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Discriminative feeding behaviour of *Anopheles gambiae s.s.* on endemic plants in western Kenya

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Abstract

Anopheles gambiae Giles *s.s.* (Diptera: Culicidae) is known to feed on plant sugars, but this is the first experimental study to consider whether it discriminates between plant species. Thirteen perennial plant species were selected on the basis of their local availability within the vicinity of human dwellings and larval habitats of *An. gambiae s.s.* in western Kenya. Groups of 100 or 200 mosquitoes were released into cages either with a cutting of one plant type at a time (single-plant assay) or with cuttings of all 13 plants simultaneously (choice assay), respectively, and left overnight. In the choice assay, direct observations of the percentages of mosquitoes perching or feeding on each plant were recorded over four 1-h periods each night. For both types of assay, mosquitoes were recaptured and the percentage that had fed on plants was assessed by testing them individually for the presence of fructose. To identify which plants the choice-assay mosquitoes had fed on, gas chromatography (GC) profiles of samples of mosquito homogenates were compared with GC profiles of extracts from relevant parts of each plant. Four of the plants that were observed to have been fed on most frequently in the choice assay (*Parthenium hysterophorus* L., *Tecoma stans* L., *Ricinus communis* L., and *Senna didymobotrya* Fresen) were also shown to have been ingested most often by mosquitoes in both types of assay, suggesting that *An. gambiae* is differentially responsive to this range of plants, regardless of whether the plants were presented singly or mixed together. Significantly more females than males fed on plants, with the exception of *P. hysterophorus* L., one of the plants most frequently fed on. For most plant species (ten of 13), GC profiles indicated that *An. gambiae* obtained sugars primarily from flowers. The exceptions were *P. hysterophorus* L., *Lantana camara* L. and *R. communis* L., on which *An. gambiae* fed more often from leaves and stems than from flowers.

Keywords

Anopheles gambiae; Kenya; malaria; nectar; plant-feeding

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Introduction

In sub-Sahara Africa, the mosquito *Anopheles gambiae* Giles *s.s*. is the primary vector of *Plasmodium falciparum* Welch, the parasite that causes the most severe form of human malaria (White, 1974; Service, 1980; Collins & Paskewitz, 1995). Understanding the role of plant-derived sugars in the biology of this particular mosquito species may be of practical importance. It is well known that the males of many mosquito species feed on plant-derived sugar (Clements, 1999). Despite being better known for their avid interest in bloodmeals (Gillett, 1971; Klowden, 1995), female mosquitoes also feed on plant-derived sugars (Yuval, 1992; Foster, 1995; Clements, 1999), but the role of plant feeding in the biology of *An. gambiae* is poorly understood.

Characteristics responsible for the dominant role of *An. gambiae s.s*. as a vector of *P. falciparum* include not only endophily, endophagy and anthropophily, but also longevity sufficient for the proliferation of sporozoites in the mosquito (White, 1974). Sugar meals may play an important role in enhancing the vectorial capacity of *An. gambiae* by extending female longevity (Okech *et al.*, 2003; Gary & Foster, 2004; Impoinvil *et al.*, 2004). Various mosquito species are known to feed on honeydew, sap, rotting or damaged fruits, leaves, and discarded plant materials such as sugar-cane waste (Foster, 1995). The primary source of plant-derived sugars, however, is nectar from flowers and extra-floral nectaries (Foster, 1995), with numerous plant species having been implicated (Sandholm & Price, 1962; Grimstad & DeFoliart, 1974; Magnarelli, 1977, 1978). That mosquitoes might show preferences for particular plant species in malarious areas of Africa is suggested by previous observations (McCrae *et al.*, 1969, 1976), yet surprisingly little is known about whether *An. gambiae* discriminates among the numerous plant species found in its natural environment

As a step toward understanding how the physiology, ecology, life history and vectorial capacity of *An. gambiae* might be shaped by associating with particular plant species, we investigated the response of this mosquito species to 13 commonly occurring plants in a region of western Kenya where malaria is holoendemic.

Materials and methods

Study area

The laboratory and field site were in Mbita Point, a village of approximately 8000 people on the shore of Lake Victoria, in Suba District, western Kenya, a region of holoendemic malaria (Mutero *et al.*, 1998; Minakawa *et al.*, 1999). Malaria from *P. falciparum* is the leading cause of morbidity for residents in the area, accounting for 50% of all clinically diagnosed illness at the local health centre (Gouagna *et al.*, 2004). Although *An. arabiensis* Patton and *Anopheles funestus* Giles are also significant malaria vectors in the region, *An. gambiae* is the primary vector of *P. falciparum* in Mbita Point. The mean minimum and maximum daily temperatures are 17°C and 34°C, respectively. The mean annual rainfall is 700–1200 mm, primarily occurring during two rainy seasons, March to May and October to November. Permanent and semipermanent larval habitats for *An. gambiae* are widespread even during the dry seasons. Vegetation, which includes a wide variety of indigenous and introduced plants, remains verdant all year.

Plant species

The high plant diversity in Mbita Point necessitated selecting a manageable subset for this study. We selected 13 of the most common species of perennial flowering plants (Table 1) based on three criteria: (1) the occurrence in the vicinity of human dwellings and nearby aquatic larval habitats of *An. gambiae* within a radius of 30 km around Mbita Point; (2) the distribution

over a wide ecological range, from the lake shore to higher elevations in the surrounding hills; and (3) the year-round availability above ground. Cuttings (branches with inflorescences) were collected from the field as required for testing with mosquitoes, care being taken not to damage the inflorescences.

Mosquitoes

The mosquitoes were laboratory-reared individuals of the Ifakara strain of *An. gambiae s.s.* (established in culture at Mbita Point in 1998 from specimens collected in 1996 at Njage, 70 km from Ifakara in south-east Tanzania). They were reared at natural ambient temperature and humidity (mean temperature and RH inside the rearing room: day, 31°C, 52% RH; night, 24° C, 72% RH), with adults being maintained on a diet of human blood three times per week, along with glucose (6% solution) continuously available on filter paper. The rearing procedures described by Benedict (1997) were used. Newly-emerged adult females and males intended for experimental use were maintained on water only (no bloodmeals and no access to sugar) and then assayed when they were 2-days old. The assays were conducted under approximately the same ambient conditions as used for rearing.

Choice assay

This was a competitive plant-feeding assay in which plants and mosquitoes were enclosed in a mesh-covered cage (3.5 × 3.5, 2.0 m high), sheltered in a glass-roofed, screen-walled greenhouse (11.5 m long × 7.1 m wide). Beneath the roof was a layer of reed mats that blocked most direct sunlight and prevented temperature extremes. Fresh cuttings of approximately 45–50 g from each of the 13 plant species were used in each trial. Each plant species was held in a separate 500-mL Erlenmeyer flask filled with distilled water, plugged with cotton wool and, to deny the mosquito access to the water, sealed with Parafilm (Pechiney Plastic Packaging, Menasha, WI). The plants were spaced evenly within the caged arena, with each plant approximately 95 cm from its nearest neighbour. The shape of the cuttings allowed observers to get a clear view of all surfaces from one or both sides of the arena.

A Latin square design (13 plant species × 13 positions in the caged arena) was followed, with 13 trials (observation nights) per block, using fresh plant cuttings and different 2-day-old adult mosquitoes for each trial. This block was repeated three times, for a total of 39 trials. Within the arena, position and nearest-neighbour effects were controlled for by changing the position of each plant species at random for each trial. Trials were carried out weekly (minimum intervening period, 6 nights). The rationale for this intertrial interval was to avoid the potential of residual odours remaining in cages and having confounding effects on successive trials and also to allow sufficient time to ensure that any mosquitoes not collected after the previous trial would die of starvation. All testing was carried out between July 2003 and April 2004.

For each trial, 200 2-day-old adult mosquitoes (100 females and 100 males) were released into the centre of the cage at 19.00 h. Observations on each plant species were carried out by two observers, each using a hand-held electric lamp (60-W red lightbulb). As a pre-caution against mosquitoes being influenced by volatiles of human origin, a transparent sheet of Perspex (150 × 60 cm) was always positioned between the mosquitoes and each observer. Observations were made at 60-min intervals (first observations, 20.00 h) for a total of four observation periods per trial. We counted the numbers of mosquitoes perching (resting on a plant, but without proboscis in contact with the plant) and feeding (when perching, applying tip of the proboscis to the plant). Initially, we tried to record instances of probing without feeding (tip of the proboscis applied to the plant, but with abdominal distension not apparent) and probing with feeding (tip of the proboscis applied to the plant, and with abdominal distension apparent) but, unfortunately, there was a continuum in degree of abdominal distension and it was not possible to discriminate between these two behaviours reliably. We also recorded perching and feeding

sites: (1) flowers (corolla, petals or calyx, the latter including sepals and receptacle); (2) leaves (midrib and surfaces); and (3) stems (main stem, branches, nodes, meris-tems and leaf petioles).

After the last observation period of the night, the plants were immediately removed from the cage and as many mosquitoes as possible (>80%; mean = 85%) were collected and immobilized with chloroform and stored at − 20°C. These mosquitoes were later crushed individually in distilled water and checked for the presence of fructose by the cold-anthrone test (Van Handel, 1972), with fructose being indicative of undigested plant sugars. A portion of the homogenate from the mosquitoes that tested positive for fructose was frozen (− 20°C) for later analysis using gas chromatography.

Single-plant assay (no-choice)

This non-competitive plant-feeding assay was used for ascertaining the mosquito's pre-disposition to ingest sugar from each plant species, with no alternative plant species present at the same time. Otherwise, the testing procedure was the same as for the choice assay, except that we used smaller cages (30 × 30 × 30 cm), and only 100 individuals of *An. gambiae* (50 males and 50 females) were released into the cage per trial. Mosquitoes were always released in the cage before 10.00 h, left for 24 h, then collected and tested with cold-anthrone, to determine the proportion positive for fructose. Each plant species was used in three trials, with fresh cuttings and 2-day old mosquitoes from a different batch being used in each trial.

Preparation of mosquito and plant samples for gas chromatography (GC)

Aliquots of homogenates from individual mosquitoes that tested positive for fructose in the choice assay and extracts of specific parts of all 13 plant species were analysed by GC at the International Centre of Insect Physiology and Ecology (ICIPE) in Nairobi, Kenya. The objective was to determine the specific plant species from which the mosquitoes acquired sugar and to identify the sugars (mono-, di- and trisaccharides). Because processing crop contents of mosquitoes for GC is time-consuming and labour-intensive, homogenates from a subsample of 80 mosquitoes (40 males and 40 females) were chosen at random from the fructose-positive mosquitoes in the last three trials. Newly-emerged mosquitoes (ten males and ten females, all reared from the same batch as the tested mosquitoes) that had received no previous blood or sugar meals were used as controls.

For each plant, flowers, leaves and, for *Ricinus communis*, stems were analysed by GC. Flowers included both nectar and floral tissue, as defined above. Using small cryo-preservation vials containing 200 μL of 50% EDTA (ethylenediamine tetraacetate) solution, approximately the same mass of flowers or leaves (or, for *R. communis*, stems) from each plant species was incubated in the dark for 2 h, after which the plant material was removed. Extracts remaining in the vials were dried under a flow of pure nitrogen.

Standards

The standards were analytical grade D-allose, D-altrose, β-D-fructose, D(+)galactose, α-D-glucose, D-gulose, D(+)mannose, D(+)raffinose and sucrose, obtained from Sigma-Aldrich Co. (U.K.). Analytical procedures were checked with α-lactose.

Trimethylsilylation of standards and samples

Sugar analysis by GC requires initial silylation for derivatization of the highly polar carboxyl and hydroxyl groups. We adopted techniques described by Hamilton & El Naiem (2000). Using a clean 2-mL reacti-vial, 1 mg of each sugar standard was trimethylsilylated by dissolving it in 100 μL of dry pyridine. An equal volume of *N*-methyl-*N*-trimethylsilyltrifluoroacetanamide (MSTFA) was then added. The mixture was placed in an oven set at 60°C and left for 60 min,

after which the sample was removed and stored at room temperature until analysis. Procedures for processing and derivatizing plant extracts were similar to those for the standards. Individual mosquito extracts were transferred into 2-mL reacti-vials, treated with 50 μL pyridine and, for removing moisture, dried under a flow of pure nitrogen in a fume hood at room temperature. Dry pyridine (2 μL) and MSTFA (2 μL) were then added to each vial, and the reaction was allowed to proceed as above.

Gas chromatography analyses

For GC, we used a Hewlett Packard (HP) 5890 series II gas chromatograph (Hewlett Packard, Waldbronn, Germany), equipped with a split-less injector system, a 50 m × 0.2 mm (i.d.) crossed-linked methylsilicone capillary column (0.33 μm film thickness), and FID-coupled to an HP 3393 A Series II integrator. The carrier gas was nitrogen, with flow set at 0.005 mL/min. The initial temperature (100°C) was increased at 30°C/min to 170°C, and then by 2°C/min to 210°C, followed by 50°C/min to a final isothermal temperature of 280°C, which was maintained for 30 min.

The derivatized sugar standards were diluted by a factor of 40 with dichloromethane (DCM 99.9+%, PRA grade). Three parts of the derivatized plant extracts were diluted with one part of the same solvent. Derivatized extracts from mosquitoes were analysed without dilution. One μL of each sample was injected into the gas chromatograph. DCM was used as the solvent to clean syringes between samples.

Plant-derived sugars were identified by comparing retention times of sugar standards with those present in the plant extracts. We concluded that the sugar meal of the mosquito had been identified when the profile of the mosquito extract matched the sugar profile of a particular plant species extract.

Statistical analysis—We looked for differences between groups (plants, plant parts and sex of mosquitoes) by using Tukey–Kramer tests for multiple comparisons of means and by using chi-square tests of independence. Repeated-measures analysis of variance was used to test for associations between the independent variables (plants, observation times) and the mosquitoes' responses to the plants. Arcsin-transformations were used for calculating percentages of mosquitoes perching and feeding (choice assay) and fructose-positive (single-plant assay) fit to the normal distribution. Data are summarized as means ± SE. All statistics were carried out using Excel 2000 (Microsoft Corp., Richmond, WA) and SAS version 8.2 for Windows (SAS Institute, Cary, NC).

Results

Choice assay

Perching ($F = 10.09$, d.f. = 2, $P < 0.001$) and feeding ($F = 16.42$, d.f. = 2, $P < 0.001$) were observed more often on flowers than on other plant parts. The percentages of mosquitoes perching were 5 ± 0.0%, 4 ± 0.0% and 2 ± 0.0%, and those feeding were 60 ± 0.1%, 4 ± 0.1% and 8 ± 0.0%, respectively, on flowers, leaves and stems. Our decision to refer to all instances of probing as 'feeding' is justified by the observation that the proportion of mosquitoes we scored as 'feeding ', regardless of the degree of evident abdominal distension was 72 ± 0.1%, and the percentages positive for fructose was 56 ± 2.6%, whereas evident abdominal distension was observed only on 10 ± 0.0%.

Despite the low percentages of mosquitoes observed perching, there were significant differences among plant species in the mean percentages of mosquitoes observed perching ($F = 2.19$, d.f. = 12, $P = 0.01$) (Fig. 1A) and feeding ($F = 6.47$, d.f. = 12, $P < 0.001$) (Fig. 1B).

It is likely that mosquitoes observed perching on a given plant had also fed on that plant, because four of the five plant species on which we most often saw mosquitoes feed (*Hamelia patens* Jacq., *Tecoma stans* L., *R. communis* L. and *Senna didymobotrya* Fresen.) were also among the seven plant species on which we most often saw mosquitoes perching. The responses to a fifth plant species, *Parthenium hysterophorus* L., were inconsistent; this is one of the plants on which mosquitoes were most often observed feeding, but also least often observed perching.

The percentage of mosquitoes perching ($F = 12.82$, d.f. = 3, $P < 0.001$) and feeding ($F = 7.28$, d.f. = 3, $P < 0.001$) (Fig. 2) varied significantly across the observation times during the night, with more perching closer to midnight, but more feeding during the early hours of the night. These trends were similar across all nights. There was a significant plant–time interaction for the proportion of mosquitoes observed feeding ($F = 2.43$, d.f. = 36, $P < 0.001$), but not for the proportion observed perching ($F = 0.86$, d.f. = 36, $P = 0.80$).

The mean number of mosquitoes recovered per trial (out of 200 released) was 169 (58% females, 42% males, total 6591 mosquitoes for all trials combined). The mean percentage of mosquitoes positive for fructose per trial was 56 ± 2.6%. Significantly more females (68 ± 2.6%) than males (44 ± 3.2%) tested positive for fructose ($\chi^2 = 45.92$, d.f. = 1, $P < 0.001$) per trial.

Single-plant assays (no-choice)

Pooling data across all plant species, a total of 3832 mosquitoes (1773 females, 2059 males) were recovered and tested for fructose, with 859 (22.5%) of these mosquitoes testing positive for fructose. Significantly more females (25%) than males (20%) were positive for fructose ($\chi^2 = 16.58$, d.f. = 1, $P < 0.001$). Again, females tended to be positive for fructose more often than males for each plant species, the exception being *P. hysterophorus*, on which more males (47 ± 4.03%) than females were positive for fructose (35 ± 12.3%) ($\chi^2 = 5.14$, d.f. = 1, $P = 0.02$).

Four of the plant species (*P. hysterophorus*, *T. stans*, *S. didymobotrya* and *R. communis*) on which mosquitoes most often fed in the choice assay (Fig. 1B) were also the four most often positive for fructose in the single-plant assay (Fig. 1C), and *Datura stramonium* L. and *Flaveria trinervia* Mohr were among the plant species on which mosquitoes least often fed in both assays.

Gas-chromatography

Standards—The purity of most standards was > 95%. Most sugar standards had a single main peak (Fig. 3), as well as several small peaks, indicating different isomeric forms of the sugar. The fructose standard, however, had four peaks, at 29.0 min (16%), at 31.2 min (24%), at 31.5 min (49%), and at 30.4 min (9%), indicating that there had been isomerization during derivatization.

Identification of sugars from plant extracts and mosquito homogenates

D-glucose, fructose, sucrose, mannose and gulose were clearly the most common sugars from whole flowers, with galactose, raffinose and altrose also being present in some plants (Table 2). These sugars were sometimes found in leaves, but in smaller amounts than for flowers (data not shown). Unidentified compounds were also present, as none of the peaks from the leaves of *H. patens*, *Ipomoea hildebrandtie* Vahl, *Cassia hirsuta* L., *Psiadia punctulata* L. and from flowers of *P. hysterophorus* L. corresponded to the retention times of derivatized standard sugars (Table 2).

Homogenates from newly-emerged mosquitoes (controls) that had never had access to the plants were similar for males and females, and the GC profiles of these mosquitoes had relatively small peaks (Fig. 4C). The small peaks discernible from homogenates of plant-fed mosquitoes (Fig. 4B) matched the small peaks from the control mosquitoes (Fig. 4C). Based on the match-ups between chromatographic profiles of mosquitoes and plants (e.g. Fig. 4A, B), we identified the five plant species from which mosquitoes in the choice assay most often ingested sugar: *R. communis* (39% of the mosquitoes matched the profile of this plant species), *H. patens* (26%), *T. stans* (25%), *S. didymobotrya* (20%), and *P. hysterophorus* (18%) (Fig. 1D). This GC-derived ranking was closely similar to the ranking derived from direct observation of feeding in the choice assay (Fig. 1B). Note that, because sugar profiles of 44% of the mosquitoes matched profiles of more than one plant species, the total for plant feeding exceeded 100% (Fig. 1D).

Mosquito profiles matched the profiles of flowers significantly more often than the profiles of leaves ($\chi^2 = 21.45$, d.f. = 1, $P < 0.001$) (Table 3). However, the composition of homogenates from 27% of the mosquitoes suggested feeding on both leaves and flowers of the same plant species. For homogenates from mosquitoes that had fed on *R. communis* in the choice assay, 21%, 26% and 35% had profiles that matched this plant' s leaves, flowers and stems, respectively. For homogenates from mosquitoes that had fed on *Lantana camara* in the choice assay, 11% and 3% had profiles that matched this plant' s leaves and flowers, respectively. Homogenates from mosquitoes that had fed on *P. hysterophorus* in the choice assay matched exclusively the profile of this plant' s leaves.

Discussion

Earlier studies have shown that feeding from plants contributes to the survival of *An. gambiae* (Gary & Foster, 2004; Impoinvil *et al.*, 2004), but this is the first study to show experimentally that *An. gambiae* discriminates among plant species, perching and feeding more often on certain species irrespective of whether the plants are presented on their own or mixed in with other species. The observations of mosquito behaviour were reasonably consistent with the analysis of the sugar content in mosquito guts; four out of 13 plants tested (*P. hysterophorus* L., *T. stans* L., *R. communis* L. and *S. didymobotrya* Fresen) were always in the group of plants that were most fed on in the choice assay and the single-plant assay, according to both behavioural observations and sugar-meal analyses. These four plant species are frequently found associated with human dwellings.

It is interesting to note that the perching frequency was generally an order of magnitude less than the feeding frequency for the choice experiment, and that the rank order of plants chosen to perch on or feed from was similar, suggesting that mosquitoes probably land on plants only to feed.

One surprising result was that females tested positive for fructose more often than males, which conflicts with a common portrayal of female mosquitoes as being primarily blood-feeders and male mosquitoes as being exclusively nectar feeders. Perhaps females feed on plants more often than males because their total energy requirements are disproportionately greater than the male' s. Another hypothesis is that males digest their sugar meals more rapidly than females, so that the cold-anthrone tests misleadingly suggested that males were ingesting plant-derived sugar less often than females. Yet another possibility is that we inadvertently chose for this study particular plant species that are more attractive to females than to males (Grimstad & DeFoliart, 1974).

The patterns of mosquito activity over the four 1-h observation periods each night suggest that *An. gambiae* is inclined to feed from plants early in the evening. Other studies (Yee & Foster,

1992; Yee *et al.*, 1992) have shown distinct diel feeding patterns of sugar feeding and blood-feeding in some other mosquito species [*Anopheles quadrimaculatus* Say, *Culex quinquefasciatus* Say, *Aedes triseriatus* (*Say*), *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse)].

The GC analyses showed that, for most of the plants investigated, the variety of sugar types in flowers is greater than in leaves and stems. Although the mosquitoes generally fed more often from flowers, there were three plant species (*R. communis*, *L. camara* and *P. hysterophorus*) on which *An. gambiae* apparently fed more often from leaves and stems than from flowers. Extra-floral nectaries are abundant on the stems and leaves of *R. communis* and, as in earlier work (Impoinvil *et al.*, 2004), in this study *An. gambiae* fed especially often on the stems and leaves of this plant. On *P. hysterophorus*, they appeared to take sugar exclusively from leaves, which were shown by GC analysis to have a greater diversity of sugar types than its flowers. On *L. camara*, more mosquitoes fed on leaves than flowers, despite higher sugar levels in the latter and the presence of only one identifiable monosaccharide (glucose) in the leaves. This is perhaps a consequence of the long corollas of *L. camara* making its nectar inaccessible to An. gambiae. In earlier studies, survival was especially low when *An. gambiae* was provided with *L. camara* as its only potential sugar source (Gary & Foster, 2004; Impoinvil *et al.*, 2004), possibly because its feeding is concentrated on this plant' s leaves, which are known to contain secondary compounds that are deleterious to insects and other organisms (Deka *et al.*, 1998; Fatope *et al.*, 2002).

In this initial study of plant discrimination by *An. gambiae*, we investigated only ingestion of sugar. Recent studies on other mosquito species (*Culex pipiens molestus* Forskal and *Aedes caspius* Pallas), including other *Anopheles* (*Anopheles sergentii* Theobald and *Anopheles claviger* Meigen) (Schlein & Müller, 1995; Müller & Schlein, 2005), as well as Schlein' s work on sandflies (Diptera: Psychodidae) (Schlein & Jacobson, 1994; Schlein & Müller, 1995; Schlein *et al.*, 2001), suggest that more than sugar might be relevant; plant tissues have also been identified from mosquito and sandfly guts. We are currently investigating how the fitness of *An. gambiae* may be influenced by the plants it feeds on, and the possibility that plant-derived components other than sugar might contribute to these fitness effects.

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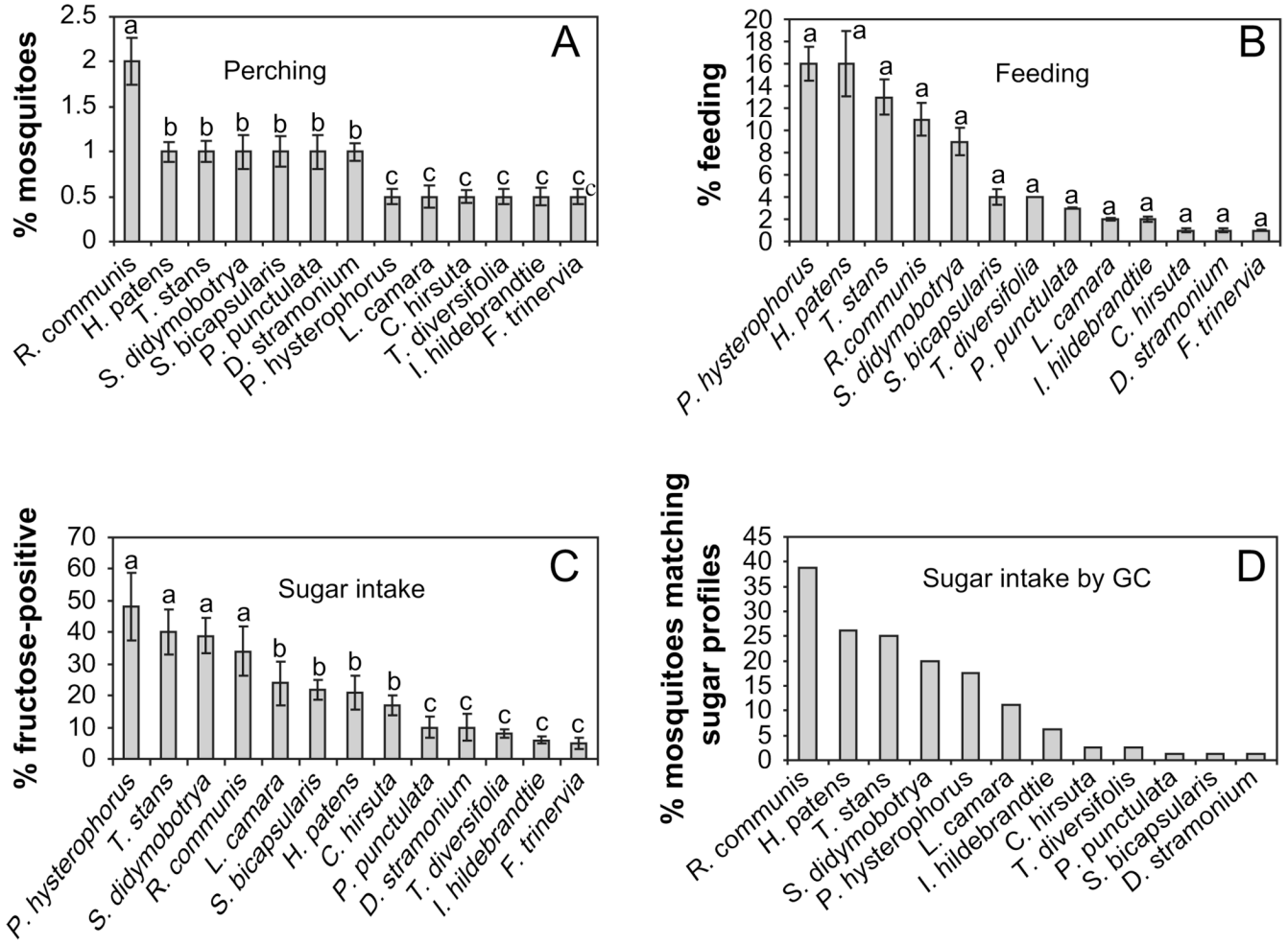

Fig. 1.

Percentages (mean ± SE) of *Anopheles gambiae* males and females that were (A) observed to be perching and (B) observed to be feeding in the choice assay (C) found to be positive for fructose after the single-plant assay and (D) found to have ingested sugars in the choice assay that matched the sugar profiles of particular plants. Columns with differ ent letters indicate significant differences and same letters indicate columns that are not significantly different (Tukey–Kramer multirange comparison, $\alpha = 0.05$). Number of replicates, 39 (A, B), 3 (C) and 80 D (40 males and 40 females). GC, gas chromatography.

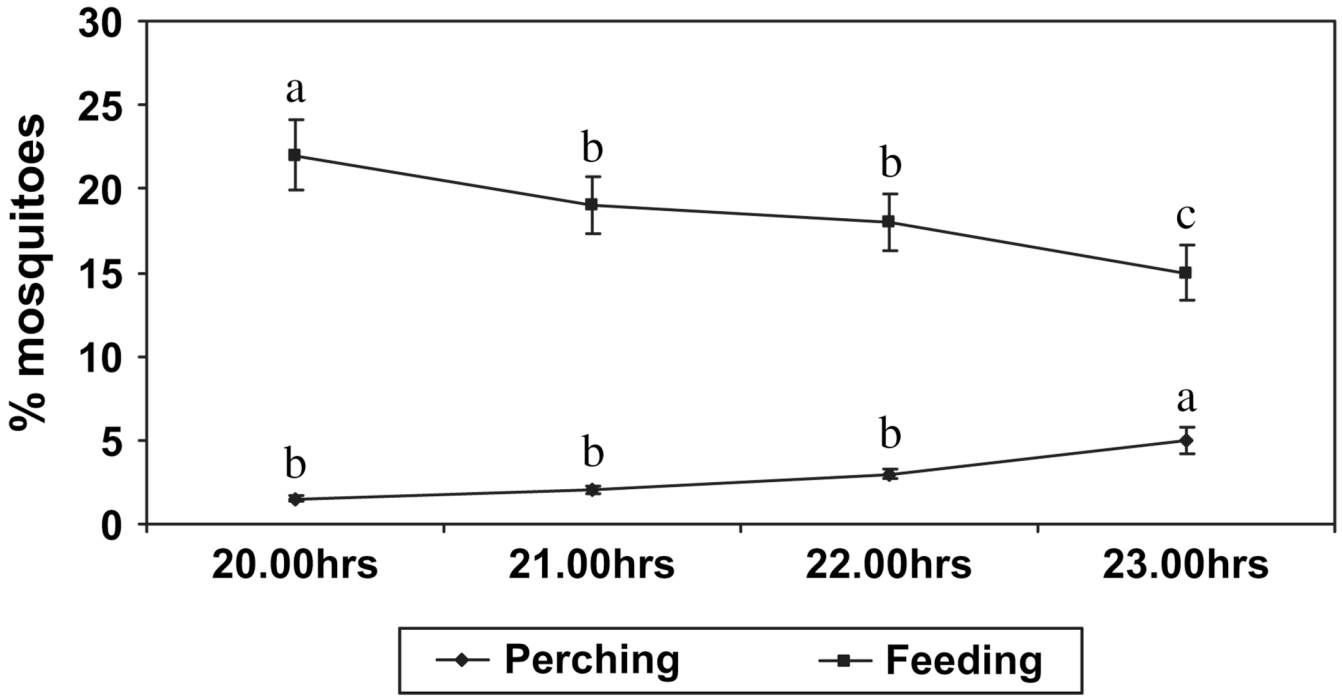

Fig. 2.

Percentages (mean ± SE) per night of mosquitoes seen perching and feeding on plants at 20.00 h, 21.00 h, 22.00 h and 23.00 h (39 replicates). Different letters in superscripts indicate significant differences (Tukey–Kramer multirange comparison, $\alpha = 0.05$).

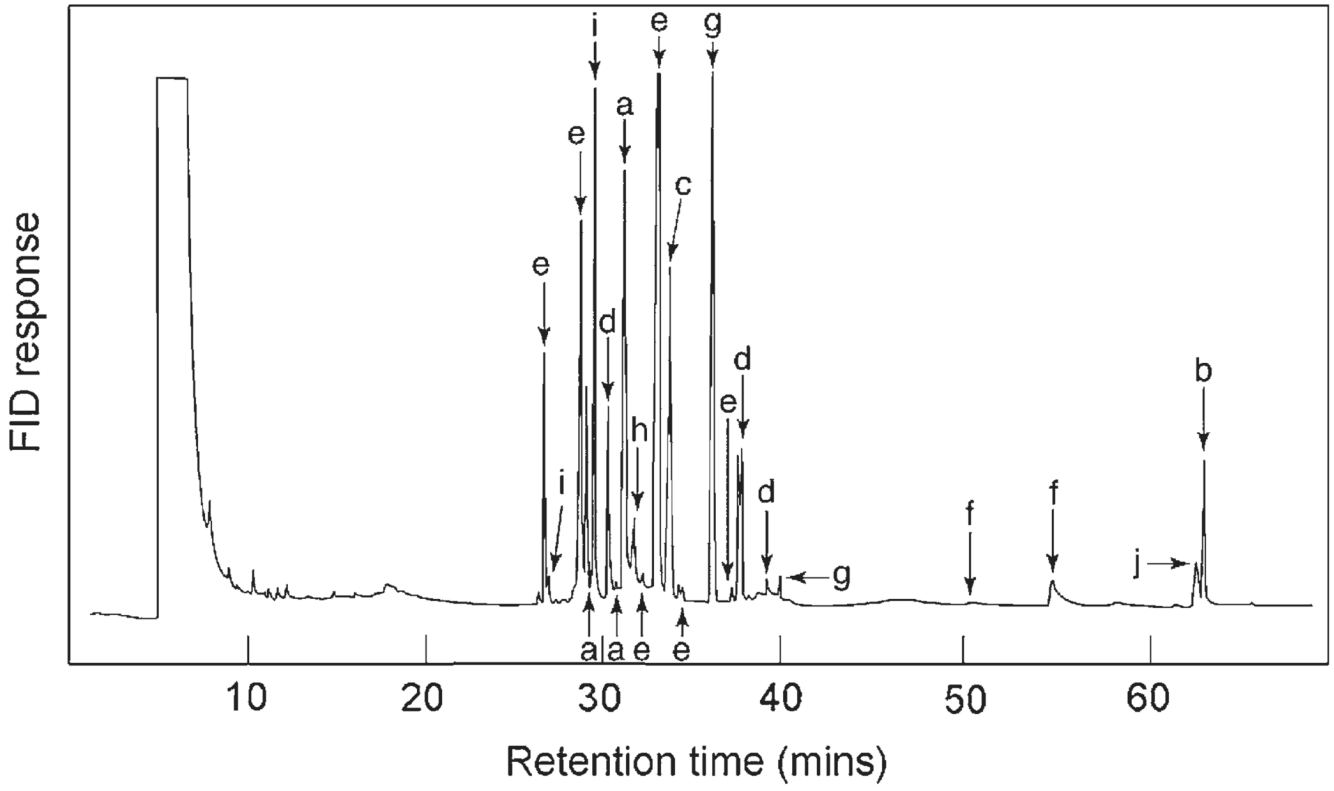

Fig. 3.

Chromatogram of sugar derivatives from extract of mixed sugar standards (multiple peaks of the same sugar, anomeric forms). a, Fructose; b, sucrose; c, galactose; d, mannose; e, gulose; f, raffinose; g, glucose; h, allose; i, altrose; j, lactose; FID, flame ionization detector.

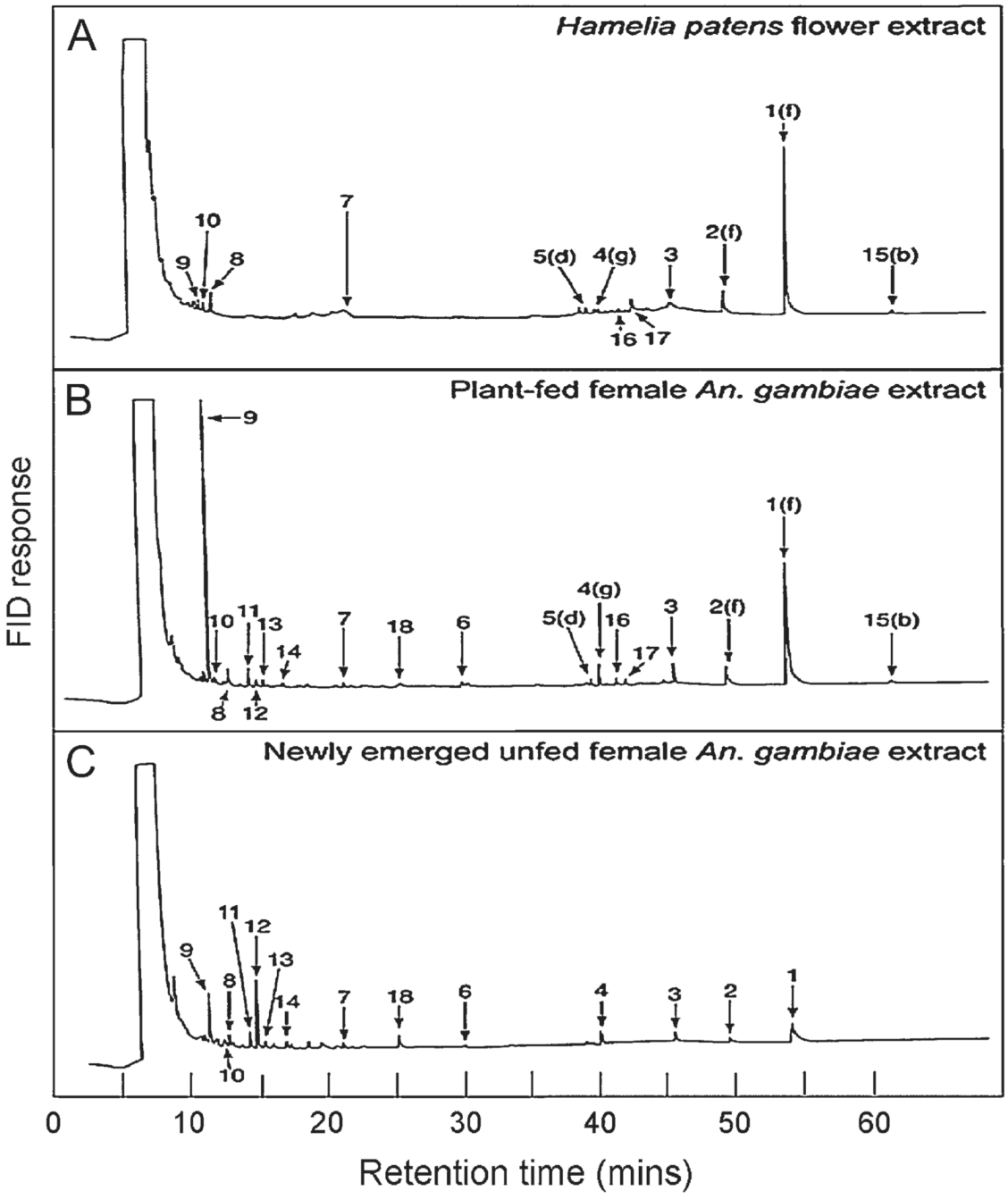

Fig. 4.

Representative chromatograms showing match between the sugar derivatives from extracts of (A) *Hamelia patens* flower and (B) one sugar-fed female *Anopheles gambiae*. (C) Representative chromatogram showing sugar profile of one newly-emerged *An. gambiae* female. Peaks with the same number or letter indicate same sugar type. Matching sugar peaks in (B) and (C) presumed to be metabolites derived from larval feeding. Extra peaks in (B), and enhanced ones also found in the unfed females (C), indicate sugars acquired from the plant (A). Letters on top of peaks represent identified types of sugars. Peaks without a letter are sugars not identified. b, Sucrose; d, mannose; f, raffinose; g, glucose; FID, flame ionization detector.

Table 1

List and common names of plant species tested for preferences of *Anopheles gambiae* in western Kenya.

Plant family	Species name	Common name
Leguminosae	*Cassia hirsuta* L.	Woolly senna
Solanaceae	*Datura stramonium* L.	Thorn-apple
Asteraceae	*Flaveria trinervia* Mohr	Clustered yellowtops
Rubiaceae	*Hamelia patens* Jacq.	Firebush
Convolvulaceae	*Ipomoea hildebrandtie* Vahl	Morning glory
Verbenaceae	*Lantana camara* L.	Wild sage
Asteraceae	*Parthenium hysterophorus* L.	Wild quinine
Asteraceae	*Psiadia punctulata* L.	Sunflowers
Euphorbiaceae	*Ricinus communis* L.	Castorbean
Leguminosae	*Senna bicapsularis* L.	Christmasbush
Leguminosae	*Senna didymobotrya* Fresen.	African senna
Bignoniaceae	*Tecoma stans* L.	Yellow bells
Asteraceae	*Tithonia diversifolia* Hemsl.	Tree marigold

Flowers and leaves were parts used in all the plant species for gas chromatography assays, except *R. communis* L. where stems were also used.

Table 2

Sugar content (obtained from gas chromatography analysis) of plant parts of each plant species.

Candidate plants	Plant parts	Glucose	Fructose	Sucrose	Mannose	Gulose	Galactose	Raffinose	Altrose	Allose	Lactose
Cassia hirsuta	Flower	+	+	−	−	+	−	+	−	−	−
	Leaf	−	−	−	−	−	−	−	−	−	−
Datura stramonium	Flower	+	+	−	−	−	−	−	−	−	−
	Leaf	+	−	−	−	−	−	+	−	−	−
Flaveria trinervia	Flower	+	−	−	+	+	−	−	−	−	−
	Leaf	−	−	−	−	−	−	−	−	−	−
Hamelia patens	Flower	+	−	+	+	−	−	−	−	−	−
	Leaf	−	−	−	−	−	−	−	−	−	−
Ipomoea hildebrandtie	Flower	+	−	−	+	−	−	−	−	−	−
	Leaf	−	−	−	−	−	−	−	−	−	−
Lantana camara	Flower	+	+	+	+	+	−	−	−	−	−
	Leaf	+	−	−	−	−	−	−	−	−	−
Parthenium hysterophorus	Flower	−	−	−	−	−	−	−	−	−	−
	Leaf	−	−	+	−	−	+	−	−	−	−
Psiadia punctulata	Flower	+	−	−	+	+	−	−	−	−	−
	Leaf	−	−	−	−	−	−	−	−	−	−
Ricinus communis	Flower	+	−	−	+	−	−	−	−	−	−
	Leaf	−	−	−	+	+	−	−	−	−	−
	Stem	+	+	−	−	−	−	−	−	−	−
Senna bicapsularis	Flower	+	+	+	−	−	+	−	−	−	−
	Leaf	+	+	+	+	+	−	+	−	−	−
Senna didymobotrya	Flower	+	+	+	+	+	+	−	−	−	−
	Leaf	+	+	+	−	+	−	−	−	+	−
Tecoma stans	Flower	+	+	+	+	+	−	+	+	−	−
	Leaf	+	−	−	−	−	−	−	−	−	−
Tithonia diversifolia	Flower	+	+	+	+	+	+	−	−	−	−
	Leaf	+	−	−	+	+	+	−	+	−	−

+, Sugar type present in the plant; −, sugar type absent in the plant.

Table 3

Percentages of *Anopheles gambiae* (data pooled for males and females) with sugar profiles matching sugar profiles of parts of each plant species (gas chromatography analysis). *n* = 80 (40 males and 40 females). NA, Not applicable.

	Percentage matching			Statistics	
Plant candidates	**Leaf**	**Flower**	**Stem**	***P*-value**	**Chi square**
Parthenium hysterophorus	17.50	0.00	NA	< 0.001*	15.34
Psiadia punctulata	1.25	0.00	NA	NA	1.00
Senna bicapsularis	0.00	1.25	NA	0.31	1.00
Tecoma stans	2.50	22.50	NA	< 0.001*	14.62
Ricinus communis	21.25	26.50	NA	0.14	3.88
Datura stramonium	0.00	1.25	NA	0.31	1.00
Cassia hirsuta	1.25	1.25	NA	1.00	0.00
Lantana camara	11.25	2.50	NA	0.02*	4.78
Senna didymobotrya	2.50	17.50	NA	< 0.001*	10.00
Tithonia diversifolia	0.00	2.50	NA	0.15	2.02
Ipomoea hildebrandtie	3.75	6.25	NA	0.46	0.52
Hamelia patens	0.00	26.20	NA	< 0.001*	15.30
Flaveria trinervia	0.00	1.25	NA	0.31	1.00

* Differences among plant-part categories significant.