = 67.5%, 95% CI [56.8 - 78.2]) and *Delonix regia* (Activation = 57.4%, 95% CI [46.8 - 68.2]) were clearly effective in eliciting more activation responses of *An. gambiae* males than other substrates tested, whereas *Cassia sieberiana* triggered the lowest activation response (Activation=30.5%, 95%CI [18.5 - 39.8]). Compared to air alone, male attraction varied among plant species (Figure 2) but independent pairwise comparisons (F -test, $p > 0.05$) of the orientation responses did not reveal any difference in mean attraction among the tested plant odors, indicating that starved *An. gambiae* males would readily start an upwind anemotaxic flight toward any air current with a plant odor rather than an airflow without odor cues.

Dual choice tests

A total of ten pairs of competing plant stimuli were conducted, during which the choice of groups of *An. gambiae* male mosquitoes was assessed in the Y-shaped olfactometer. The levels of activation and flight orientation were used to assess plant sugar source preferences. Figure 3 summarizes the results obtained from six replicates of each pairing. Generalized Linear Models were first carried out to see whether there was a significant difference in the overall attractiveness of plants for the whole series. The plants tested differed in their attractiveness to male *An. gambiae* s.s. ($F_{5,115} = 761.3$, $p < 0.001$). On different test series, combinations of plant blends of *M. indica* and/or *D. regia* elicited the highest activation response in male mosquitoes, whereas those involving *C. sieberiana* showed the lowest responses (Figure 3A). *M. indica* and, to a lower extent, *D. regia* systematically and consistently attracted more mosquitoes than any other plant tested. These two plants did not show a significant difference in their attractiveness (Figure 3B). Thus, the overall ranking of these potential sugar sources was the same as in the plant-air combinations (above).

No-choice feeding assay

Figure 4 provides the cumulative average percentage (from three replicates per plant species) of mosquitoes

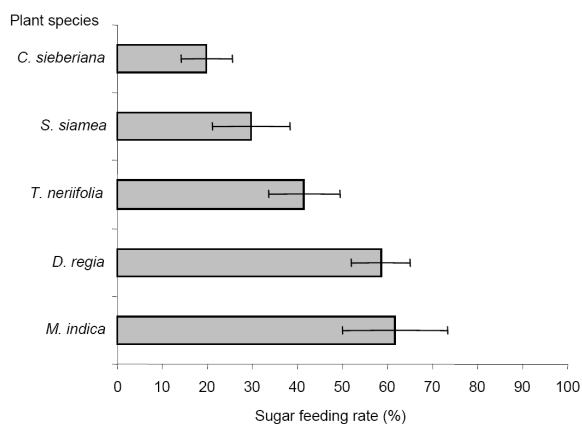


Figure 4. Proportions of sugar-fed mosquitoes recorded in cage feeding assays of *An. gambiae* males exposed to selected flowering plants for three h.

that tested positive for fructose in the no-choice assay. Significant differences were observed in the proportion of sugar-fed males after exposure to individual plants ($\chi^2 = 7.14$, $df = 1$, $P = 0.02$). Again, a higher proportion of sugar-fed males was recorded on *M. indica* ($64.2 \pm 3.2\%$) and *D. regia* ($63.6 \pm 8.3\%$) compared to *T. neriifolia* ($48.6 \pm 8.1\%$), *S. siamea* ($44.2 \pm 2.0\%$), and *C. sieberiana* ($38.8 \pm 3.1\%$).

DISCUSSION

The present study attempted to identify the main sources of sugar meals for anopheline males under both natural and experimental conditions. Almost all (60-80%) adult males of the *An. gambiae* complex caught in clay pots suspended in flowering trees had fed on plant sugar and > 50% of indoor-resting males were fructose positive. Both the no-choice and dual-choice olfactometer assays indicated that adult male *An. gambiae* can locate natural sources of plant sugar readily and utilize them effectively, with a stronger response towards *Mangifera indica* and *Delonix regia*. The results of laboratory evaluations based on the limited number of plants examined certainly support our field observations based on one-season trapping, which revealed relatively high quantitative differences in wild males feeding on various flowering plants. This is the first evidence for an interaction between males of *An. gambiae* and natural sugar sources obtained from both field observations and laboratory experiments.

Field observations give information on local plant species that are used by male mosquitoes as resting shelters and/or as carbohydrate sources. *An. gambiae* males concentrated mainly on five nectar-feeding centers, namely flowering *Mangifera indica* L. (Anacardiaceae), *Delonix regia* (Fabaceae), *Thevetia neriifolia* Juss (Apocynaceae), *Senna siamea*, and *Cassia sieberiana* (both of the Fabaceae family) compared to grass, but the preference of some wild males for grass may depend on there being adequate resting sites because they alternate between sugar feeding and mating. Even when a large variety of flowers is available, the

number of sugar sources is limited by the preferences of the mosquitoes (Martinez-Ibarra et al. 1997, Foster and Eischen 1987). Under field conditions, males may search for a plant either to feed upon or to gain shelter. However, a previous experimental study by Manda et al. (2007) suggested mosquitoes land on plants only to acquire a sugar meal. Thus, we assume that the number of wild males caught per plant reflects the preference of male mosquitoes to feed on the individual sugar sources. Our survey, however, did not exhaust all the plant species from which *An. gambiae* males could obtain a sugar meal in the field. Indeed, sampling in the dry season probably led to a bias caused by the lack of certain plant species and different tree phenologies across seasons. An extensive survey of host plants in both dry and wet seasons would probably find additional host plants from which *An. gambiae* males could be acquiring sugar meals.

Under laboratory conditions, we found upwind anemotaxic flights of male *An. gambiae* in a Y-shaped olfactometer in response to plant stimuli. For a given experiment, the presence of plant material in the olfactometer increased the likelihood that males would fly toward the odor source. They responded actively to plant odors in comparison to air alone and odors from *M. indica* were the most favored when compared to all other sugar sources. These differential responses observed in the olfactometer assays were consistent with inherent differences in the proportions of sugar-fed males recorded in no-choice experiments. Critically these results obtained in the laboratory assays imply that there is a correlation between where the males are caught and feeding activity on naturally occurring plants with flowers. Male preference for some flowering plants over others as reported both under laboratory and field conditions may be inferred on the basis of active chemicals or other constituents as has previously been shown for other insects (Jepson and Healy 1988). Overall, this finding is consistent with the assumption that plant volatiles are important in the location of flowers as food sources by male *Anopheles* mosquitoes. Although we did not demonstrate the exact structures serving as sugar sources on the preferred plants, we cannot rule out the possibility that the volatile blend emitted by preferred flowering plants may be an indicator of its suitability for males. Among the conceivable benefits of feeding on certain plants are nectar concentration, composition, and floral structure. However, a previous study by Manda et al. (2007) showed that female *An. gambiae*'s preference for different plants correlated with the relative fitness-related benefits the mosquito derives from feeding on the selected plant species. This may also suggest that *An. gambiae* males preferentially feed on plants with a high sugar content, which in turn will confer a high fitness advantage. However, we lack convincing evidence that sugar concentration in the plants tested varies sufficiently to affect male attractiveness. Overall, sugar feeding could have multiple effects on a male's ability to disperse, mate, survive, and proliferate throughout its adult life (Foster 1995).

This study did not examine either the chemical composition of the plant odor or nutritional differences

among plant cuttings. The majority of known volatiles from plants can be placed in the following four groups: terpenoids, fatty acid derivatives, benzenoids, and nitrogen-containing compounds (Dobson 1994, Raguso 2004). However, the dominant volatiles of flowers, terpenoids, and aromatics have been previously shown to elicit both chemosensory and behavioral responses in mosquitoes (Foster and Hancock 1994). Although the involvement of visual cues seems plausible (Allan et al. 1987), previous studies have demonstrated that odors alone without visual cues could trigger mosquito foraging choices (Foster and Hancock 1994, Raguso 2008, Goyret et al. 2007).

The results of the current study demonstrate that *An. gambiae* can express selective preferences in its feeding behavior and that it uses odor cues to regulate its preference for sugar sources, which probably accounts for the spatial variation of males on a large number of flowering plants. The proportion of fructose-positive males (60-80%) recorded in field collections was higher than that of the anthropophilic females of the same species (c.a. 20%) in a previous study within the same locality (Gouagna et al., unpublished data) and elsewhere (Beier 1996). Another study by Huho et al. (2007) found that the majority (70%) of *An. gambiae* males collected in Tanzania had no detectable sugar. This discrepancy between studies of males and females suggests the existence not only of temporal and geographical fluctuations, but also sex-specific variation in mosquito energetic demands. Yuval (1992) noted that males may require a broader range of nutritional resources to maximize their energetic reserves than females.

Conceivably, males should be used as the prime example in exploring sugar feeding behavior of mosquitoes in their natural environment. Although it is too early to draw conclusions about the potential impact of plant-derived sugar on male biology, what we report so far about this neglected area of male behavior certainly encourages further studies. Despite the significant expansion in our knowledge of male *An. gambiae* feeding behavior as reported here, many questions regarding nutritional correlates and patterns of behavior in wild populations of adult *An. gambiae* males remain unknown. Future studies should expand the scope of this research to look into specific behavior of male *An. gambiae* to various constitutive plant-derived chemicals and to investigate the fitness benefit gained from these interactions. Such information could lead both to a better understanding of the ability of mosquitoes to locate food resources in nature and to develop new strategies for mosquito control. For example, current research in this area focuses on the possibility of exploiting facets of plant-mosquito interactions to develop synthetic attractants (Schlein and Müller 2008) or effective ways of suppressing mosquito populations with the use of natural sugar sources as baits with pour-on insecticides (Müller and Schlein 2006, Müller and Schlein 2008).

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