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Contribution of Fatty Acids to Olfactory Host Finding of Female *Aedes aegypti*

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Abstract

Single carbon to 18 carbon *n*-aliphatic carboxylic acids were tested for their attractive effects on female *Aedes aegypti* in a Y-tube olfactometer. Each acid was tested over a wide range of concentrations together with L-(+)-lactic acid, the indispensable synergist for other attractive components emitted from human hosts. The attractiveness of lactic acid was significantly augmented when combined with fatty acids of chain length C_1–C_3, C_5–C_8 and C_{13}–C_{18}, respectively. The addition of the C_9 and C_{11} acids reduced the attractive effect of lactic acid. According to experiments showing a further increase of attractiveness by adding a second fatty acid, we suggest two groups of attractive carboxylic acids: C_1–C_3 and C_5–C_8. The addition of a fatty acid from one group to a mixture of lactic acid and an acid from the other group augmented the attraction to the mixture. Together with ammonia, a previously demonstrated attractant for *Aedes aegypti*, lactic acid plus two fatty acids from the different groups formed the hitherto most attractive, artificially composed blend. Two of the carboxylic acids which were found to be attractive together with lactic acid were also tested alone and in combination with CO_2, the major attractant in human breath. In both cases no attractive effect of the carboxylic acids could be observed.

Introduction

For host finding and selection, female mosquitoes use odours of their prey (Bowen, 1991; Takken, 1991, 1996; Davis and Bowen, 1994; Davis, 1996). One important kairomone is CO_2, a major component of breath that has been shown to activate and to attract a number of mosquito species (Gillies, 1980; Takken, 1991; Eiras and Jepson, 1994; Geier *et al.*, 1999a). Another is L-(+)-lactic acid, a component present in human breath as well as on human skin (Acree *et al.*, 1968; Smith *et al.*, 1970; Eiras and Jepson, 1991). This compound is only slightly effective by itself, but acts synergistically with CO_2 and other components from human skin, at least for the yellow fever mosquito *Aedes aegypti* (Geier *et al.*, 1996). Interestingly, the latter components were found to be attractive only in combination with lactic acid. This might be one reason for the not always conclusive and in some case even contradictory data reported earlier on the attractive effects of single odorants [reviewed in (Hocking, 1971; Takken, 1991)]. In contrast to single compounds, odour mixtures such as human sweat, skin residues, breath and even extracts from Limburger cheese were found to be highly attractive to mosquitoes of different species (Hocking, 1971; Takken, 1991; Knols and De Jong, 1996; Knols *et al.*, 1997). This also indicates that an odour blend rather than a single compound represents the attractive principle in the host odour for mosquitoes. Recently, ammonia, which is present on human skin as well as in breath, was identified as an attractant for *A. aegypti* (Geier *et al.*, 1999b) which also increases the attractive effect of a lactic acid stimulus alone. The combination of the two compounds, however, did not attract as many *A. aegypti* as did an extract from human skin residues (Geier *et al.*, 1999b). Therefore, additional components of the human body odour must play a role. Promising candidates are the fatty acids which are present in significant amounts on human skin (Stoddart, 1990). Electrophysiological studies have shown that grooved peg sensilla on the antennae of yellow fever mosquitoes house olfactory receptor cells which respond to fatty acids (Lacher, 1967; Pappenberger *et al.*, 1996). Recently, Knols *et al.* showed that a mixture of 12 aliphatic fatty acids, identified in the head space of Limburger cheese, were attractive for *Anopheles gambiae* (Knols *et al.*, 1997). Roessler (Roessler, 1961) obtained a weak attraction of *A. aegypti* to a blend of several aliphatic and amino acids, and Carlson *et al.* (Carlson *et al.*, 1973) reported C_2–C_5 carboxylic acids to be attractive for this species when presented together with CO_2. But so far, no behavioural tests have been done with single fatty acids in combination with lactic acid as the synergist. Moreover, no attempt has been made to create a synthetic blend of host odours by adding attractive volatiles step by step, in order to understand the role of each single substance. All these findings led us to undertake a broader screening for attractants among fatty acids, keeping in mind the role of lactic acid as an essential synergist. In a modified Y-tube olfactometer we

tested first the responses of *A. aegypti* to C_1–C_{18} aliphatic acids in combination with lactic acid. The effective fatty acids were then tested alone as well as in combination with CO_2, ammonia and the other effective fatty acids.

Materials and methods

Animals

Ten- to 40-day-old female *A. aegypti* from cultures of the Centre for Plant Research (Pflanzenschutzzentrum) at Bayer AG in Monheim (Germany) were used in experiments. The larvae were reared under standard conditions. About 300–500 adults were kept in containers (50 × 40 × 25 cm) at 26–28°C, 60–70% relative humidity and a light–dark regime of 12:12 h. The insects had access to a 10% glucose solution on filter paper.

Olfactometer

The olfactometer consisted of a long Plexiglas® tube with an exchangeable release chamber at the downwind end. The tube was attached to a rectangular box from which two arms of the Y-olfactometer leave the opposite side (Figure 1). Each arm was connected to a PVC® tube (stimulus chamber) at the upwind end into which the stimuli were injected. A constant airstream of 80 l/min from the laboratory's pressurised air system was purified with activated charcoal, then heated and humidified before being passed through the olfactometer. For more details of this set-up see (Geier, 1995; Geier *et al.*, 1999b). The temperature in the olfactometer was 28 ± 1°C, relative humidity 70 ± 5%, and wind speed 0.2 m/s in both test and control chambers and 0.4 m/s in the long tube. The olfactometer was placed on a white table and covered on both sides with white 20 cm high cardboard shield. Two 40 W light bulbs provided overhead illumination.

Application of the odour stimuli

Skin odour was produced using an ethanolic extract of human skin residues. After taking 5 µl of the extract into a glass pipette, it was heated to 60°C and the odour was blown out by air at a flow rate of 1600 ml/min [for details see (Geier and Boeckh, 1999; Geier, 1995)]. Pure CO_2 (AGA, Hamburg, Germany) at a flow rate of 1600 ml/min was mixed with olfactometer air to a final concentration of 4% CO_2 in the test chamber. The other stimuli were delivered as described elsewhere (Ough and Stone, 1961). Therefore, charcoal-filtered compressed air at flow rates between 0.03 and 300 ml/min passed through a 250 ml Erlenmeyer flask over the surface of a solution of the test substance at room temperature. Flow meters (Rota GmbH, Germany, for flow rates of at least 3 ml/min) or a precision tubing pump (Masterflex, Novodirect GmbH, Kehl/Rhein, Germany, for flow rates of <3 ml/min) were used to control the air flow. To cover a wide range of concentrations, two different dilutions (5 and 500 µl in 50 ml of deionized water) of

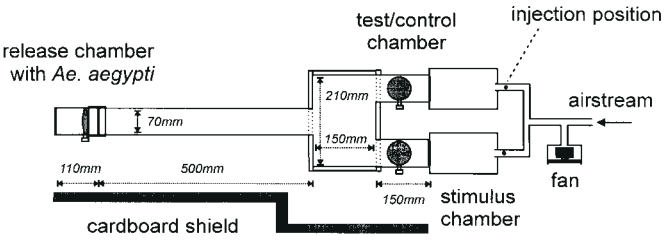


Figure 1 Schematic drawing of the olfactometer. For details on function see Material and methods.

C_1–C_9 fatty acids were tested at flow rates of 3, 30 and 300 ml/min, respectively. The C_{10}–C_{18} fatty acids were melted and 2 ml of the pure substance was distributed over the bottom of the Erlenmeyer flask over which air swept at flow rates of 0.03, 0.3, 3, 30 and 300 ml/min. L-(+)-Lactic acid was tested in an amount of 3 µg/min [20 ml of pure lactic acid, flow rate 15 ml/min; see (Geier *et al.*, 1999a)] and ammonia at 5 µg/min [50 ml of 0.13 mol/l ammonia in distilled water, flow rate 3 ml/min; see (Geier *et al.*, 1999b)].

Chemicals were purchased from Merck (Darmstadt, Germany), Sigma-Aldrich (Deisenhofen, Germany), Riedel-de Haën (Seelze, Germany) or Roth (Karlsruhe, Germany). Except for nonanoic acid (97%), tridecanoic acid (98%) and heptadecanoic acid (95%) they were >99% pure. Air purified by a charcoal filter and passed through an Erlenmeyer flask filled with deionized water was used as a control stimulus.

Odour distribution

To check the spatial distribution of odours in the olfactometer, titanium tetrachloride smoke was used to visualize the distribution of odours. In the arms of the olfactometer, smoke was found to be distributed as in the homogeneous plumes reported elsewhere (Geier *et al.*, 1999a). More turbulent odour eddies, i.e. odour clouds and filaments, appeared in the rectangular Plexiglas® chamber into which the two airstreams flew.

Bioassay

Groups of 18–22 female mosquitoes were used for the tests. Using the odour of the experimenter's hand the animals were lured out of the container into the release chamber, which was then attached to the downwind end of the olfactometer. Before stimulation, the mosquitoes were given 20 min to acclimate. At stimulus onset, the release chamber was opened and the mosquitoes were allowed to enter the olfactometer. After 30 s of stimulation, the screen doors of the release-, control- and test chambers were closed and the distribution of the animals in the olfactometer was measured. After the experiment the mosquitoes were lured back into the release chamber by reversing the airflow in the olfactometer and using the hand as a natural attractant. A given group of animals was tested up to 10 times with an interval of at least 20 min between subsequent tests. In order

to avoid possible side effects such as learning or habituation, every stimulus was tested only once in each group of mosquitoes. Furthermore, the test and control chambers alternated in each experiment.

The different concentrations of a given fatty acid were tested within one series of tests. Each stimulus combination and the control stimulus was tested with at least eight different groups of mosquitoes, each exposed to every combination in a random order. Between the tests, a constant flow of fresh air flushed the olfactometer. The experiments ran from 8:00 a.m. to 6:00 p.m.

Evaluation

In each test, three different types of responses were analysed. (i) The percentage of mosquitoes found outside of the release chamber after 30 s was taken as a measure of activation, which included taking off and short upwind progress. (ii) The percentage of mosquitoes trapped in the test chamber was a measure for attraction to the treatment. (iii) The percentage of mosquitoes in the control chamber measured the response to pure air. The values were averaged from at least eight experiments with different groups of individuals. Since the data are percentage values, angular transformation was made for further statistical analysis (Sachs, 1997). The transformed means were analysed independently by a one-way ANOVA using the Duncan test *post hoc* for comparison of treatments. All calculations and statistics were performed with the statistics program SPSS 8.0 for Windows.

Results

Responses to clean air

Control tests with clean air stimulation were done at random intervals throughout the experiments. All control studies (n = 400 repetitions) were pooled based on the similarity of their evoked responses. The activation of the mosquitoes was 32.1 ± 0.7% (mean ± SE), the attraction to the test chamber was 1.4 ± 0.1% and that to the control chamber was 0.8 ± 0.1%. These low levels of attraction to the pure olfactometer air indicate no contamination effects during the experiments.

Responses to single fatty acids in combination with lactic acid

Different doses of a given fatty acid were tested in combination with lactic acid at a dose of 3 µg/min (Figures 2 and 3). The lactic acid dose was in the range of evaporation rates from human hands (Smith *et al.*, 1970) and was also tested alone as a standard in each series of tests. These tests were performed to see whether the attractive effect of lactic acid could be increased by adding a single fatty acid. In all tests, the attractiveness of lactic acid varied between 15 and 25%, and the attractiveness was significantly increased by addition of certain concentrations of C_1–C_3, C_5–C_8 and C_{13}–C_{18} saturated fatty acids. No increase was observed with butyric acid as well as the C_9–C_{12} fatty acids over a range of various concentrations. In contrast, when nonanoic acid was added to lactic acid from a solution of 1:10 000 and a flow rate of 300 ml/min or from a 1:100 dilution and a flow rate of 30 ml/min a slight decrease of attractiveness was recorded compared with the effect of lactic acid alone. The same was observed for undecanoic acid at a flow rate of 300 ml/min. In general, the attractive effect of the C_{13}–C_{18} fatty acids increased with higher concentrations, whereas the short chain fatty acids were most effective at low (C_1–C_3) or medium (C_5–C_8) concentrations.

Responses to combinations of fatty acids with lactic acid

To test the possibility of a combinatorial effect of mixtures including short-, medium- and long chain fatty acids, each fatty acid in the C_1–C_{18} series was added to the following mixtures: lactic acid plus C_3, lactic acid plus C_5, and lactic acid plus the combination of C_3 and C_5 (Table 1).

The combination of lactic acid plus propanoic acid became significantly more attractive by adding fatty acids from the C_5–C_8 series, and no other fatty acid could increase the attractiveness of this base mixture. The attractive effect of the combination of lactic acid plus valeric acid was augmented by adding either short chain fatty acids from the C_1–C_3 series or stearic acid. The attractiveness of the combination of lactic acid plus propanoic and valeric acid could not be increased by adding other fatty acids, but addition of C_{11} and C_{14} caused a significant decrease of attraction for this base combination.

Since a group of mosquitoes was used several times for the experiments, we proved whether possible side effects through habituation or fatigue influence the behavioural responses of the mosquitoes. We thus pooled the data of all experiments with the mixture of lactic acid and propionic acid. When the mixture was tested as the first odour stimulus 31.2 ± 0.7% (mean ± SE) of the mosquitoes were attracted and 73.8 ± 1.2% were activated (n = 22 repetitions). Other mosquito groups which were tested first with two other stimuli and then with the mixture attracted 32.5 ± 1.2% and activated 74.3 ± 0.9% (n = 22 repetitions). Even when the odour mixture was presented as the fifth stimulus in a series (31.7 ± 0.9% attracted, 74.9 ± 1.1% activated; n = 26 repetitions) or the seventh stimulus (29.6 ± 0.9% attracted, 72.4 ± 1.1% activated; n = 25 repetitions), no significant differences were observed (ANOVA, $P > 0.1$).

Responses to mixtures of two fatty acids, ammonia and lactic acid

To determine whether the efficacy of several attractive compounds add up to higher levels of attractiveness when combined with lactic acid, mixtures of lactic acid, ammonia, propanoic acid and valeric acid were tested (Figure 4). A stepwise increase of attractiveness was observed with successive addition of each component (up to 68.1%). Most

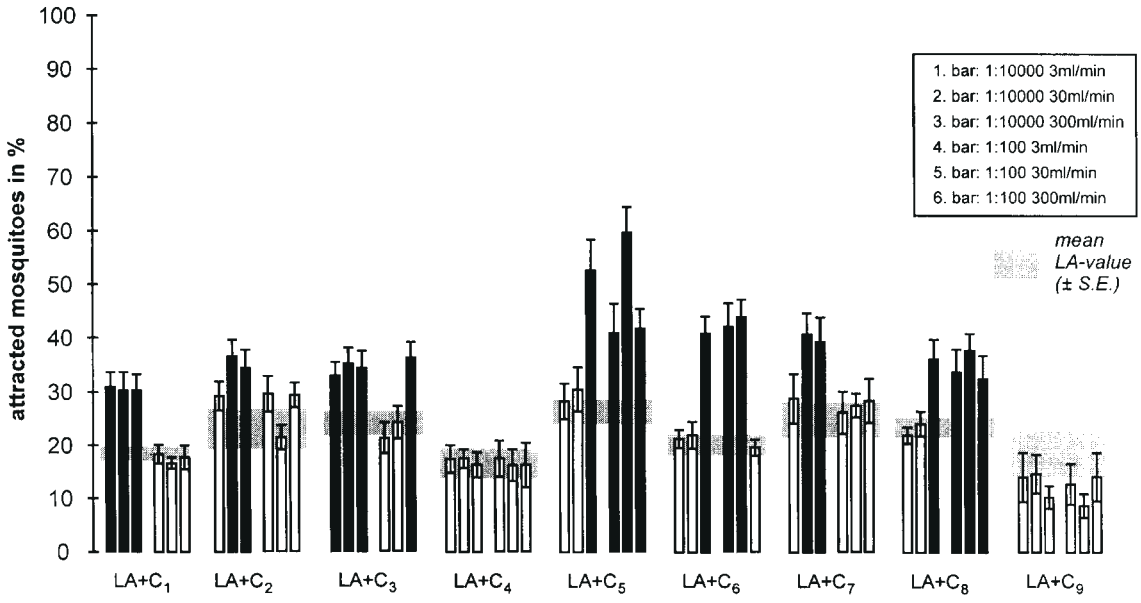


Figure 2 Effect of C_1–C_9 fatty acids added singly at different concentrations on the attraction of lactic acid for *A. aegypti*. The columns represent the mean percentage of mosquitoes trapped in the test chamber. Since no mosquitoes were trapped in the control chamber, those data are not shown in the diagram. Horizontal bars show the mean attractiveness ± SE (n = 16 repetitions) of lactic acid for each experiment. Black bars indicate a significant difference from the mean value of lactic acid at $P = 0.05$. Means were compared with each other using the Duncan test *post hoc* for a one-way ANOVA.

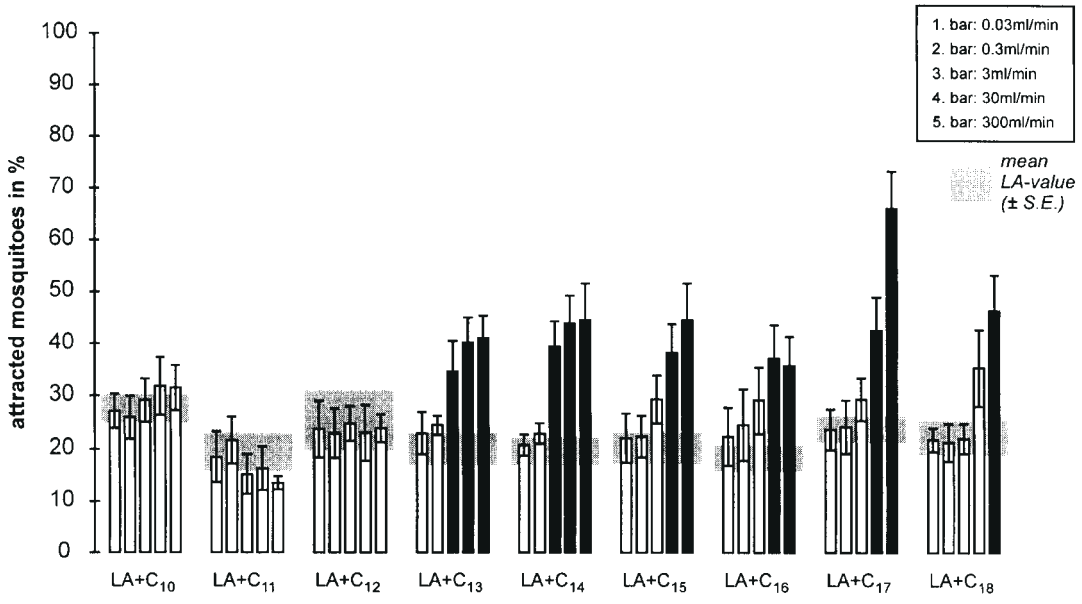


Figure 3 Effect of C_{10}–C_{18} fatty acids added singly at different concentrations on the attraction of lactic acid for *A. aegypti*. For an explanation see the legend to Figure 2.

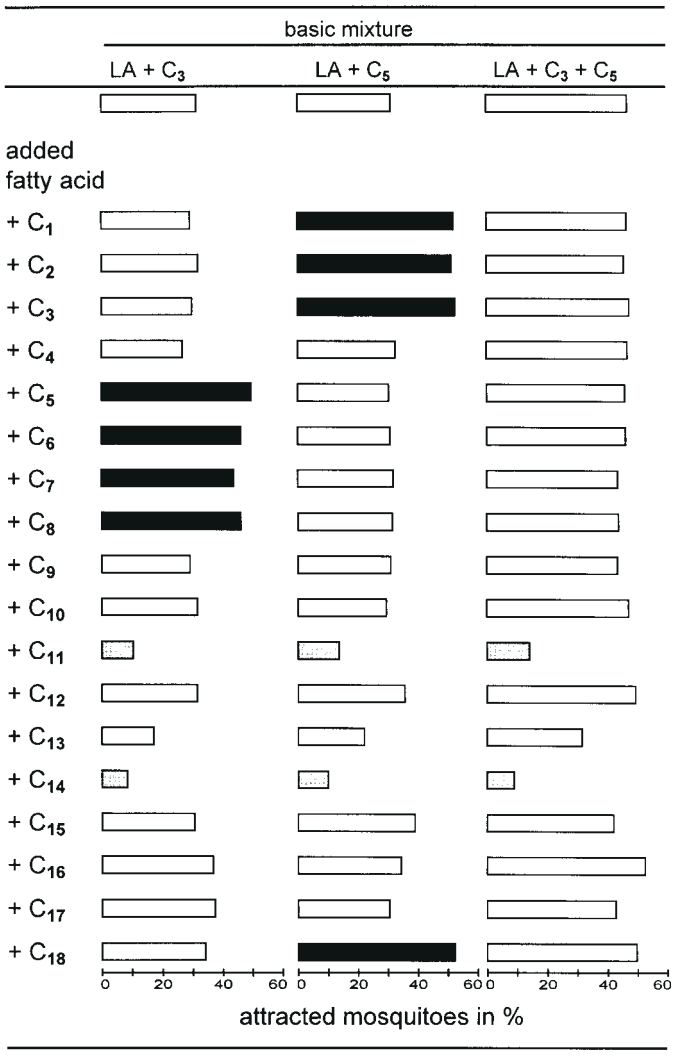
attractive was the blend which included all four synthetic components. This blend almost reached the effect of an extract from human skin (77.9%).

Responses to single fatty acids in combination with CO_2

Propanoic and valeric acid, which both increased the attractiveness of lactic acid, were also tested alone at their most effective concentration and in combination with CO_2. Neither of the test substances had an effect on the behaviour of the mosquitoes when presented alone (Figure 5A,B): both, activation (ranging from 21 to 33%, Figure 5A) and attraction (maximum 1%, Figure 5B) were in the range of the control groups with clean air (first columns in Figure 5A,B).

In combination with 4% CO_2 none of the fatty acids changed the responses compared with the CO_2 stimulus

Table 1 Effects of combinations of lactic acid (LA) with fatty acids on attraction of *A. aegypti*



Every stimulus was tested at least eight times. Bars show the percentage of attracted mosquitoes when another fatty acid (left column) was added to the basic mixture (top line). The level of attraction of the base mixtures alone is shown in the first row. The tested concentration for C_1–C_3 and C_7 fatty acids was a dilution of 1:10 000 at a flow rate of 30 ml/min, for C_4–C_9 (except C_7) a dilution of 1:100 at a flow rate of 30 ml/min and for C_{10}–C_{18} acids undiluted at a flow rate of 30 ml/min. Means were compared with each other using the Duncan test *post hoc* for a one-way ANOVA. Black, respectively grey shaded bars indicate a significant difference to the base mixture at $P = 0.05$.

alone (activation 91.5%, attraction 10%; Figure 5A,B). In contrast, lactic acid significantly increased the attractiveness of CO_2, corroborating the synergistic effect of the CO_2 plus lactic acid mixture (Figure 5B).

Discussion

Our experiments demonstrate that the attractive effects of fatty acids in host finding of *A. aegypti* depend on two factors: their chain length and their specific combination in the blend. A possible role for fatty acids as attractants for mosquitoes has already been proposed by several authors as many of these substances are present on human skin (Roessler, 1961; Carlson *et al.*, 1973; Knols *et al.*, 1997). This has been confirmed in our present study and, moreover, since we have tested the fatty acids in more detail, we can now precisely state the effectiveness of these compounds. The mosquitoes are able to discriminate between fatty acids of distinct chain length when combined with lactic acid. The attractiveness of mixtures of fatty acids varies greatly with the specific composition of the blend. With regard to the interpretation of our findings, it is essential that we can exclude possible effects like habituation or fatigue on the behavioural responses of the insects, since we used every group of mosquitoes several times in a series of tests. Our data show clearly that preceding stimuli have no effect on subsequent ones. These results are also in agreement with earlier findings (Geier and Boeckh, 1999).

The attractive effect of the fatty acids was only apparent when lactic acid was present. Carbon dioxide could not replace lactic acid as the synergist, confirming the crucial role of lactic acid in host finding by the yellow fever mosquito. Lactic acid acts as a synergist with CO_2 in breath (Acree *et al.*, 1968) and with components from human skin (Geier *et al.*, 1996) in attracting mosquitoes. Ammonia, present in both host odour sources, is only attractive in combination with lactic acid (Geier *et al.*, 1999b). In accordance with the data of Geier *et al.* (Geier *et al.*, 1999b), our results demonstrate that the attractive host odour for mosquitoes consists not of a single substance but rather of a blend of odorants which are not or only slightly attractive by themselves. The idea of a complex blend as the attractive olfactory signal in host finding of mosquitoes has already been mentioned (Galun, 1977; Takken, 1991).

Our data indicate that the following compounds contribute to the attractive blend emitted from human hosts: a short chain fatty acid, a medium-length fatty acid and ammonia, with lactic acid as the indispensable synergist. Whether fatty acids with a longer carbon chain (C_{13}–C_{18}) also play a role in host finding is not yet clear. When tested as pure compounds, these fatty acids significantly increase the attraction to lactic acid, but their addition to mixtures of lactic-, propanoic- and valeric acid did not cause any further increase in attractiveness. Interestingly, both the fatty acid C_{14}, which is the most abundant in human sweat (Cork and Park, 1996), and C_{11} even reduced the attractiveness of any combination of lactic acid with another fatty acid. These findings point in a direction similar to those reported by Skinner *et al.* (Skinner *et al.*, 1965, 1967), who found a repellent effect of skin-surface lipids. The authors suggested that the attraction to a given host depends upon a balance between naturally occurring attractants and repellents. It still remains open whether some long chain fatty acids of human skin odour play a role in the mosquito's preference

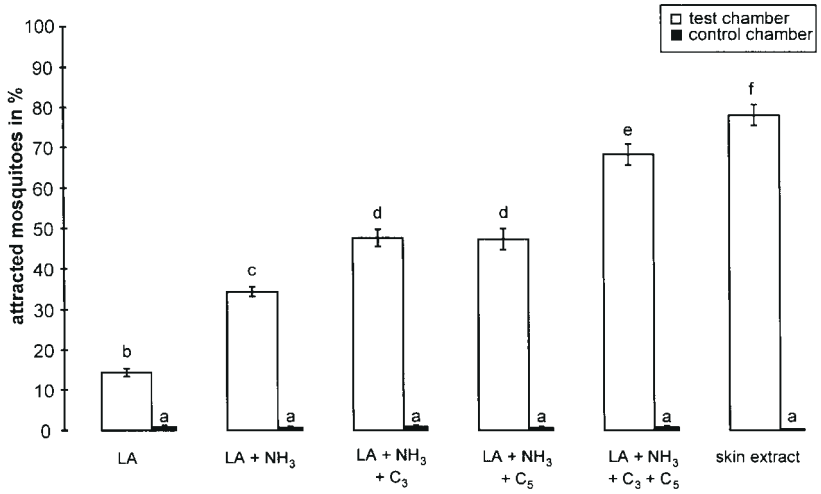


Figure 4 Attractiveness of single human skin odour mixtures to *A. aegypti*. Every stimulus was tested eight times. Left bars: percentage of mosquitoes attracted to the mixture in the test chamber; right bars: percentage of mosquitoes found in the control chamber. Letters on top of columns indicate significant differences to the control at $P = 0.05$; means with no letters in common are significantly different. Means were compared with each other using the Duncan test *post hoc* for a one-way ANOVA.

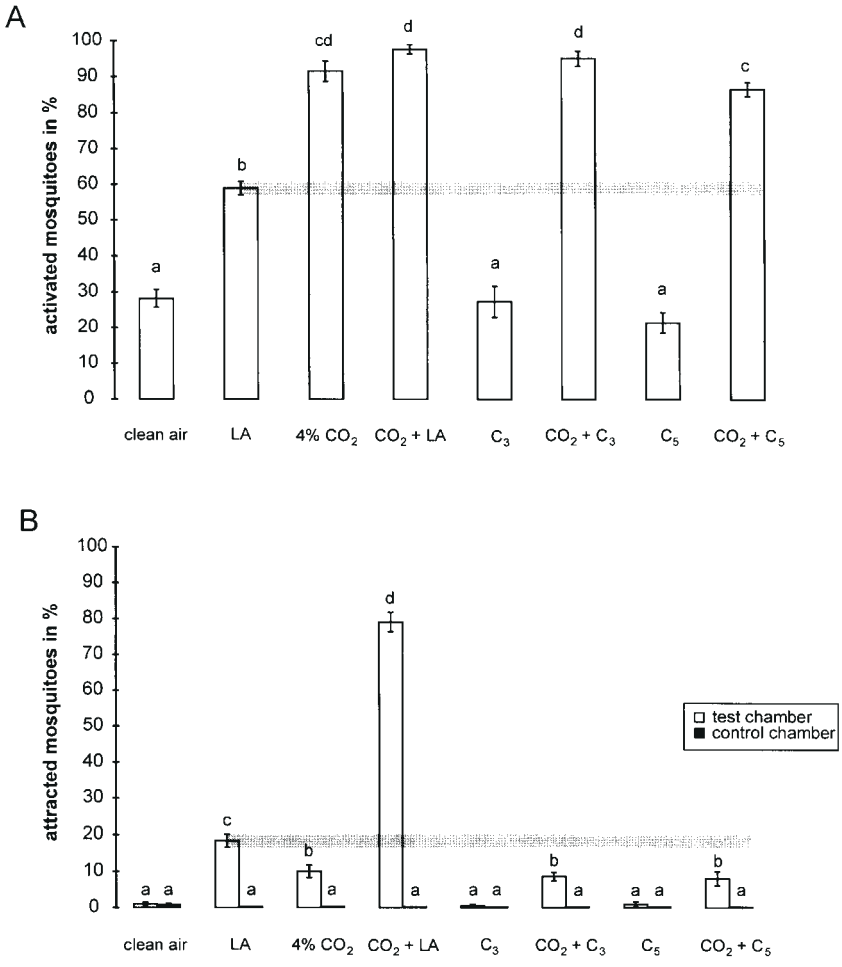


Figure 5 Responses of *A. aegypti* to single fatty acids in combination with CO_2. Columns represent the mean percentage ± SE ($n = 8$ repetitions) of responding mosquitoes. For a further explanation see the legend to Figure 4. **(A)** Activated mosquitoes; the horizontal bar shows the mean level of activation ± SE with lactic acid. **(B)** Left bars: percentage of mosquitoes attracted to the stimulus; right bars: percentage of mosquitoes found in the control chamber; the horizontal bar shows the mean attraction ± SE to lactic acid.

for certain persons [reviewed in (Khan, 1977)]. Since only a single concentration per stimulus has been tested so far in our experiments with odour mixtures, we cannot exclude effects at other concentrations.

Lactic acid and ammonia are attractive in doses which are supposed to evaporate from a human host (Smith *et al.*, 1970; Geier *et al.*, 1999b). In the case of fatty acids, however, we know neither their evaporation rate from human skin nor the absolute concentrations in our olfactometer. In order to confirm the attractive role of fatty acids, both the behavioural thresholds and the emitted doses from the host have to be determined. Although the artificial blend, composed of only four components, was almost as attractive as the whole skin extract, the slight difference in attractiveness might indicate that some other attractants have still to be identified. It is, however, also conceivable that the concentrations and proportions of the synthetic compounds tested so far were not optimal.

The behavioural effects of fatty acids in *A. aegypti* are also supported by electrophysiological studies (Pappenberger *et al.*, 1996). These authors found receptor cells in antennal A3 sensilla that responded to fatty acids. These receptor cells differed with regard to their maximum sensitivity to fatty acids of different chain length, but revealed overlapping response spectra. Such receptors could enable the mosquitoes to detect both the combinations as well as the relative proportions of these fatty acids in host odour.

Acknowledgements

We thank the Bayer AG (Leverkusen, Germany) for supply of the mosquito eggs.

Erratum

In the paper 'Ammonia as an attractive component of host odour for the yellow fever mosquito, *Aedes aegypti*' (Chem. Senses 24: 647–653, 1999) the information about the concentration of ammonia, 0.13 mmol/l NH_3, is wrong. 0.13 mol/l is correct.

References

Acree, F. Jr, Turner, R.B., Gouck, H.K., Beroza, M. and **Smith, N.** (1968) *L-Lactic acid: a mosquito attractant isolated from humans*. Science, 161, 1346–1347.

Bowen, M.F. (1991) *The sensory physiology of host-seeking behavior in mosquitoes*. Annu. Rev. Entomol., 36, 139–158.

Carlson, D.A., Smith, N., Gouck, H.K. and **Goodwin, D.R.** (1973) *Yellow fever mosquitoes: compounds related to lactic acid that attract females*. J. Econ. Entomol., 66, 329–331.

Cork, A. and **Park, K.C.** (1996) *Identification of electrophysiologically-active compounds for the malaria mosquito,* Anopheles gambiae*, in human sweat extracts*. Med. Vet. Entomol., 10, 269–276.

Davis, E.E. (1996) *Olfactory control of mosquito behaviour*. In Cardew, G. and Goode, J. (eds), Mosquito Olfaction and Olfactory-mediated Mosquito–Host Interactions. Ciba Foundation Symposium 200. John Wiley & Sons, New York, pp. 48–53.

Davis, E.E. and **Bowen, M.F.** (1994) *Sensory physiological basis for attraction in mosquitoes*. J. Am. Mosq. Control Assoc., 10, 316–325.

Eiras, A.E. and **Jepson, P.C.** (1991) *Host location by* Aedes aegypti *(Diptera: Culicidae): a wind tunnel study of chemical cues*. Bull. Entomol. Res., 81, 151–160.

Eiras, A.E. and **Jepson, P.C.** (1994) *Responses of female* Aedes aegypti *(Diptera: Culicidae) to host odours and convection currents using an olfactometer bioassay*. Bull. Entomol. Res., 84, 207–211.

Galun, R. (1977) *Responses of blood-sucking arthropods to vertebrate hosts*. In Shorey, H.H. and McKelvey, J. Jr (eds), Chemical Control of Insect Behavior. John Wiley & Sons, New York, pp. 103–115.

Geier, M. (1995) Verhaltensversuche mit Gelbfiebermücken Aedes aegypti zur Aufklärung des attraktiven Reizmusters bei der olfaktorischen Wirtsfindung. PhD Thesis, Universität Regensburg.

Geier, M. and **Boeckh, J.** (1999) *A new Y-tube olfactometer for mosquitoes to measure the attractiveness of host odours*. Entomol. Exp. Appl., 92, 9–19.

Geier, M., Sass, H. and **Boeckh, J.** (1996) *A search for components in human body odour that attract females of* Aedes aegypti. In Cardew, G. and Goode, J. (eds), Mosquito Olfaction and Olfactory-mediated Mosquito–Host Interactions. Ciba Foundation Symposium 200. John Wiley & Sons, New York, pp. 132–144.

Geier, M., Bosch, O.J. and **Boeckh, J.** (1999a) *Influence of odour plume structure on upwind flight of mosquitoes towards hosts*. J. Exp. Biol., 202, 1639–1648.

Geier, M., Bosch, O.J. and **Boeckh, J.** (1999b) *Ammonia as an attractive component of host odour for the yellow fever mosquito,* Aedes aegypti. Chem. Senses, 24, 647–653.

Gillies, M.T. (1980) *The role of carbon dioxide in host-finding by mosquitoes (Diptera: Culicidae): a review*. Bull. Entomol. Res., 70, 525–532.

Hocking, B. (1971) *Blood-sucking behavior of terrestrial arthropods*. Annu. Rev. Entomol., 16, 1–26.

Khan, A.A. (1977) *Mosquito attractants and repellents*. In Shorey, H.H. and McKelvey, J. Jr (eds), Chemical Control of Insect Behavior. John Wiley & Sons, New York, pp. 305–325.

Knols, B.G.J. and **De Jong, R.** (1996) *Limburger cheese as an attractant for the malaria mosquito* Anopheles gambiae *s.s.* Parasitol. Today, 12, 159–161.

Knols, B.G.J., van Loon, J.J.A., Cork, A., Robinson, R.D., Adam, W., Meijerink, J., De Jong, R. and **Takken, W.** (1997) *Behavioural and electrophysiological responses of the female malaria mosquito* Anopheles gambiae *(Diptera: Culicidae) to Limburger cheese volatiles*. Bull. Entomol. Res., 87, 151–159.

Lacher, V. (1967) *Elektrophysiologische Untersuchungen an einzelnen Geruchsrezeptoren auf den Antennen weiblicher Mosquitos (*Aedes aegypti *L.)*. J. Insect Physiol., 13, 1461–1470.

Ough, C.S. and **Stone, H.** (1961) *An olfactometer for rapid and critical odor measurement*. J. Food Sci., 26, 452–456.

Pappenberger, B., Geier, M. and **Boeckh, J.** (1996) *Responses of antennal olfactory receptors in the yellow fever mosquito* Aedes aegypti *to human body odours*. In Cardew, G. and Goode, J. (eds), Mosquito Olfaction and Olfactory-mediated Mosquito–Host Interactions. Ciba Foundation Symposium 200. John Wiley & Sons, New York, pp. 254–263.

Roessler, H.P. (1961) *Versuche zur geruchlichen Anlockung weiblicher Stechmücken (*Aedes aegypti *L., Culicidae)*. Z. Vgl. Physiol., 44, 184–231.

Sachs, L. (ed.) (1997) Angewandte Statistik. 8. Aufl. Springer-Verlag, Berlin.

Skinner, W.A., Tong, H., Maibach, H.I., Khan, A.A. and **Pearson, T.** (1965) *Repellency of skin-surface lipids of humans to mosquitoes*. Science, 149, 305–306.

Skinner, W.A., Tong, H., Pearson, T. and **Maibach, H.** (1967) *Repellency of human skin-surface lipid hydrocarbons to the yellow-fever mosquito*. J. Econ. Entomol., 60, 927–929.

Smith, C.N., Smith, N., Gouck, H.K., Weidhaas, D.E., Gilbert, I.H., Mayer, M.S., Smittle, B.J. and **Hofbauer, A.** (1970) *L-Lactic acid as a factor in the attraction of* Aedes aegypti *(Diptera: Culicidae) to human hosts*. Ann. Entomol. Soc. Am., 63, 760–770.

Stoddart, D.M. (ed.) (1990) The Scented Ape. Cambridge University Press, Cambridge.

Takken, W. (1991) *The role of olfaction in host-seeking of mosquitoes: a review*. Insect Sci. Appl., 12, 287–295.

Takken, W. (1996) *Synthesis and future challenges: the response of mosquitoes to host odours*. In Cardew, G. and Goode, J. (eds), Mosquito Olfaction and Olfactory-mediated Mosquito–Host Interactions. Ciba Foundation Symposium 200. John Wiley & Sons, New York, pp. 302–312.

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