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Nectar-related vs. human-related volatiles: behavioural response and choice by female and male *Anopheles gambiae* (Diptera: Culicidae) between emergence and first feeding

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Abstract

The close association of *Anopheles gambiae* Giles with humans and its females' ability to live on human blood alone suggest that females may ignore sources of sugar in favour of human blood as a source of energy. They have limited energy reserves at emergence, and at 27°C both sexes generally die if they do not feed during night 1, 24–36 h after emergence. Food preferences during this critical period were tested by measuring responses to volatiles from honey and soiled socks, which served as surrogates for nectar-related and human-related volatiles in a wind-tunnel olfactometer. Both sexes responded more strongly to honey than to human volatiles, and given a choice, preferred honey over human volatiles. After 5 days of sugar access and maturation, males continued to prefer honey volatiles, whereas females changed behaviour, responding almost exclusively to human volatiles. Night 1 experiments also demonstrated that: (i) females previously having had sugar during the night of emergence responded more strongly to human volatiles; (ii) large-bodied mosquitoes of both sexes responded more strongly to honey than small-bodied ones; and (iii) females were equally responsive to honey in both early and late scotophase but were slightly more responsive to human volatiles in late scotophase. These results indicate that for a female's first meal, sugar is a viable option and is preferred when nectar-related stimuli are strong. This supports field evidence that sugar-feeding is a significant component of *A. gambiae* female behaviour.

Introduction

Anopheles gambiae Giles *sensu stricto* (Diptera: Culicidae) is the principal vector of malaria in tropical Africa, where most malaria death and illness occur (Sachs & Malaney, 2002). The mosquito is attuned to humans, developing in surface water close to human habitations, often mating in the vicinity, feeding primarily on human blood, and usually resting inside houses (Gillies & Coetzee, 1987). This anopheline species is, in some respects, an ecological homologue of the culicine *Aedes aegypti* (Linnaeus) (Diptera: Culicidae), which also is synanthropic and anthropophilic and is the principal vector of dengue and urban yellow

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fever. The similarity between these disparate species is great enough to suggest that they are physiologically convergent, sharing a common diet and metabolism.

One aspect of the physiological convergence between *A. gambiae* and *A. aegypti*, their ability to derive all energy requirements from human blood, may allow the females to dispense with sugar-feeding altogether. This is due partly to human blood itself, which is limited in isoleucine and thus yields fewer mosquito eggs and more energy reserves (Briegel, 1985, 1990a,b). When humans are readily available and their blood can be taken frequently, *A. aegypti* females feed on them almost exclusively, and few contain plant sugar (Macfie, 1915; Edman, *et al*., 1992; Van Handel *et al*., 1994; Costero *et al*., 1998a). In the laboratory, they live long and reproduce well without sugar when offered human blood daily (Scott *et al*., 1997; Naksathit & Scott, 1998; Costero *et al*., 1998b; Harrington *et al*., 2001). Likewise, a relatively low proportion of *A. gambiae* s.l. females taken around houses contain plant sugar (Beier, 1996), from which it has been inferred that they sugar-feed infrequently. Furthermore, in the laboratory they reproduce well enough on human blood alone to offset a shorter life span without sugar (Gary & Foster, 2001).

Yet, even in these synanthropic species, sugar-feeding persists. The proportion of female *A. aegypti* containing plant sugar increased from 8% to 21% with increasing distance from urban centres in southern Mexico, perhaps because of a greater diversity of sugar sources in outlying zones (Martinez-Ibarra *et al*., 1997), and sugar-containing females comprised 50–75% of the population in rural areas of Florida, USA, where humans may be less readily available (Nayar, 1981; Van Handel *et al*., 1994). In *A. gambiae* s.l., even around houses the fructose-positive rate was 17.5% of host-seeking females in one village in western Kenya (Beier, 1996), and in The Gambia up to 30% of females resting indoors contained fluid in their crops (A.W.R. McCrae, personal communication). Analysis of recent samples in Tanzania also demonstrated a moderate frequency of recently sugar-fed *A. gambiae* s.l. (R.E. Gary, B.G.J. Knols & W.A. Foster, unpublished). Thus, sugar-feeding apparently continues to give both domestic species a competitive advantage under some circumstances, despite its presumed infrequent occurrence in the field and optional status in the laboratory.

The first few days of *A. gambiae* adult life may be particularly important for obtaining energy. Even after developing under optimum larval conditions, both sexes emerge with a very small energy reserve (e.g. Briegel, 1985, 1990b). At 27°C unfed females die 2–3 days after emergence (Takken *et al*., 1998). When larval conditions are poor, which is typical in the field, the survival period is shorter. A female may require at least one blood meal just to become gonoactive, then another to start developing eggs (Gillies, 1954; Briegel, 1990b; Takken *et al*., 1998). Whether or not females generally rely exclusively on blood both for energy and for egg development, their ability to obtain food during the first 1–2 days after emergence is critical to their success. In the laboratory *A. gambiae* females will respond to human volatiles and take blood as early as 24 h after emergence (W.A. Foster, unpublished), and the sibling species *Anopheles arabiensis* Patton will respond to floral volatiles at the same age (Healy & Jepson, 1988). Sources of sugar near breeding sites or close to human habitations may be just as valuable to females as blood, if not more so, for surviving this initial period of maturation and flight. Yet, it is not known whether either blood or sugar is taken at this early age in the field. Young adults undergo a rapid, energy-demanding dispersal prior to blood feeding, as indicated by release-recapture experiments in which both males and females were provided with sugar from the time of emergence, prior to release (Gillies, 1961). This suggests that sugar-feeding might have high priority for females on the night after emergence. Males may be completely dependent on plant sugar, dying without ever joining a mating swarm if they do not receive it during this period (R.E. Gary & W.A. Foster, unpublished).

The primary objective of the present study was to measure responses of unfed female and male *A. gambiae* to nectar-related and human-related olfactory stimuli during the critical period between emergence and death, to test whether attraction to human hosts always is the dominant food-related activity of females. Honey was used as a surrogate for nectar-related volatiles, and soiled socks provided human-derived volatiles. Their use is justified below (Materials and methods). The critical period proved to be primarily the first night after emergence. Responses to the same olfactory stimuli after 5 days of sugar-feeding and maturation served as a comparison. Sources of variation in the response of mosquitoes to honey volatiles were examined by rearing and testing small-bodied and large-bodied adults. Mosquitoes receiving a sugar meal on the night of emergence were tested the following night, to detect the effect of digested sugar, independent of age, on their response to human volatiles. Tests were conducted during the first and second halves of the scotophase to determine whether time of peak responsiveness differs for honey and human volatiles.

Materials and methods

Rearing and handling procedures

The Suakoko strain of *A. gambiae*, derived from Suakoko, Liberia, by M. Coluzzi in 1987, was used in all experiments. Colony and experimental mosquitoes were reared at Wageningen University at unregulated density in 1-litre of demineralized water in 20 × 10.5 × 8 cm pans, providing ~200 cm^2 of surface area, and fed powdered Tetramin® fish food according to routine methods that produced a wide range of adult body sizes, as occurs under field conditions. Ambient air was maintained at 27 ± 1°C, 80 ± 5% r.h., and all stages were exposed to a 12:12 light:dark cycle with 1-h crepuscular periods at both ends of the scotophase. Virtually all emergence occurred during scotophase, and adults were inactive during photophase unless disturbed. Female insemination status was not verified, because previously it had been determined to have no effect on response to host volatiles (W. Takken, unpublished). Studies of sexual maturation (Verhoek & Takken, 1994; Gary & Foster, 2001) indicated that neither sex tested on night 1 after emergence had engaged in mating activity prior to testing, but that most tested on night 5 had mated. In most experiments, adults emerging during the previous scotophase in a cage with two water wicks were transferred during photophase to cylindrical releasing cages, 7-cm inside diameter and 15-cm long, with two water wicks, screens at both ends, and a butterfly valve at the end that would fit into the downwind end of the flight section of a wind-tunnel olfactometer. Three

to five hours prior to onset of the next scotophase, releasing cages were transferred to a clear box in the olfactometer room, which provided mosquitoes with approximately the same physical conditions and photoperiod regime but which were exposed, throughout scotophase, to the low light intensity at which olfactometer tests would be conducted.

Olfactometer description and operation

A 2-port wind-tunnel olfactometer was used to measure the oriented flight response of adult mosquitoes to volatile chemical stimuli in an air current (see Knols *et al*., 1994). The bilaterally symmetrical flight section, 164 cm long × 60 cm wide × 60 cm high, was constructed of Lexan® on the side walls and ceiling, white formica on the floor and upwind end, and wirescreen on the downwind end. In the centre of the downwind screen was a round hole circumscribed by a mounting ring to hold a releasing cage. The side walls of the flight section were opaque white on the lower half, transparent on the upper half; the ceiling was translucent white. Diffuse overhead lighting was provided by four 30-watt incandescent light bulbs mounted at intervals above the ceiling, which at the voltage setting during tests produced a dim light, approximately 2.5 lux half-way between floor and ceiling of the flight section. The flight section contained two modifications from earlier uses (Knols *et al*., 1994; Braks & Takken, 1999; Braks *et al*., 2001; Pates *et al*., 2001): (i) its floor was lined in dark grey plastic sheet, covered at intervals by wet white paper towels and by white pans holding wet paper towels, providing more visual structure to the space and greatly increasing the humidity within the flight section, and (ii) all but a narrow vertical section (5–8 cm wide) of the screen at the rear of the flight section was covered in clear plastic film, restricting possible air contamination with volatiles from the person handling the releasing cage at the start of each test.

At the upwind end of the flight section, two round 5-cm ports, 30 cm apart and equidistant from the midline and from floor and ceiling, allowed the snugly fitting cylindrical openings of two glass traps to protrude slightly into the flight section. The trap section of the olfactometer matched the width and height of the flight section and housed the two traps, their supports, and their air supply. A light bulb identical to those over the flight section was positioned above each trap over the translucent ceiling. The traps were custom-made clear glass bottles with two elements: a large, 1.5-litre container with a screened lid at the upwind end, and at the downwind end a small trapping device that protruded through the port, consisting of a glass cylinder (5 cm diameter) downwind and a centrally positioned screen baffle (6 cm diameter) upwind, inside the container (see Knols *et al*., 1994). The latter allowed air to flow freely both through and around the baffle, but mosquitoes entering the cylinder could reach the container only by crawling around the baffle and were unlikely to leave by that route. Pressurized air was passed through a charcoal column, then through a large bottle of heated water to provide both heat and humidity. The conditioned air was split into two streams, passed into larger conduits, then conducted through the screened lids of the trap containers. Air speed at the ports varied from 14 to 22 mm s^{-1} in various regions of the effluent stream, as measured by electronic anemometer 5 mm from the protruding trap apertures. Air at this point (measured by telethermometer and hygrometer between tests) fluctuated between 25 and 28°C in different tests, subject to ambient fluctuations in the olfactometer room, and humidity was always above 90% r.h. At the rear of the flight section, temperature was the same or slightly lower, and the humidity generally was 80–90%.

Stimulus sources were held in stainless steel wirescreen lenticular enclosures (7 cm diameter), placed within the container elements of the traps. Human-host volatiles were provided by crumpled nylon socks, which were never washed and had been worn with shoes on one or more days before each series of tests. Previous studies had shown that *A. gambiae* has a predilection for human feet and for the volatiles from them, generated by the action of skin bacteria (e.g. De Jong & Knols, 1995; Knols & De Jong, 1996; Knols *et al*., 1997; Braks *et al*., 2000). Used (i.e. soiled) nylon socks have a similar effect and are at least as attractive as live human hands (Pates *et al*., 2001; Dekker *et al*., 2001; W. Takken, unpublished) and therefore served as an effective surrogate for a human host. Clean socks were found to have neither positive nor negative effects on mosquito trapping (Pates, 2002). Four socks were worn and used, one per test, repeatedly by the same person (W.A.F.), during a 12-week period of testing, and the complex of chemicals emanating from them was demonstrated to be attractive and hereafter is designated 'human volatiles' or 'human'. Use of just one human, upon whom *A. gambiae* readily feed, minimized extraneous sources of variability that would confound comparisons. Controls consisted of identical wirescreen enclosures containing a sheet of dry, crumpled paper towel, which also was behaviourally neutral and impeded airflow to the same degree as a sock. Nectar-related stimuli were provided by commercial multifloral honey from a single source (de Traay, Lelystad, The Netherlands), about 10 g being spread over a paper towel, which was then crumpled. The complex of chemicals from honey previously has been used as an effective surrogate for sources of nectar (Wensler, 1972; Vargo & Foster, 1982; Hancock & Foster, 1989, 1997, 2000). Honey was used because the natural sources of *A. gambiae* sugar meals are unknown, and honey contains many of the plant volatiles attractive to pollinators and utilized by mosquitoes to locate flower nectar (Foster & Hancock, 1993; Knudsen *et al*., 1993; Foster, 1995). Honeys differ widely in volatile composition (e.g. Bicchi *et al*., 1983; Bouseta *et al*., 1992; Verzera *et al*., 2001), depending on their plant sources. They may yield higher concentrations of some semiochemicals produced by plants from which the honeys are derived, lack others altogether, or include some bee-derived and microbe-produced volatiles. Furthermore, the attractive properties of extrafloral nectars, which are likely sources of sugar for *A. gambiae*, have yet to be identified for any insect. Nonetheless, honey volatiles provide effective, generic cues for the presence of sugar to mosquitoes, including *A. gambiae*, as demonstrated in the current study. In early tests, controls for honey consisted of crumpled paper towel containing syrupy, saturated sucrose solution. Mosquitoes were found not to respond even to the aqueous component of sucrose syrup, and subsequent tests employed crumpled dry paper as controls.

Experimental procedures

Four kinds of tests were conducted with the two olfactometer ports as alternatives: honey vs. blank, human vs. blank, blank vs. blank, and honey vs. human (see table 1

Table 1. Summary of two-choice olfactometer experiments on *Anopheles gambiae*, showing number of replicates of a test series, total sample sizes, and ranges of results. Blank-trap-adjusted trap totals are presented in figures and text.

Experiment or treatment	Night of test	Type of test[b]	Sex	No. replicate nights	Total no. tests	Total no. mosquitoes flying (n)	Total no. mosquitoes trapped (and % of total plus range among replicates)	
Age and maturation	1						Blank	Honey
		Honey	Male	4	15	578	3 (<1: 0–2)	133 (23: 15–38)
			Female	4	15	600	1 (<1: 0–1)	226 (38: 23–47)
							Blank	Human
		Human	Male	8	15	520	31 (6: 1–12)	80 (15: 0–31)
			Female	8	15	546	20 (4: 1–11)	132 (24: 4–34)
							Blank	Blank
		Double blank	Male	2	4	141	3 (2: 0–4)	2 (1: 0–3)
			Female	2	4	150	1 (<1: 0–1)	2 (1: 0–3)
							Honey	Human
		Honey/human choice	Male	4	12	440	168 (38: 29–43)	3 (<1: 0–2)
			Female	4	12	471	214 (45: 26–52)	19 (4: 1–9)
	5						Blank	Honey
		Honey	Male	3	6	170	3 (2: 0–5)	62 (36: 20–54)
			Female	3	6	223	16 (7: 0–18)	11 (5: 1–9)
							Blank	Human
		Human	Male	3	7	218	9 (4: 3–6)	30 (14: 1–24)
			Female	3	7	268	3 (1: 0–3)	147 (55: 5–87)
							Blank	Blank
		Double blank	Male	3	5	137	3 (2: 2–4)	5 (4: 0–11)
			Female	4	6	227	11 (5: 0–10)	3 (1: 0–3)
							Honey	Human
		Honey/human choice	Male	3	8	225	64 (28: 26–35)	3 (1: 1–2)
			Female	3	8	252	12 (5: 0–13)	163 (65: 32–86)
Sugar on night 0[a c]	1						Blank	Human
		Human	Male	7	14	497	47 (9: 4–17)	86 (17: 8–20)
			Female	7	14	521	32 (6: 3–12)	204 (39: 30–61)
Larval density	1						Blank	Honey
High		Honey	Male	2	7	268	0 (0: 0–0)	60 (22: 18–32)
			Female	2	7	279	0 (0: 0–0)	91 (46: 31–38)
Low		Honey	Male	2	7	271	0 (0: 0–0)	125 (33: 42–56)
			Female	2	7	279	1 (<1: 0–1)	165 (59: 59–61)
Testing time	1						Blank	Honey
Early		Honey	Female	3	9	275	1 (<1: 0–1)	160 (58: 53–63)
Late			Female	3	9	261	0 (0: 0–0)	135 (52: 36–72)
							Blank	Human
Early		Human	Female	3	9	263	1 (<1: 0–1)	15 (6: 2–13)
Late			Female	4	15	431	2 (<1: 0–1)	53 (12: 3–20)

[a] Night 0 was the night of emergence, and night 1 was the latter half of the following night, approximately 30 ± 5 h after emergence. Night 5 occurred 4 days (about 96 h) after that. All mosquitoes tested on night 5 were provided with 10% sucrose between the day after emergence and the day preceding the test on night 5.

[b] Tests that contrasted catches in a trap containing volatiles with a blank trap are measures of the strength of response to one volatile complex, whereas tests that contrasted honey volatiles with human volatiles are measures of the strength of choice between the two. Tests employing two blank traps were used to detect left-right bias and served as a baseline for detecting any influence of the presence of volatiles in the olfactometer flight section on the blank-trap catches in blank-vs.-volatile experiments.

[c] The human-response tests on night 1 of the age and maturation experiment, presented above, served as water-only controls for tests of the effect of sugar provided on night 0. The 7 replicates of tests of the latter were conducted on the same dates as 7 of the 8 replicates of the former, and within each replicate night the tests alternated between water-only and sugar-supplied groups.

for overview). Except where stated otherwise, each test was conducted as follows: 40 males and 40 females were transferred from an emergence cage to a releasing cage during the middle portion of the first photophase following emergence. A series of 30-min tests was carried out during the second half of the subsequent scotophase, designated night 1, within a 4-h period corresponding to approximately 0100–0500 h, which is when peak biting activity is reported to occur in the field (e.g. Haddow, 1954; Hamon, 1963; Lindsay *et al*., 1989). Because nearly all emergence occurred in the first half of scotophase (1800–2400 h) of night 0, in accord with the results of Reiter & Jones (1975), mosquitoes had emerged 30 ± 5 h prior to the time of testing on night 1. As an external control for olfactory response tests on night 1, some groups of mosquitoes were retained in the 7-l cages and allowed access to one wick each of 10% sucrose solution and of water during the following 5 days and 4 nights (nights 1–4) and were tested on night 5 after emergence. At this age, females of this strain of *A. gambiae* are known to respond strongly to the presence of human-host volatiles, including soiled socks (e.g. Knols *et al*., 1994, 1997; Braks *et al*., 2000; Dekker *et al*., 2001; Pates *et al*., 2001).

Fewer mosquitoes were trapped in the olfactometer during the last hour of scotophase (0500–0600 h), consistent with field data, so tests generally were discontinued before this time. During each testing period, a series of two to six tests was conducted, each test lasting 30 min, starting when the releasing-cage valve was opened and ending when it was closed and the traps were removed. Throughout the testing period, all olfactometer parts were handled only with rubber gloves, and procedures minimized chances of cross-contamination between volatiles in successive tests. In several test series, more than one kind of test was conducted, e.g. honey vs. blank and blank vs. blank, in alternation. During a 10–15 min interval after each test's completion, mosquitoes yet to be tested were shrouded, the lights in the flight section increased to maximum intensity, and the mosquitoes remaining in it removed by vacuum. Then the light was returned to its lowest setting, the untested mosquitoes unshrouded, new traps installed in the trap section, and a new releasing cage inserted in the flight section. The positions of baited and blank traps, or of honey-baited and human-baited traps, were alternated between tests within a single test series (= replicate), to minimize possible left–right bias. At the end of a test series, the mosquitoes in the traps and releasing cages from each test were anesthetized, removed, and counted.

Three other kinds of experiments were conducted to explore dimensions of the response to honey or human volatiles (see table 1), with modifications of the standard night-1 protocol:

1. To determine whether sugar-feeding would increase the response to human volatiles, independent of aging and maturation, as experienced by mosquitoes fed for five days (see above), emerging mosquitoes were provided with two wicks of either 10% sucrose (two replicates, four tests) or 50% honey solution (honey diluted with equal volume of water) (five replicates, ten tests) on night 0. Sugar sources were removed the next morning, and mosquitoes were tested on night 1. The control groups, which also served as the standard of comparison for 5-day-old sugar-fed mosquitoes, had access to two water wicks between emergence and testing. Despite the weak response of mosquitoes to honey in the olfactometer on night 0 (see Results), direct observation (at about 0400 h) and the experimental results themselves demonstrated that large numbers did feed on honey in 7-l cages within 12 h of emergence.

2. To determine whether small body size reduced the response to nectar volatiles, as suspected in experiments described above, large and small mosquitoes were obtained by rearing larvae at low (100 per pan) and high density (300 per pan), respectively, in 1-l pans containing 0.5 l of demineralized water (surface area ~180 cm^2), and providing them different food regimens. Low-density pans were given 20 mg of food per day for three days, then 30 mg per day; high-density pans were given 30 mg per day for three days, then 60 mg per day. Large and small individuals were tested on night 1 both in separate series and in a combined series, as necessitated by their availability after emergence. An approximation of body size (Briegel, 1990b; Armbruster & Hutchinson, 2002; cf. Siegel *et al*., 1994) was obtained from samples of 22–47 extra males and females of each cohort tested, by measuring wing length to the nearest 0.027 mm from alular notch to wing tip.

3. To determine which time of night was most appropriate for comparing responses to human and nectar stimuli, female responses were measured in the first and second halves of the night. Thirty females were used per test, housed in releasing cages half the length of those described above. For each stimulus, six 20-min tests were conducted between approximately 1930 and 2300 h, and another six tests between approximately 0130 and 0500 h. These series of tests were replicated on three and four different nights, respectively.

Tests of responsiveness of unfed *A. gambiae* to volatiles had been planned for nights 0, 1 and 2. However, response to honey volatiles in the windtunnel was minimal on the night of emergence and, by the latter half of night 2, mortality was high, and many mosquitoes still alive either failed to leave the releasing cage or appeared weak and came to rest on the floor of the flight section. To quantify the survival of adults without food under these laboratory conditions during the critical 3-day period after emergence, mortality was recorded for three replicate cohorts of males and females emerging during the first 7 h of three consecutive scotophases (i.e. 1800–0100 h), by which time *c* 95% of the pupae placed in a cage each afternoon had emerged. They were segregated by sex in 7-l acrylic cages (two cohorts) or 30 cm^3 netting cages (one cohort), totalling six cages, each containing two water wicks. Three cages had 100 females each, two cages had 100 males each, and a sixth cage had 79 males. Starting at 0900 h in the next photophase (i.e. when all individuals were 8–15 h old), and every 8 h thereafter, dead mosquitoes were removed and counted. If still moving, they were scored as dead if unable to right themselves when turned upside down. Due to escape, possible miscount, and accidental death in cage sleeves, the final count of mosquitoes dying of food deprivation was 295 females and 264 males.

Data analysis

The number of mosquitoes leaving the releasing cage and entering the flight section of the wind tunnel comprised the basic sample size (n), the total number of mosquitoes flying in each test. The numbers caught in each trap (N = honey-baited, H = human-baited, B = blank) were used to derive

the proportional trap catch (N/n, H/n, and B/n). In analyses of direct behavioural choice (volatiles vs. blank, honey volatiles vs. human volatiles, and blank vs. blank), raw trap-catch values were analysed with 1:1 goodness-of-fit χ^2 tests. In statistical comparisons of strength of response to volatiles (i.e. relative attraction) when tested against a blank trap, χ^2 tests of independence were used, employing blank-adjusted trap values that were contrasted with the numbers flying but not responding. The resulting honey response (N-B) and human response (H-B) were measures adjusted to more accurately reflect the number attracted to the volatile, and the numbers not attracted were n-(N-B) or n-(H-B). The adjustment was based on this assumption: mosquitoes that entered blank traps did so without regard to the volatile stimulus, so a similar proportion of mosquitoes probably entered baited traps, and these should be considered part of the flying-but-not-attracted category. All replicates were pooled for analysis by Statistica (StatSoft, 1999). Ranges of results among replicates are presented in table 1, to reflect heterogeneity. Fisher's Exact test and an exact binomial test (Sokal & Rohlf, 1981) were applied to contingency tables with small expected values, as substitutes for independence and goodness-of-fit χ^2 tests, respectively, but the results were similar and are not presented. Significant interactions between factors affecting response were detected by log-linear analysis of multi-way frequency tables (StatSoft, 1999), which considered three behavioural categories of raw data: entered left trap, entered right trap, and remained in flight section. Survival analyses and tests were based on Statistica life-table computations. Differences in wing length of mosquitoes reared at low and high density were detected by Mann-Whitney *U*-test. Confidence limits for percentages presented in the figures were calculated from total proportions according to Snedecor & Cochran (1980).

Trapping efficiency, the measure of response strength to volatiles, often was below 50%, and bias for the baited trap was often not unanimous, yet probably all mosquitoes in a test would have benefited from feeding. Thus, samples were large and each replicate consisted of several tests. Furthermore, trapping efficiency was subject, potentially, to several uncontrolled variables. Tests within a series on the same day (i.e. same replicate) gave more consistent results that those performed on different days. These replicate differences probably were caused by day-to-day fluctuations both in average body size and in physical conditions within the olfactometer, so multiple replicates were conducted.

Results

Survival with access only to water from emergence

Estimates of the numbers of pupae and adults of *A. gambiae* in the emergence cage of the first replicate of the survival experiment at 2400 h indicated that approximately 95% of pupae collected the previous day emerged as adults during the first 6 h of scotophase, and only these were used. In the two subsequent replicates, an additional hour of emergence time was allowed. The mortality pattern was similar in both sexes and all three replicates, but males tended to die sooner (Cox's F Test: $F = 1.415$, $P < 0.0001$). Mortality was negligible until the second half of night 1, before they were 24–39 h old, and mortality peaked between 0900 and 1700 h the next day, before mosquitoes were 40–47 h old. Fewer than 18% of males and 42% of females were alive by the beginning of night 2. By the third hour of the following day, those values were 8% and 4%, respectively, indicating a precipitous drop in living females during night 2. The last two individuals of both sexes died before they were 72–79 h old, during night 3. Assuming median emergence time to be in the middle of the 7-h emergence period, mean survival was as follows: males, 42.9 h (log-likelihood median age: 40.9 h), females, 46.6 h (log-likelihood median age: 45.6 h).

Response to honey volatiles on night of emergence: night 0

To determine whether mosquitoes would respond to honey-source volatiles in the wind-tunnel olfactometer on the night they emerge (night 0), on each of two nights >100 pupae were placed in an emergence cup at the rear of the flight section prior to 1600 h. On the first night, the cup was open to the flight section, and throughout the scotophase adults could enter traps baited with honey or with sucrose (the control), respectively. No males or females were caught in either trap but were scattered throughout the flight section at onset of photophase. On the second night, the mosquitoes emerged within a 7-l cage and its 12.2 cm door was opened at about 0230 h, allowing them the last 3.5 h of scotophase to leave the cage, fly the length of the flight section, and enter the traps. At dawn, the honey trap contained three males and five females; the sucrose trap contained three females. Most mosquitoes were aggregated around the rear of the flight section. It was concluded that under these conditions, neither sex could exhibit sustained, oriented, upwind flight toward honey at < 12 h after emergence.

Effect of age and maturation on response to honey and human volatiles: night 1 (water only) vs. night 5 (sugar fed)

Strength of response to either honey or human volatiles alone (paired with blank control)

Night 1. When honey was presented opposite a blank on night 1 (1 day after emergence), a substantial proportion of both sexes entered the baited trap within 30 min. The proportion of males caught in honey traps, out of all males flying, varied moderately among replicate series (different days) (table 1), with a combined unadjusted representation of 23%, whereas <1% entered blank traps. A significantly larger proportion of females than males responded to honey ($\chi^2 = 31.5$, $P < 0.001$), with a combined honey-trap female representation of 38%, whereas < 1% entered blank traps. When human was presented in the baited trap, catches of both sexes were smaller than when honey was presented (males: $\chi^2 = 34.3$, $P < 0.001$; females: $\chi^2 = 39.7$, $P < 0.001$) (fig. 1a), the blank catches were higher (males: $\chi^2 = 27.0$, $P < 0.001$; females: $\chi^2 = 19.4$, $P < 0.001$), and there was much greater variation among replicate series (table 1). Nonetheless, among males, a combined representation of 15% were in the human trap and 6% in the blank trap, a significant bias for human ($\chi^2 = 21.6$, $P < 0.001$). Among females the bias was stronger, with 24% in the human trap and 4% in the blank trap ($\chi^2 = 82.5$, $P < 0.001$). The difference between males and females in their response to human also was significant ($\chi^2 = 25.6$, $P < 0.001$). Log-linear analysis of a three-way frequency table of the above data by mosquito location in the olfactometer detected highly significant

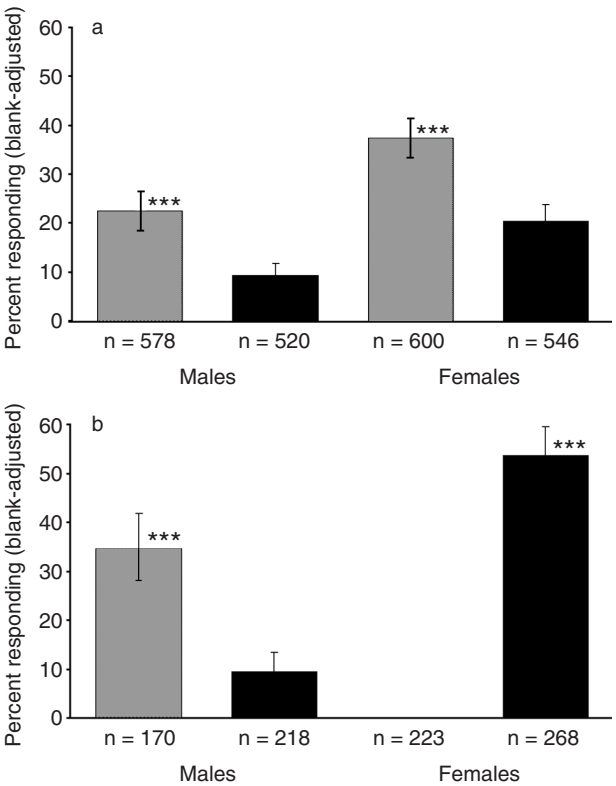

Fig. 1. Response of *Anopheles gambiae* to honey (■) and human (■) volatiles presented separately, with a baited trap opposite a blank trap, ± 95% confidence limits. Attractive response is based on proportion caught in baited trap out of total mosquitoes flying, but adjusted to account for catch in blank trap. a) Night 1 (1 day old), water only. b) Night 5 (5 days old), after continuous access to sucrose. *** Denotes significantly greater ($P < 0.001$) than counterparts of the same sex. (For other statistical comparisons, see text.)

interactions both between sex and location (partial assoc: $\chi^2 = 44.6$, $P < 0.001$; marginal assoc: $\chi^2 = 43.4$, $P < 0.001$) and between kind of volatile and location (partial assoc: $\chi^2 = 79.0$, $P < 0.001$; marginal assoc: $\chi^2 = 77.8$, $P < 0.001$).

Two series of double-blank tests yielded < 2% per trap of the total mosquitoes flying (table 1) and no significant difference between them for males ($\chi^2 = 0.2$, $P > 0.6$) or females ($\chi^2 = 0.3$, $P > 0.5$). This indicated a minimal behavioural response to the current, humidity, or temperature of the effluent air in the absence of designated baits, and a lack of bias between ports.

Night 5. After five days of continuous sugar availability, maturation, and mating, the unadjusted proportions of males collected in honey traps had nevertheless increased moderately above that on night 1 (from 23% to 36%) ($\chi^2 = 10.4$, $P < 0.002$), but that of females had dropped steeply (from 38% to 5%) ($\chi^2 = 115.1$, $P < 0.001$) (fig. 1b, cf. fig. 1a). When blank-trap adjusted, the female honey catch was zero. The proportions of males in human traps on night 5 remained about the same as on night 1 (15% and 14%, respectively) ($\chi^2 = 0.01$, $P > 0.9$), but that of females increased steeply from 24% to 55% ($\chi^2 = 92.0$, $P < 0.001$). As on night 1, the 5-day-old males exhibited a significantly stronger response to honey (paired with a blank) than to human ($\chi^2 = 36.7$, $P < 0.001$), but unlike females on night 1, 5-day-old females exhibited a much stronger response to human than to honey ($\chi^2 = 169.5$, $P < 0.001$) (fig. 1b). A direct comparison between 5-day-old males and females showed that although females again outperformed males in their response to human ($\chi^2 = 104.3$, $P < 0.001$), males at this age had the stronger honey response ($\chi^2 = 91.1$, $P < 0.001$). Three-way log-linear analysis by location, pitting volatiles against blanks, indicated that there were significant interactions both between kind of volatile and location (partial assoc: $\chi^2 = 36.7$, $P < 0.001$; marginal assoc: $\chi^2 = 36.0$, $P < 0.001$) and between sex and location (partial assoc: $\chi^2 = 9.2$, $P < 0.01$; marginal assoc: $\chi^2 = 8.4$, $P < 0.014$). In double-blank tests, trap catches averaged < 5%, but among females there was a significant left-right bias ($\chi^2 = 4.5$, $P < 0.033$). The latter was the effect of one test of 40 females, in which the catches were one and eight mosquitoes, respectively, probably representing normal sampling error.

Choice between honey and human volatiles presented simultaneously

Night 1. In a direct comparison of attraction to honey and human, when a choice was presented, there was a strong bias for honey in both sexes (males: $\chi^2 = 171.3$, $P < 0.001$; females: $\chi^2 = 114.7$, $P < 0.001$) (fig. 2a). Out of all individuals flying, males were trapped in only slightly smaller proportions than females both in honey traps (37% and 41%, respectively) and human traps (< 1% and 8%, respectively), but the choice was influenced significantly by sex ($\chi^2 = 24.2$, $P < 0.001$), with males showing the stronger honey bias. This interaction was confirmed by 2-way log-linear analysis (partial and marginal assoc. both $\chi^2 = 530.6$, $P < 0.001$). In a striking exception to the general result, in one early replicate consisting of a single test of 50 males and 50 females, most females caught were in the human trap (48%), very few in the honey trap (2%), though when presented with honey vs. blank in this same test series, nearly all trapped females entered the honey trap (33%), very few in the blank (2%). In a seemingly identical human vs. honey test two days later, female catches were reversed: 9% in the human trap, 26% in the honey trap, similar to all 13 tests with males and all 11 subsequent tests with females. The anomalous first test is not included among the summary data of table 1 but was included in the statistical analysis. When the two early testing dates are removed from consideration, the result is essentially the same: honey, 38% of males, 48% of females; human, < 1% and 4%, respectively. The sex difference in the volatile-based response remained significant ($\chi^2 = 4.6$, $P < 0.032$), and the female preference for honey over human was even stronger. The competitive nature of the honey–human choice is evident in a comparison of the total human catches when human was presented alone and when pitted against honey in a choice test: male response to human alone, 15% (blank-adjusted, 9%), to human in a choice, < 1%. Female response to human alone, 24% (blank-adjusted, 21%), to human in a choice, 8% (figs. 1a vs. 2a).

Night 5. When 5-day-olds were presented with honey and human simultaneously, males showed a significant bias for honey over human (28% vs. 1%) ($\chi^2 = 55.5$, $P < 0.001$), similar to their bias on night 1 (37% vs. <1 %) (difference between nights 1 and 5: $\chi^2 = 1.0$, $P > 0.3$). Females with the same

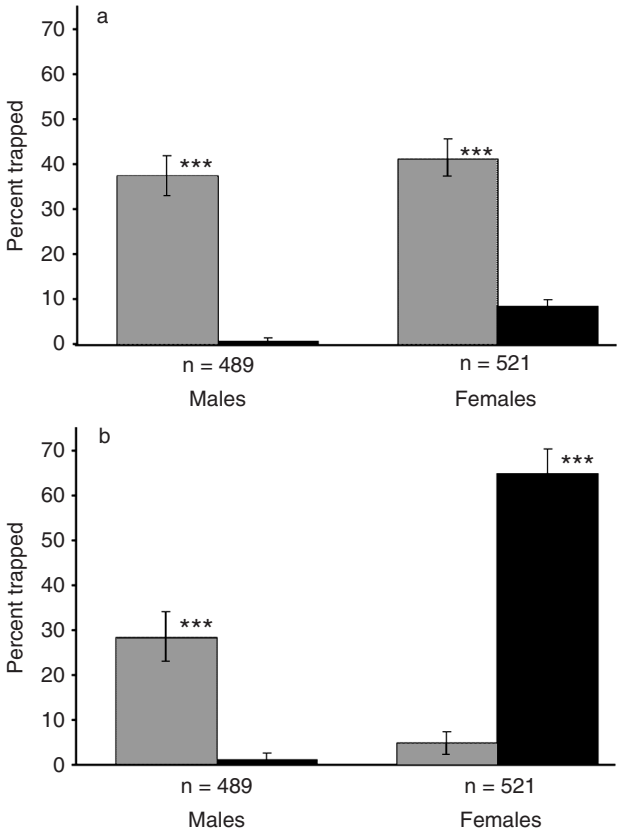

Fig. 2. Response *Anopheles gambiae* to honey(■) and human (■) volatiles presented simultaneously in separate traps, ± 95% confidence limits. Attraction bias (choice) is based on proportion caught in each trap out of total number of mosquitoes flying. a) Night 1 (1 day old), water only. b) Night 5 (5 days old), after continuous access to sucrose. *** Denotes significantly greater ($P < 0.001$) than counterparts of the same sex. (For other statistical contrasts, see text.)

choice were biased in the opposite direction (5% vs. 65%) ($\chi^2 = 130.3$, $P < 0.001$), the reverse of night 1 (41% vs. 8%) (difference between nights 1 and 5: $\chi^2 = 244.5$, $P < 0.001$) (fig. 2b). A direct comparison of males and females in this choice test demonstrated a significant sex difference in bait bias ($\chi^2 = 176.8$, $P < 0.001$) at this age and physiological state. As in 1-day-olds, the competitive nature of the honey–human interaction was illustrated by the sharp reduction in the human catch of 5-day-old males, from 14% opposite a blank (blank-adjusted, 10%) to 1% opposite honey (figs 1b vs. 2b). A similar competitive effect of honey on the response to human or vice versa, was not evident in 5-day-old females, suggesting complete dominance of human volatiles in females under both conditions at this age.

Effect of sugar-feeding during night 0 on strength of response to human volatiles on night 1

When provided with sucrose or honey on the night of emergence, males and females commonly fed on it before the onset of photophase in the 7-l emergence cages, but the exact proportion taking sugar meals was unknown. Among males, the proportion caught in human traps on night 1 was not significantly different between those offered sugar (17%; blank-adjusted, 8%) and those with access only to water (15%; blank-adjusted, 9%) ($\chi^2 = 0.8$, $P > 0.3$). Among females, however, significantly more were trapped when previously offered sugar (39%; blank-adjusted, 33%) than when provided with water only (24%; blank-adjusted, 21%) ($\chi^2 = 21.3$, $P < 0.001$) during the same test series (see table 1). This difference between sugar-access and water-only females was particularly wide in three of the seven replicates, involving 194 sugar-offered females and 174 water-only females, six tests per replicate for each treatment. In these three replicates combined, exceptionally few water-only females entered human-baited traps (12% human, 5% blank), and exceptionally large numbers of sugar-offered females entered them (49% human, 7% blank). As in other replicates of this experiment, the sugar and water groups were tested in alternate succession, and the human ports were switched for each pair of tests. Therefore, the pronounced differences in three replicates could be attributed only to an unusual physiological status of the particular cohorts of mosquitoes or to undetected physical conditions. In the other four replicates, sugar-offered females were caught in human traps only 0–12% more frequently than water-fed females. Both sugar-offered and water-only mosquitoes were caught more often in blank traps during these tests with human volatiles than they were during tests with honey volatiles in other experiments (see table 1). Two-way and three-way log-linear analyses of all mosquitoes by location, comparing sugar-offered and water-fed mosquitoes, confirmed the presence of a significant interaction between diet and location among females. Among both sexes combined they detected significant interactions both between sex and location (partial assoc: $\chi^2 = 69.1$, $P < 0.001$; marginal assoc: $\chi^2 = 68.5$, $P < 0.001$) and between diet and location (partial assoc: $\chi^2 = 33.9$, $P < 0.001$; marginal assoc: $\chi^2 = 33.3$; $P < 0.001$).

Effect of body size on strength of response to honey volatiles on night 1

Measures of responsiveness to volatiles on night 1 indicated that small-appearing mosquitoes were less often caught in baited traps. This suggested that size fluctuation in the experimental material might account for some of the large differences in response among replicates (table 1). Preliminary experiments to test for this effect were made by sorting males and females by eye according to apparent body size and testing them separately on night 1 in the age and maturation experiments described above. Their responses to human were weak in both sexes and both size classes, but in a first test the response of large-bodied females to honey was more than twice that of small-bodied ones. In a second test, the difference was three-fold. These results suggested that inadvertent variation in body size, generated by methods of mosquito production, might strongly affect performance in the olfactometer.

In a controlled test of the effect of body size on attraction to honey, mosquitoes were reared at high and low density, to obtain adults of large and small sizes and energy reserves, as indicated by wing length (table 2). High-density mosquitoes started pupating earlier than low-density mosquitoes, as observed previously by Lyimo *et al*. (1992). Within each sex, those reared at high density had significantly shorter wing lengths, and at both rearing densities males were significantly smaller than females. Mosquitoes of the same sex and rearing density, but emerging on different days, also

Table 2. Effect of *Anopheles gambiae* body size on honey-baited and blank-trap catches in two-choice olfactometer, comparing 1-day-old males and females, reared at high or low density and maintained on only water after emergence.

Sex	Larval density (body size)	Replicate dates	Mean wing length (mm) ± SE[a]	No. mosquitoes with wing measured (n)	No. tests per replicate	Total mosquitoes flying (n)	No. mosquitoes trapped[a]			
							Blank Trap	Blank trap % of total flying	Honey trap	Honey trap % of total flying[b]
Males	High (Small)	1-II	2.66 ± 0.015	35	5	190	0	0	35	18
		3-II	2.61 ± 0.013*	42	2	78	0	0	25	32
		Total	2.63 ± 0.010	77	7	268	0	0	60	22
	Low (Large)	2-II	2.76 ± 0.017	31	5	193	0	0	81	42
		3-II	2.79 ± 0.023	26	2	78	0	0	44	56
		Total	2.77 ± 0.014	57	7	271	0	0	125	46
Females	High (Small)	1-II	2.75 ± 0.022	22	5	199	0	0	61	31
		3-II	2.71 ± 0.013	47	2	80	0	0	30	38
		Total	2.73 ± 0.011	69	7	279	0	0	91	33
	Low (Large)	2-II	2.89 ± 0.015	37	5	200	0	0	117	59
		3-II	2.98 ± 0.019*	37	2	79	1	<1	48	61
		Total	2.93 ± 0.013	74	7	279	1	<1	165	59

* For both sexes, mosquitoes reared at high density but emerging later tended to have shorter wings, whereas those reared at low density but emerging later tended to have longer wings. Among high-density males and low-density females, this effect of emergence date was significant ($U = 514$, $P < 0.024$, and $U = 385$, $P < 0.002$, respectively).

[a] Differences in wing length between males and females were significantly different at both high ($U = 1172$, $P < 0.0001$) and low ($U = 658$, $P < 0.0001$) rearing densities. Differences in wing length between mosquitoes reared at high and low densities were significant in both males ($U = 679$, $P < 0.0001$) and females ($U = 402$, $P < 0.0001$). [b] Differences in honey-trap catch between small and large mosquitoes were highly significant for both sexes. (See text for statistical analysis.)

had different wing lengths. Among high-density males and low-density females, those emerging earlier had significantly longer wings and shorter wings, respectively, than their same-sex counterparts emerging later (see table 2). Approximately twice as many large (i.e. low-density, long-winged) mosquitoes as small mosquitoes of each sex responded to honey (table 2) (males: $\chi^2 = 33.7$, $P < 0.001$; females: $\chi^2 = 38.5$, $P < 0.001$), and within each size class, more females than males responded (small: $\chi^2 = 7.2$, $P < 0.008$; large: $\chi^2 = 8.8$, $P < 0.003$). Three-way log-linear analysis of mosquito numbers by location detected significant interactions both between body size and location (partial assoc: $\chi^2 = 74.8$, $P < 0.001$; marginal assoc: $\chi^2 = 73.5$, $P < 0.001$) and between sex and location (partial assoc: $\chi^2 = 17.0$, $P < 0.001$; marginal assoc: $\chi^2 = 15.7$, $P < 0.001$). The difference in body size according to day of emergence appeared not to affect the proportions responding to honey (table 2).

Effect of early or late scotophase of night 1 on strength of response to honey or human volatiles

In the series of tests comparing responses of females during the first and second halves of night 1, more than half of flying females were captured in honey traps within 20 min, in approximately equal proportions (58% and 52%, respectively) in the two time periods ($\chi^2 = 2.0$, $P > 2.0$). Responsiveness to human was much lower than to honey in both time periods. Fewer females were captured in human traps during the first half of the night (6%) than the second half (12%), a pooled significant difference ($\chi^2 = 8.2$, $P < 0.004$), with statistically insignificant (but nonetheless considerable) heterogeneity among replicate series (see table 1). Further experiments are needed to establish with certainty the late-night bias of responsiveness to human. The low attraction to human volatiles may be attributed partly to fluctuations in flight-section temperature, which during some series reached 29.9°C, while during others did not exceed 26.8°C. Small body size of some cohorts also may have contributed to low trap catches.

Discussion

Survival after emergence

The rearing and maintenance conditions employed here permitted survival of both sexes for about 2–3 days without food, similar to other studies (Takken *et al.*, 1998). Most males were dead before the second full night of adult life, and most females were dead by the following morning. This short life demonstrates that the night of emergence (night 0) and the first full night of life (night 1), when both sexes are 24–36 h old, are crucial for obtaining energy sufficient to proceed with adult activity. Natural temperatures fluctuate between higher and lower levels than those used here. At lower median temperatures, which would extend survival, the first few days probably nevertheless comprise a critical time during which either sugar or blood must be taken for survival and reproduction. It appears that even at high temperatures feeding is unlikely to occur on the night of emergence unless sugar is accessible in the immediate vicinity, because airborne orientation toward honey was weak at this age (< 12 h), and blood is never taken on the night of emergence (W. Takken, unpublished). Most likely, in nature males must find sugar by the following night, and

females must find sugar or blood on that night or early part of the night after that (night 2). Mosquitoes that develop under suboptimal larval conditions are particularly vulnerable to this stringent timetable, because both foraging performance, as shown in this study, and survival time are compromised by reduced body size and energy reserve (Briegel 1990a,b; Takken *et al*., 1998).

Time-of-night differences in responsiveness to honey and human volatiles

The comparison of responses to honey and human during early and late scotophase revealed no strong temporal bias for either behaviour. Some mosquito species are reported to segregate blood-feeding and sugar-feeding activities in the field (Foster, 1995). *Anopheles gambiae* appears to concentrate its blood-feeding activity in the field during the second half of the night (e.g. Lindsay *et al*., 1989), whereas spontaneous flight activity in the laboratory is concentrated in the early part of the night among virgin females and throughout the night in inseminated females (Jones & Gubbins, 1978). The weak response to human at the age tested here provided insufficient data to demonstrate a strong late-night bias even for blood feeding, though a weak one was detected. The substantial data on the honey response suggest that sugar-feeding is equally likely to occur before or after midnight. Therefore, the second half of the scotophase was a suitable time for testing responses of 1-day-old mosquitoes to both kinds of volatiles in the olfactometer in the present study.

Predominance of honey responsiveness among teneral females and all males

Olfactometer tests of responsiveness to honey or human 24–36 h after emergence demonstrated a strong female response to both kinds of food when they were presented alone, though the response to honey was stronger. The male response to both foods was weaker than that of females. It is doubtful that the motivation (i.e. behavioural responsiveness, *per se*) to obtain sugar was lower in males than in females; their poorer performance is attributed to some aspect of the ability to negotiate the windtunnel and enter the traps. The lower but nonetheless significant male response to human may indicate either a broadening attraction to unspecific organic chemicals as energy reserves dwindle or an alternative mating tactic, in which males are attracted to the vicinity of human hosts (see Yuval, 1994), where virgin females converge. This is consistent with the result that even among 5-day-old sugar-fed males, there was a moderate response to human when a honey alternative was not offered. Yet, the responses of both 1-day-old and 5-day-old males to honey were stronger than those to human, whether honey was paired with a blank or with human, indicating that the energy-driven attraction of males to sugar was always dominant in the second half of scotophase.

The response to human on night 1 contrasts with the results of Takken *et al*. (1998), who found no response to a hand 24 h after emergence, among either small or large females. Possible explanations include differences between human hosts, between foot and hand volatiles, between mosquito strains, or between physical testing conditions. It might be expected that sugar, available from emergence in the Takken *et al*. (1998) study, would inhibit the host response on night 1, but present results indicate that the opposite is true.

The relative responsiveness of 5-day-old mosquitoes to honey and human supports the expectation that males respond most strongly to sugar-related stimuli, whereas females' behaviour is dominated by blood-related stimuli. It may seem unexpected that males continued to respond strongly to honey after 5 days of sugar feeding. However, in view of their intense flight activity in the early part of each night, when they normally would swarm, their reserves probably already were depleted by the time of the late-scotophase olfactometer tests. For *Anopheles freeborni* Aitken, it has been calculated that males lose about half of their glycogen, the primary flight substrate, during each night and therefore must feed frequently (Yuval *et al*., 1994).

Competition and preference between honey and human volatiles

When honey and human were offered as simultaneous choices on night 1, the dominance of honey over human was decisive in both sexes. The suppression of the response to human volatiles by honey volatiles (fig. 2a) in this choice test was evident by comparison to the strength of response to each stimulus when presented separately (fig. 1a). This suggests that these stimuli competed with one another, rather than drawing upon mosquitoes in different states. The difference between separate and simultaneous presentations was absent among 5-day-old females, whose response to honey was low-to-nil even in the absence of human volatiles, probably because of extensive sugar-feeding and accumulation of energy reserves. Thus, both energetic state and external stimulus situation appear to determine the feeding decision.

The priority of response to honey over human in choice tests might be used to infer that sugar sources generally are more attractive than humans during the first foraging periods of females. This conclusion is premature, because both the qualities and relative stimulus intensities of the two combinations of volatiles, as well as other kinds of plant-host and human-host stimuli, influence the decision process (Hancock & Foster, 1997). For example, in the case of a sleeping human, both the particular combinations and concentrations of volatiles being released from feet and other body parts and the distance of the mosquito from the human will contribute to the chemical component of his stimulus strength. The sources of sugar for *A. gambiae*, and their distribution in relation to breeding sites and human habitations, remain unknown. It is to be expected that a plant's proximity to the mosquito and the quantity and quality of its stimulus output will determine the competitive status of nectar-related volatiles. Nevertheless, in the olfactometer the overwhelming dominance of honey on the behaviour of 1-day-old unfed females, followed by the dominance of human in 5-day-old females that had aged, matured, mated, and accumulated reserves, demonstrated that both honey and human volatiles were intrinsically attractive and that the distinct shift in the nectar-human bias was the result of a changed physiological state.

Promotion of response to human volatiles by sugar-feeding, independent of maturation

Sugar-feeding on night 0 promoted the response of females to human volatiles the following night. This

indicates that enhanced energy status itself, independent of age, maturation, mating status, or competing stimuli, was responsible for heightened responsiveness to human (or at least ability to enter human-baited traps) and probably contributed to the shift in preference from honey to humans after five days. The ability of sugar-feeding to enhance the response toward blood sources has previously been observed in the mosquitoes *Culex nigripalpus* Theobald (Hancock & Foster, 1997, 2000), *Ochlerotatus cantans* (Meigen) (Renshaw *et al*., 1994), and *Aedes vexans* (Meigen) (Briegel *et al*., 2001). The differences recorded between sugar-offered and water-fed *A gambiae* females varied widely from one test series to another, and some were not significant. The test series exhibiting the widest differences between water-fed and sugar-fed females occurred in three replicates whose water-fed females had very weak responses to human, so these cohorts may have been compromised by small body size for which a sugar meal somehow compensated (but see below). Among males, an overall difference did not occur between those with and without access to sugar at emergence, suggesting that male attraction to human volatiles may not be related to food directly.

Poor response of small-bodied mosquitoes to honey volatiles

Large-bodied males and females responded to honey in greater numbers than small-bodied ones. This result is similar to that obtained with blood-host stimuli presented to *A. aegypti* (Klowden *et al*., 1988), *C. nigripalpus* (Hancock & Foster, 1997), *O. cantans* (Renshaw *et al*., 1994), and *A. gambiae* (Takken *et al*., 1998). In the field, a cohort of *A. gambiae* was found to suffer a successive loss of smaller females between the times of emergence, the gonoactivating (i.e. 'pregravid') blood meal, and the ovigenic blood meal (whether it was the first or second meal) (Lyimo & Takken, 1993). Some aspect of body size may be connected to the ability to respond to stimuli and to behave effectively both in the olfactometer and in nature. Smaller individuals contain a lower energy reserve (Briegel 1990a,b; Takken *et al*., 1998), which might account for the poor performance, were it not that the opportunity to feed on sugar does not alleviate the size-effect (Klowden *et al*., 1988; Takken *et al*., 1998). The negative influence of size now extends to males and to nectar-feeding behaviour, broadening the conclusion that inadequate larval nutrition makes adults especially vulnerable to starvation.

Conclusions

The principal conclusion to be drawn from this study is that during the critical period of energy stringency that follows emergence of *A. gambiae*, the nectar response can be stronger than the human response in both sexes. It can be inferred, then, that under some sets of field circumstances, sugar-feeding is the dominant feeding mode for females. In nature, the actual response strengths and food choices will be determined by the combinations and concentrations of chemicals emanating from specific human and plant hosts and their proximity to the mosquito, and also by the various physical features of these hosts that contribute to mosquito attraction. But it now seems probable that situations commonly arise in which teneral females, as well as males, give priority to sugar. This helps to explain the moderately high proportions of females containing plant sugars in some collections of *A. gambiae* s.l., despite its reputation for doing well on human blood alone.

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