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Synergism between ammonia, lactic acid and carboxylic acids as kairomones in the host-seeking behaviour of the malaria mosquito *Anopheles gambiae sensu stricto* (Diptera: Culicidae)

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Abstract

Host odours play a major role in the orientation and host location of blood-feeding mosquitoes. *Anopheles gambiae* Giles *sensu stricto*, which is the most important malaria vector in Africa, is a highly anthropophilic mosquito species, and the host-seeking behaviour of the females of this mosquito is guided by volatiles of human origin. Ammonia, lactic acid and several carboxylic acids are known to be present in the human odour blend. We investigated the effect of these compounds on naïve female mosquitoes using a dual-port olfactometer. Ammonia was an attractant on its own, whereas lactic acid was not attractive. Carboxylic acids, offered as a mixture of 12 compounds, were repellent at the concentration tested. The addition of ammonia to the carboxylic acid mixture overruled the repellent effect of the latter. Combining ammonia with either lactic acid or the carboxylic acids did not enhance the attractiveness of ammonia alone. However, a synergistic effect was found when ammonia, lactic acid and the carboxylic acids were applied as a blend. Our findings indicate that *An. gambiae s.s.* relies on the combination of ammonia, lactic acid and carboxylic acids in its orientation to human hosts. The role of lactic acid in this tripartite synergism differs from that reported for the yellow fever mosquito *Aedes aegypti*.

Key words: dual-port olfactometer, host-odours, human volatiles, olfaction, sweat

Introduction

Females of the mosquito *Anopheles gambiae* Giles *sensu stricto* (Diptera: Culicidae) (henceforth termed *An. gambiae*) are important vectors of malaria in Africa. The dominance of this mosquito as a malaria vector is largely due to its preference for human blood (White, 1974; Coluzzi *et al.*, 1979; Pates *et al.*, 2001a). Because female mosquitoes use host odours to find their blood-hosts (Takken, 1991; Takken and Knols, 1999) and because *An. gambiae* females are highly anthropophilic, it is likely that the latter use human-specific odour compounds for orientation.

Field studies in Africa showed that carbon dioxide (CO_2), a major component of breath, accounts for only a minor part of the attractiveness of a human host to *An. gambiae* females. Human body odour appeared to play a larger role (Costantini *et al.*, 1996, 1998; Mboera *et al.*, 1997). This is in accordance with the hypothesis mentioned above since all warm-blooded vertebrates exhale CO_2 and it is unlikely that *An. gambiae*, which has a preference for humans, will be guided to its host by this compound alone.

Laboratory studies aimed at elucidating the compounds constituting human-produced odour blends that mosquitoes use for host location have yielded several active mixtures and individual substances. Human sweat was found to be attractive to *An. gambiae* (Braks *et al.*, 1997; Braks and Takken, 1999; Healy and Copland, 2000). One of its components is ammonia and it was shown in a dual-port olfactometer that ammonia is a kairomone for *An. gambiae* (Braks *et al.*, 2001). Carboxylic acids make up an important part of human sweat (Cork and Park, 1996). However, an artificial blend of 22 aliphatic carboxylic acids did not elicit landings of *An. gambiae* females whereas 2-oxopentanoic acid did, although the latter compound was not attractive from a distance (Healy and Copland, 2000). In contrast, Knols *et al.* (1997) found that a synthetic mixture of 12 aliphatic carboxylic acids was attractive to females of *An. gambiae* in a dual-port olfactometer. Human sweat also contains lactic acid (Cork and Park, 1996; Healy and Copland, 2000) and humans seem to have uniquely high levels of this compound on their skin compared to other animals (Dekker *et al.*, 2002). Although Healy and Copland (2000) found that *An. gambiae* females land on filter papers impregnated with human sweat, the concentration of lactic acid that was present in sweat did not elicit landing responses. Braks *et al.* (2001) showed that this compound was slightly attractive to *An. gambiae* females in an olfactometer.

Lactic acid is known to play an important role in the host-seeking behaviour of another anthropophilic mosquito species, the yellow fever mosquito, *Aedes aegypti* L. (Acree *et al.*, 1968; Smith *et al.*, 1970; Geier *et al.*, 1996). Ammonia was also identified as an attractant for *Ae. aegypti*; it is not attractive when tested alone, but it enhances the attractiveness of lactic acid (Geier *et al.*, 1999). Fatty acids of chain length C1–C3, C5–C8 or C13–C18 had the same effect when mixed with lactic acid. The combination of lactic acid, ammonia and two fatty acids appeared to be almost as attractive as an extract of human skin residues (Bosch *et al.*, 2000).

Building upon the results of Geier *et al.* (1999) and Bosch *et al.* (2000) with *Ae. aegypti* and with the knowledge that the human sweat compounds ammonia, L-lactic acid and several carboxylic acids were attractive to *An. gambiae*, we examined the effects of blends of these compounds on the behaviour of this mosquito. Initially, because the amount released by humans is to our knowledge unknown, the concentration range within which ammonia is attractive to *An. gambiae* females was examined. Subsequently experiments were done to examine whether ammonia might cause synergism when combined with lactic acid and/or a mixture of carboxylic acids.

Materials and Methods

Mosquitoes

The *Anopheles gambiae* Giles *sensu stricto* colony at Wageningen University, The Netherlands, originated from Suakoko, Liberia. The mosquitoes have been cultured in the laboratory since 1988 with blood meals from a human arm twice a week. The adult mosquitoes were maintained in 30 × 30 × 30 cm gauze cages at 27 ± 1°C, 80 ± 5% relative humidity, and a photo-scotophase of 12:12 light:dark. They had access to a 6% glucose solution on filter paper. The larvae were reared in tap water in plastic trays and fed daily with Tetramin® baby fish food. Pupae were collected daily and placed in adult cages for emergence.

Olfactometer

A dual-port olfactometer (see Pates *et al.*, 2001b), consisting of a Perspex flight chamber of 1.60 × 0.66 × 0.43 m, was used to study the behavioural responses of female mosquitoes to different odour stimuli. Pressurized air was charcoal filtered, humidified and led through two Perspex mosquito trapping devices, which were linked to two ports (diameter 4 cm, 28 cm apart), into the flight chamber with a speed of 0.22 ± 0.02 m/s. The light of one tungsten light bulb (75 W) was filtered and scattered through a screen of yellow cloth hanging ~1 m above the flight chamber. This resulted in dim light of ~1 lux in the olfactometer. The experimental room was maintained at a temperature of 28 ± 1.5°C and a relative humidity of 60 ± 6%. The temperature inside the flight chamber was equal to that of the room and the relative humidity was maintained at 65 ± 6%. The relative humidity of the air flowing out of the ports was maintained above 80% and the temperature was 28 ± 1.5°C.

Odour stimuli

Compounds were tested singly and in combinations with each other. They were tested either against clean air or against one or two of the test stimuli. The bioassay methods described by Braks *et al.* (2001) were used for the preparation of ammonia and lactic acid; the mixture of carboxylic acids was made following the method of Knols *et al.* (1997).

Ammonia

One day before the experiments, a specified amount of an aqueous ammonia solution (Stock: Merck, min. 25%, 1 l = 0.91 kg) was injected in a 80 l dual stainless steel fitted Tedlar air sample bag (SKC Inc., USA). Subsequently, the bag was filled with 60 l of humidified and filtered warm, pressurized air at least 17 h prior to the experiments to allow the ammonia to evaporate. This procedure resulted in ammonia concentrations of 13.6, 136, 1,364, 13,637, 27,274 or 136,371 p.p.m. in different bags (Table 3). Another 80 l air sample bag filled with corresponding volumes of distilled water and 60 l air was prepared in a similar way to be used as the control stimulus. During the experiments, the air was pumped at 230 ml/min (MG-4 Ametek air pump, Ametek, USA) from the air sample bags through silicon tubes (diameter 7 mm; Rubber BV, The Netherlands) into one or both trapping devices where it mixed with the main airstream (Braks *et al.*, 2001). Based on the response of the mosquitoes to these six concentrations of ammonia (Figure 1), an ammonia concentration of 13.7 p.p.m. was used in subsequent experiments.

Lactic acid

L-(+)-Lactic acid sodium salt (98%, L-7022, Sigma, St Louis, MO, USA) was dissolved in ethanol (Merck, absolute ethanol, pro analysis) following the method used by Braks *et al.* (2001) and Geier *et al.* (1999) who found certain concentrations of this compound to be attractive to *An. gambiae* and *Ae. aegypti*. For each experiment 100 µl of a lactic acid solution was applied on filter paper (5 × 2 cm; Whatman 2 or Schleicher & Schuell 595) which was placed in an iron clip. The ethanol was allowed to evaporate before the clip with the filter paper was put in a trapping device. For the control stimulus an equivalent amount of ethanol was applied in a similar way and placed in the other trapping device (Braks *et al.*, 2001).

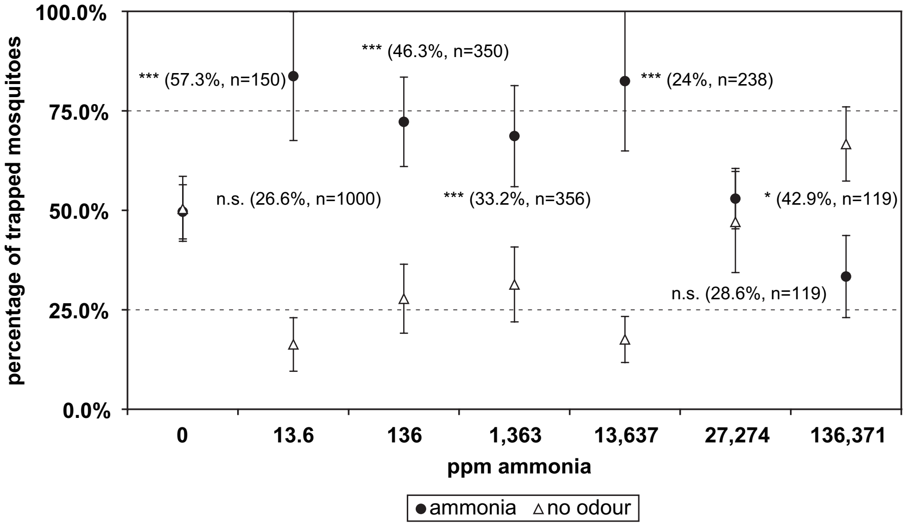

Figure 1 Responses of *An. gambiae s.s.* females to different concentrations of gaseous ammonia (p.p.m.), applied from air sample bags, plotted on a logarithmic scale. The percentage of mosquitoes that flew into either trap is shown. Vertical bars show standard errors of the mean. Asterisks mark significant differences between the total number of mosquitoes trapped in both trapping devices; with ammonia and no odour respectively (χ^2 test: NS, not significant; $*P < 0.05$; $***P < 0.001$). Between brackets with each datapoint, the percentage of mosquitoes that entered one or two of the trapping devices is given together with the total number of mosquitoes that left the release cage (*n*).

Initially, five different concentrations of lactic acid (0.1, 0.01, 0.001, 0.0001 and 0.00001 g/ml) were tested in combination with gaseous ammonia (136 p.p.m.) and against gaseous ammonia only (136 p.p.m.) to obtain the highest attractiveness of the mixture of these two compounds together. Based on the results of this experiment and the results previously obtained by Braks *et al.* (2001), the concentration of lactic acid used in the subsequent experiments was 0.001 g/ml.

Mixture of carboxylic acids

A mixture of carboxylic acids was dissolved in diethyl ether (Merck, min. 98%) based on the relative amounts of each acid in the acid extracts of Limburger cheese samples, which were shown to be attractive to *An. gambiae* (De Jong and Knols, 1995; Knols *et al.*, 1997). A sandblasted glass slide (5×2 cm) with 100 μl of the 10^8 times diluted synthetic mixture of the 12 carboxylic acids (Sigma, min. 99%) was placed in a trapping device. A control glass slide with an equivalent amount of diethyl ether was placed in the opposite trapping device after the diethyl ether had evaporated.

Experimental procedure

Thirty female mosquitoes, 5–8 days old, which had not received a blood meal, were randomly collected from their cage 14–18 h before the start of the experiments and placed in a cylindrical release cage (diameter 8 cm, height 10 cm) with access to tap water from damp cotton wool placed on top of the cage.

The experiments were performed during the last 4 h of the dark period, when *An. gambiae* is normally active. In each trial test odours were released in the airstream before a group of mosquitoes was set free from a cage which was placed at the downwind end of the flight chamber, 1.60 m from the two ports. Mosquitoes were left in the flight chamber for 15 min. The female mosquitoes that had entered either trapping device were counted at the end of the experiments, after anaesthetization with 100% CO_2. Mosquitoes remaining in the flight chamber were removed with a vacuum cleaner.

Each trial started with new mosquitoes, clean trapping devices and new stimuli. Experiments were repeated at least six times on different days. The sequence of test odours was randomized on the same day and between days. Test stimuli were alternated between right and left ports in different replicates to rule out any positional effects. Experiments with clean air only in either port were done to test the symmetry of the trapping system. Surgical gloves were worn by the experimenter to avoid contamination of the equipment with human volatiles.

Statistical analysis

For each two-choice test a χ^2 test was used to analyse whether the total (i.e. sum of all replicates) number of mosquitoes that was trapped in the treatment trapping device and the total number that was trapped in the control trapping device differed from a 1:1 distribution. A generalized linear model (GLM; binomial, linked in logit; Genstat, release 4.2) was used to investigate the effect of the different odour stimuli on the total response (i.e. total number of mosquitoes that entered both the treatment and control trapping device as fraction of the total number of mosquitoes that left the release cage). Two-sided *t*-probabilities were calculated to test pairwise differences between means. Effects were considered to be significant at $P < 0.05$ (Oude Voshaar, 1994; Sokal and Rohlf, 1998).

Results

Symmetry of trapping system

An. gambiae females responded to a humidified odourless airstream as was shown previously by Knols *et al.* (1994). In all our experiments in which we tested clean air coming from both ports, equal numbers of mosquitoes were caught in both trapping devices (χ^2 test; P ≥ 0.50), which demonstrated that the olfactometer was symmetrical (Figure 1).

Dose–response effects of ammonia

Figure 1 shows the dose-dependent response of *An. gambiae* to gaseous ammonia. Odorous air from sample bags containing 13.6, 136, 1,364 or 13,637 p.p.m. ammonia attracted significantly more mosquitoes than clean air (χ^2 test; $P < 0.05$). Catches with air containing 27,274 p.p.m. ammonia did not differ from the catches in the trapping device with clean air (χ^2 test; P ≥ 0.05). The mosquitoes preferred the clean air to the highest concentration of ammonia tested, 136,371 p.p.m. (χ^2 test; P = 0.02), indicating that this concentration is repellent for *An. gambiae*.

The percentage of mosquitoes responding in the experiments with 13,637 (24%) and 27,274 (28.6%) p.p.m. ammonia was not significantly different from the control (clean air against clean air) (26.6%), whereas the response (varying from 33.2 to 57.3%) was significantly higher when the other concentrations were tested (GLM; Figure 1).

Different concentrations of lactic acid in combination with ammonia

None of the concentrations of lactic acid that were tested against ammonia alone (136 p.p.m.) increased the attractiveness of ammonia (χ^2-test) (Table 2). The concentration of 0.0001 g/ml of lactic acid combined with ammonia was repellent.

Responses to blends of ammonia, lactic acid and a synthetic mixture of 12 carboxylic acids

Gaseous ammonia at a dose of 136 p.p.m. alone was attractive when tested against clean air (χ^2 test; P = 0.02), whereas lactic acid alone was not (χ^2-test; P = 0.10). The combination of ammonia and lactic acid attracted significantly more mosquitoes than clean air (χ^2 test; P = 0.0003; Table 3A).

The synthetic mixture of the 12 carboxylic acids was repellent when tested alone and in combination with lactic acid (against the solvents only) (χ^2-test; P = 0.0002 and P = 0.047 respectively). However, the combination of the carboxylic acid mixture with ammonia and with both ammonia and lactic acid was significantly more attractive than clean air (χ^2 test; P = 0.03 and P = 0.00005 respectively; Table 3A).

The attractiveness of ammonia was not increased by the addition of lactic acid or by the carboxylic acid mixture (χ^2 test; P = 0.41 and P = 0.59 respectively; Table 3B). However, the combination of ammonia, lactic acid and carboxylic acids was significantly more attractive than ammonia alone (χ^2 test; P = 0.02).

A mixture of carboxylic acids and lactic acid or a mixture of ammonia and lactic acid did not attract more mosquitoes than lactic acid alone (χ^2 test; P = 1.000 and P = 0.26 respectively). But as with the previous series of experiments (Table 3B), the combination of all chemicals together attracted more mosquitoes than only one of the compounds, lactic acid (χ^2-test; P = 0.004; Table 3C).

Lactic acid combined with the carboxylic acids was not more attractive than the latter alone (χ^2 test; P = 0.78). Adding ammonia only or ammonia together with lactic acid overruled the repellency of the carboxylic acid mixture (χ^2 test; P = 0.03 and P = 0.0006 respectively; Table 3D).

No significant differences were found between the number of mosquitoes attracted to the combination of ammonia, lactic acid and the carboxylic acid mixture and the numbers attracted to the mixture of ammonia and lactic acid or the combination of ammonia and the carboxylic acid mixture (χ^2 test; P = 0.66 and P = 0.35 respectively; Table 3E). The combination of lactic acid and the carboxylic acid mixture was significantly less attractive than the mixture of all chemicals together (χ^2 test; P = 0.002).

Discussion

Ammonia, lactic acid and several carboxylic acids have been detected in human sweat and in human skin emanations (e.g. Eiras and Jepson, 1991; Cork and Park, 1996; Bernier *et al.*, 1999, 2000; Braks *et al.*, 2001). Our experiments clearly show that the combination of these components affects the host-seeking behaviour of the highly anthropophilic females of the malaria mosquito *An. gambiae s.s.*.

As was shown before by Braks *et al.* (2001), ammonia alone is attractive to *An. gambiae* females. Concentrations of 13.6–13,637 p.p.m. of ammonia in the sampling bag attracted 25–57% of the mosquitoes. Higher concentrations produced repellency (Figure 1). These data confirm that ammonia is an important kairomone derived from incubated sweat, an attractive complex odour source containing higher amounts of ammonia (49.4 mM) than fresh sweat (6.3 mM), which is not or only slightly attractive (Braks and Takken, 1999; Meijerink *et al.*, 2000; Braks *et al.*, 2001). By our calculations the dilution of ammonia from the air sample bags in the surrounding airstream was 100-fold, but due to the turbulence of the plume, causing heterogeneous mixing of odour components, it is likely that instantaneous peak concentrations of ammonia may have been present, similar to the ammonia concentration in the sample bags (Murlis *et al.*, 1992), to which the mosquitoes were exposed, starting with the undiluted concentrations as released from the bags (Table 1).

Assuming a 100-fold dilution, the lowest concentration for which we found attractiveness (136 ppb) is within the range found in human breath (between 40 and 3170 ppb; Larson *et al.*, 1979). This concentration is above the threshold for attractiveness reported with *Ae. aegypti* (between 2 and 17 ppb; Geier *et al.,* 1999). Another crucial difference between the two species is that in *Ae. aegypti* ammonia was only active in combination with lactic acid as an essential synergist. Conversely, lactic acid alone attracts *Ae. aegypti* (Geier *et al.*, 1996, 1999), but not *An. gambiae.* In *An. gambiae* the mixture of ammonia and lactic acid does not attract more mosquitoes than ammonia alone (Tables 2 and 3B). The synthetic mixture of the 12 carboxylic acids did not increase the attractiveness of ammonia either (Table 3B). However, only the combination of ammonia, lactic acid and carboxylic acids was significantly more attractive than any of the three components alone (Table 3B–D). Therefore, enhanced attractiveness of the combined components by definition implies a tripartite synergistic effect between these stimuli. This tripartite synergism was not revealed for *Ae. aegypti* as the carboxylic acids have only been tested in combination with lactic acid (Bosch *et al.*, 2000). These data also demonstrate the important role of ammonia in the blend, as the addition of lactic acid or carboxylic acids to binary blends of ammonia and carboxylic acids or ammonia and lactic acid, respectively, did not result in enhanced attractiveness, whereas addition of ammonia to a blend of lactic acid and carboxylic acids clearly caused significantly enhanced effects (Table 3E).

Although in our experiments lactic acid alone was not significantly more attractive than clean air (Table 3A), contrary to what was found by Braks *et al.* (2001) and Smallegange *et al.* (2002), but corresponding to Dekker *et al.* (2002), the data presented here show that lactic acid is necessary to evoke the synergistic effect with ammonia and carboxylic acids that we observed (Table 3).

It was remarkable that ammonia suppressed the repellent effect of the carboxylic acid mixture (Table 3A, B). When tested alone, the carboxylic acid mixture was repellent (Table 3A). This result appears in contrast with the first report of olfactory activity of carboxylic acids to *An. gambiae* from our laboratory, where a similar mixture of carboxylic acids at the same concentration (diluted by a factor of 10^8) was reported attractive (Knols *et al.*, 1997). Impurities in the (commercially) obtained carboxylic acids may be one explanation for the differences in results between those of Knols *et al*. and the present study. The repellent effect of carboxylic

Table 1 Conversion table used for the calculation of the NH_3 concentration (p.p.m.) in the air sample bags

Concentration of NH_3 in watery solution (%)	Volume of NH_3 solution (ml) added to air sample bag	Concentration of NH_3 in air sample bag (p.p.m.)*
0.25	0.25	13.6
2.5	0.25	136.4
25	0.25	1363.7
25	2.5	13,637.1
25	5	27,274.2
25	25	136,370.9

*We calculated the concentration of ammonia as follows:
$(C \times V \times d \times V_m)/(A \times MW)$
C: concentration of ammonia solution (%)
V: volume of ammonia solution added to the air sample bag (ml)
d: density of ammonia solution (g/ml)
V_m: molar volume of gas at 25°C and one atmosphere pressure (24.5 l/mol)
A: volume of the air in the sample bag (60 l in our experiments)
MW: molecular weight of ammonia (17.03 g/mol)

Table 2 Responses of *An. gambiae s.s.* females to gaseous ammonia (NH_3, released at 136.4 p.p.m.) combined with lactic acid (LA) at five different concentrations (1: lowest concentration; 5: highest concentration tested, see Materials and methods)

Stimuli		No. released[a]	Response[b]		χ^{2} [c]	Total response[d]
Treatment	Control		Treatment	Control		
NH_3	NH_3	145	17	25	NS	29.0
NH_3 + LA conc. 1	NH_3	169	29	20	NS	29.0
NH_3 + LA conc. 2	NH_3	173	15	32	*	27.2
NH_3 + LA conc. 3	NH_3	168	15	13	NS	16.7
NH_3 + LA conc. 4	NH_3	173	23	14	NS	21.4
NH_3 + LA conc. 5	NH_3	162	19	16	NS	21.6

[a]The total number of mosquitoes that left the release cage.
[b]The response is given as the total number of mosquitoes caught in either the treatment or control trapping device.
[c]Significant differences (*$P < 0.05$) or no significant differences (NS: $P \geq 0.05$) between the total number of mosquitoes caught in the treatment and control trapping device (χ^2 test).
[d]The total number of mosquitoes that entered both the treatment and control trapping device as fraction of the total number of mosquitoes that left the release cage (%).

Table 3 Responses of *An. gambiae* s.s females to combinations of gaseous ammonia (NH_3), lactic acid (LA) and a synthetic mixture of 12 carboxylic acids (CAmix)

	Stimuli		No. released[a]	Response[b]		$\chi^{2\ c}$	Total response[d]
	Treatment	Control		Treatment	Control		
A	Clean air	clean air	921	42	44	NS	9.3
	NH_3	clean air	489	59	37	*	19.6
	LA	clean air	732	54	38	NS	12.6
	CAmix	clean air	592	38	79	***	19.8
	NH_3 + LA	clean air	375	57	24	***	21.6
	NH_3 + CAmix	clean air	169	31	16	*	27.8
	LA + CAmix	clean air	378	28	45	*	19.3
	NH_3 + LA + CAmix	clean air	381	57	21	***	20.5
B	NH_3	NH_3	107	7	6	NS	12.1
	NH_3 + LA	NH_3	119	5	8	NS	10.9
	NH_3 + CAmix	NH_3	116	14	17	NS	26.7
	NH_3 + LA + CAmix	NH_3	115	17	6	*	20.0
C	LA	LA	110	2	4	NS	5.5
	NH_3 + LA	LA	139	5	2	NS	5.0
	LA + CAmix	LA	138	6	6	NS	8.7
	NH_3 + LA + CAmix	LA	140	19	5	**	17.1
D	CAmix	CAmix	85	0	2	NS	2.4
	NH_3 + CAmix	CAmix	131	11	3	*	10.7
	LA + CAmix	CAmix	130	7	6	NS	10.0
	NH_3 + LA + CAmix	CAmix	128	17	2	***	14.8
E	NH_3 + LA + CAmix	NH_3 + LA	180	22	25	NS	26.1
	NH_3 + LA + CAmix	NH_3 + CAmix	172	17	12	NS	16.9
	NH_3 + LA + CAmix	LA + CAmix	178	26	8	**	19.1

[a,b,d]See descriptions under Table 2.
[c]Significant differences (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$) or no significant differences (NS: $P \geq 0.05$) between the total number of mosquitoes caught in the treatment and control trapping device (χ^2 test).
NH_3 = ammonia mixed at 136.4 p.p.m. with main airstream, see Materials and methods.
LA = lactic acid released from a solution of 0.001 g/ml in ethanol.
CAmix = mixture of 12 carboxylic acids applied at 10^{-8} dilution in diethyl ether. Composition given in Knols *et al*. (1997).

acids alone is also illustrated by the very low response (2.4%) of mosquitoes when the acids were released from both odour ports of the olfactometer (Table 3D). Whether only one or several carboxylic acids caused the repellency of the 12-component mixture needs to be investigated. For *Ae. aegypti*, Bosch *et al*. (2000) found that especially C1–C3 and C5–C8 carboxylic acids enhanced attractiveness of lactic acid, whereas C9 and C11 reduced attractiveness. We already know from electroantennographic (EAG) studies that *An. gambiae* females can detect saturated carboxylic acids. These studies showed that short-chain carboxylic acids elicit higher EAG responses than less volatile, long-chain, acids (Cork and Park, 1996; Knols *et al.*, 1997). Experiments with *An. gambiae* females that were done in Y-tube olfactometers showed that the synergistic effect could also be achieved when combining ammonia and lactic acid with only one of the short-chain carboxylic acids that was present in our synthetic mixture: hexanoic acid (Smallegange *et al.*, 2002). In addition, Costantini *et al.* (2001) reported attractiveness of an unsaturated carboxylic acid, 7-octenoic acid, which is a human-specific component secreted from the apocrine sweat glands in the axillary regions (Zeng *et al.*, 1991).

Our results show that a synthetic blend of volatiles, all naturally present in human odour (Bernier *et al.*, 2000; Healy and Copland, 2000; Braks *et al.*, 2001), provides a synergistic effect on olfaction-based trap-entry responses of *An. gambiae* mosquitoes. It is clear from field studies that *An. gambiae* is strongly attracted to natural human odours (Haddow, 1942; Ribbands, 1950; Costantini *et al.*, 1996; Mboera *et al.*, 1997), and the development of traps baited with highly attractive synthetic blends simulating human odours appears realistic.

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