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The Effect of Lactic Acid on Odour-Related Host Preference of Yellow Fever Mosquitoes

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Abstract

In a behavioural study we have investigated the role of lactic acid for the host preferences of yellow fever mosquitoes (*Aedes aegypti*) by comparing the attractiveness of rubbings from the hands of different human individuals and extracts obtained from skin rubbings from different mammals (*Bos primigenius* f. *taurus*, *Capra aegagrus* f. *hircus*, *Felis silvestris* f. *catus* and *Homo sapiens*). Certain human individuals were consistently more attractive to mosquitoes than others. Addition of lactic acid markedly increased the degree of attractiveness of formerly less attractive human odour samples and they were preferred over those which were originally the most attractive. There was almost no response to animal odour samples. In contrast to human samples, which contain a high amount of lactate, this compound could not be detected in samples from animals. When skin emanations from animals were combined with lactic acid, however, as many mosquitoes responded to odour samples of *B. primigenius* f. *taurus* and *C. aegagrus* f. *hircus* as did to human odours. All these data demonstrate that olfactory-based host preference of the anthropophilic mosquito *A. aegypti* is to a large extent due to differences in the amount of lactic acid in the odour samples.

Introduction

Female yellow fever mosquitoes suck blood mostly from humans (Scott *et al.*, 1993). They are the main vector of yellow and dengue fevers. Not all human hosts are likely to attract mosquitoes equally [reviewed by Kahn (Kahn, 1977)]. Individual differences in attractiveness could be due to various factors such as body odour, temperature, moisture or visual cues. However, odour emanating from the skin of hosts is regarded as a main reason for inter-host differences [reviewed by Clements (Clements, 2000)]. *Aedes aegypti* responds well to human skin odours [summarized by Takken and Knols (Takken and Knols, 1999)], whereas the effect of skin emanations from animals is less clear (McIver, 1968; Geier, 1995; McCall *et al.*, 1996). Although progress has been made in identifying attractive substances [reviewed in Takken and Takken and Knols (Takken, 1991; Geier *et al.*, 1999; Takken and Knols, 1999; Bosch *et al.*, 2000)], it is poorly understood why mosquitoes prefer skin odours of certain host species or host individuals to those of others. One of the substances that attract yellow fever mosquitoes is lactic acid. As a single stimulus this compound is only a weak attractant, but it acts as a powerful synergist together with other compounds from human skin odour (Geier *et al.*, 1996). Earlier studies on responses of *A. aegypti* to lactic acid indicate that there might be a relationship between the attractiveness of humans and the quantity of lactic acid on their skin (Acree *et al.*, 1968; Smith *et al.*, 1970). More recent studies have focused on differences in host odour composition: Cork and Park assumed that anthropophilic mosquitoes might respond to unidentified, particular human-specific chemicals (Cork and Park, 1996). Knols and Meijerink suggested that the blend of fatty acids plays a role in the attractiveness of humans to the malaria mosquito *Anopheles gambiae* s.s. (Knols and Meijerink, 1997).

So far no attempt has been made to investigate whether mosquitoe preferences for host odours can be altered by adding lactic acid. In our study on *A. aegypti* we increased the amount of lactic acid in skin odour samples from humans and other mammals to investigate whether the quantity of this compound affects the attractiveness of host odours.

Materials and methods

Yellow fever mosquitoes [*A. aegypti* (L.)] were raised from eggs obtained from the Centre of Plant Research at Bayer AG (Mohnheim, Germany). The larvae were fed with Tetramin fish food. The adults were maintained in containers (50 × 40 × 25 cm) and had access to filter paper soaked in 10% glucose solution. The culturing room was maintained at 26–28°C, 60–70% relative humidity and on a 12:12 h light:dark photoperiod. Experiments were conducted with 10- to 30-day-old female mosquitoes.

Wind tunnel

A Y-shaped wind tunnel was used as designed by Geier [for a detailed description see Geier and Boeckh (Geier and Boeckh, 1999; Geier *et al*., 1999b)]. The air stream (flow rate 80 l/min) was purified with an activated charcoal filter, heated (28 ± 1°C), humidified (70 ± 5%) and passed constantly through the wind tunnel at a speed of 0.2 m/s in the arms and 0.4 m/s in the stem of the Y-tunnel. The tunnel device was placed on a white table with white cardboard shields (height 20 cm) which prevented visual stimulation by the experimenter from both sides. Overhead illumination was provided by two 40 W daylight bulbs.

Odour stimuli

Odour samples from the hands of different human subjects were obtained using the method described by Geier *et al*. (Geier *et al*., 1999a). By intensely rubbing glass test tubes (16 mm outer diameter, length 160 mm) between both hands for 30 s, skin residues were transferred to the glass surface. The hands were cleaned with tap water and a pH neutral perfume-free washing liquid (EUBOS med.; Dr Habein & Co., Meckenheim, Germany) about 1 h before the rubbing and care was taken to avoid any contact with cosmetics or perfumes. The glass tube with skin residues was inserted into a Teflon tube (inner diameter 17 mm, length 150 mm) and charcoal-filtered air was passed through the narrow space between the Teflon tube and the glass tube at a flow rate of 2.8 l/min. Human donors were of similar age (26–32 years); individuals A and B were male, C and D female. To compare the lactate content of skin residues, tubes with rubbings from donors A and C were rinsed with ethanol. The total lactate content (salt and free acid) of the dissolved samples was measured enzymatically (Hohorst, 1970) using lac- tate dehydrogenase (Boehringer Mannheim, Mannheim, Germany) and NAD (Boehringer Mannheim).

Odour samples from different mammals

Samples were collected by rubbing the skins of live mammals (dorsal and lateral parts) with 1 g cotton pads. Samples were taken from calf (*B. primigenius* f. *taurus*), goat (*C.aegagrus* f. *hircus*), cat (*F. silvestris* f. *catus*) and from a human (subject C). After 10 min of rubbing each pad was placed in a glass column (0.5 × 8 cm) and extracted with ethanol (Merck). Each column filling yielded 0.5 ml of extract. For each behaviour test 5 µl of the extract was applied to the inner surface of a glass cartridge (inner diameter 0.5 cm). After the solvent had evaporated the glass cartridge was placed in a heating element on top of the stimulus chamber of the wind tunnel and air was blown through the cartridge at a rate of 2.8 l/min. The total lactate content (salt and free acid) of the extracts was determined enzymatically using lactate dehydrogenase (Boehringer Mannheim) and NAD (Boehringer Mannheim). According to calculations given in Geier and Boeckh (Geier and Boeckh, 1999), ~7% of the total lactate content in a human skin rubbing extract is free lactic acid.

Lactic acid

Charcoal-filtered compressed air was passed through a 250 ml Erlenmeyer flask filled with 10 ml l-(+)-lactic acid (90% aqueous solution, extra pure; Merck). The output of lactic acid depended on the flow rate. Calibration values were taken from Geier *et al*. (Geier *et al*., 1999a). By leading an airflow of 15 ml/min over the surface of the solution, a stimulus of 3.1 µg/min lactic acid (18 nmol/min, a concentration of 0.45 nmol/l air in the arm of the wind tunnel) was generated. This value is within the range of lactic acid amounts given off from human hands (Smith *et al*., 1970). In order to determine response characteristics to different doses, three different amounts of lactic acid were tested: air flows of 0.3, 3 and 30 ml/min resulted in outputs of 0.06, 0.6 and 6.3 µg/min (0.009, 0.09 and 0.91 nmol/l) lactic acid, respectively. The lowest flow rate was generated by a precision tubing pump (Masterflex; Novodirect, Kehl/Rhein, Germany). For the other flow rates flow meters were used to control the air flow.

Bioassay

For the behaviour tests groups of 17–23 female mosquitoes were lured out of the holding container into the release chambers using a piece of filter paper with 0.5 ml extract from skin rubbings of 50 humans. The bioassays were performed as previously described in detail (Geier and Boeckh, 1999). The percentage of mosquitoes trapped in the test chambers at the two upwind ends of the wind tunnel were taken as a measure for attractiveness of the odour samples. For each stimulus the means (± SE) of attractiveness were calculated. Since the data are percentage values, arc-sine transformation was applied to the data (Sokal and Rohlf, 1981) before statistical analysis. Values for the two-choice tests were compared using a *t*-test for paired samples. In the experiment with odours from different mammals the transformed means were analysed for statistical differences between treatments by one-way ANOVA and the LSD method as a *post hoc* test ($P < 0.05$). All calculations were performed with the statistics program SPSS 8.0 for Windows.

Results

Inter-individual differences in the attractiveness of human odour samples

Odour samples taken from the hands of four human subjects (A–D) were tested against each other, two at a time (Figure 1). This revealed differences among individuals regarding their attractiveness to yellow fever mosquitoes. The order of attractiveness was A ≥ B > D ≥ C. Rubbings from A and C differed in their attractiveness by 35% absolute.

Figure 2 demonstrates that the difference between these two donors was quite consistent over almost 1 year. Odour samples were taken on 28 different days over a period of 11 months. In 75% of the experiments the difference was significant. Differences in attractiveness were up to 56% absolute. The lactate content of the rubbings was tested on three different days with significant differences in attractiveness each time. The lactate content of skin residues from donor A was 2.9–4.2 times higher than that from C (A mean ± SD 58.4 ± 15.9 µg lactate per rubbing; C mean ± SD 16.2 ± 4.1 µg lactate per rubbing).

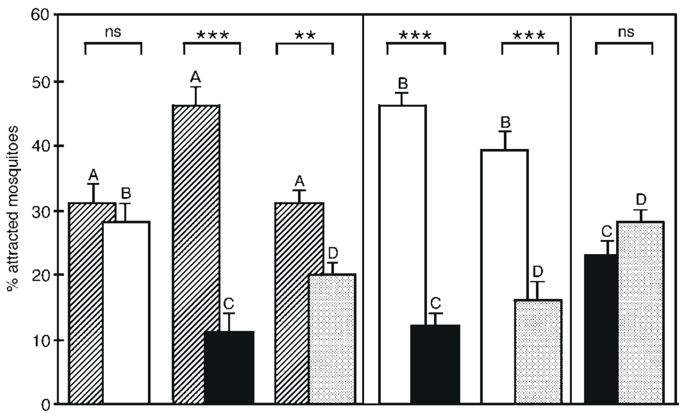

Figure 1 Comparison of odour samples obtained from four humans (A–D) with respect to their attractiveness for yellow fever mosquitoes. Values are means ± SE of 56 repeated bioassays with 20 (± 3) mosquitoes, respectively. Significance levels: ***, $P < 0.001$; **, $P < 0.01$; ns, not significant.

The effect of lactic acid on inter-individual differences in attractiveness

Skin rubbings from three different human subjects were tested, two at a time, for their attractiveness to *A. aegypti* (Figure 3). Again, subjects A and B were found to be substantially more attractive than C. However, when lactic acid (3.1 µg/min) was added to the olfactory less attractive skin rubbing from C its attractiveness increased far beyond that of A and B.

We determined the dose–response characteristics of lactic acid by adding three different doses (ranging from 0.06 to 6.3 µg/min) to odour samples from subject C and testing them against subject A (Figure 4). The unaltered samples as well as those modified with the lowest dosage of 0.06 µg/min lactic acid were significantly less attractive than subject A. When lactic acid was added in a 10-fold higher amount (0.6 µg/min) the attractiveness of the modified stimulus was doubled and the altered rubbings were significantly preferred to those of subject A. The addition of a higher amount of lactic acid (6.3 µg/min) increased the attractiveness even further: more than three times as many mosquitoes chose the modified odour compared with the unaltered odour sample of subject A. In general lactic acid enhanced the attractiveness of subject C in a dose-dependent manner.

The attractiveness of mammalian odour samples

Odour samples of different mammals were tested as unaltered odours and in combination with lactic acid. All odour samples were presented to the mosquitoes in successive trials and tested against air. Without addition of lactic acid clear differences were found between the attract-

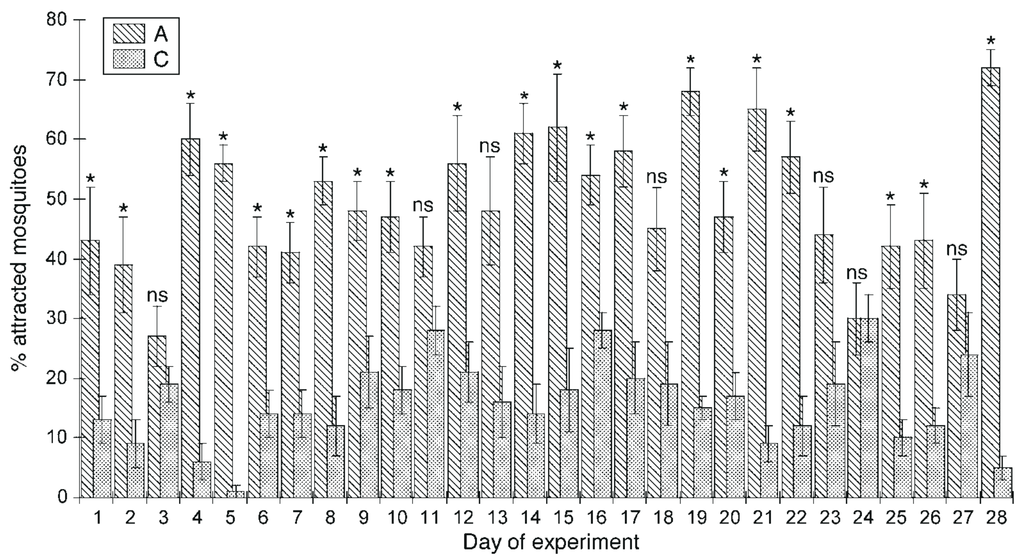

Figure 2 Comparison of odour samples from two humans (A and C). Series of experiments on 28 different days over a period of 11 months. Values are means ± SE of eight repeated bioassays with 20 (± 3) mosquitoes, respectively. *, significantly different ($P < 0.05$); ns, not significant.

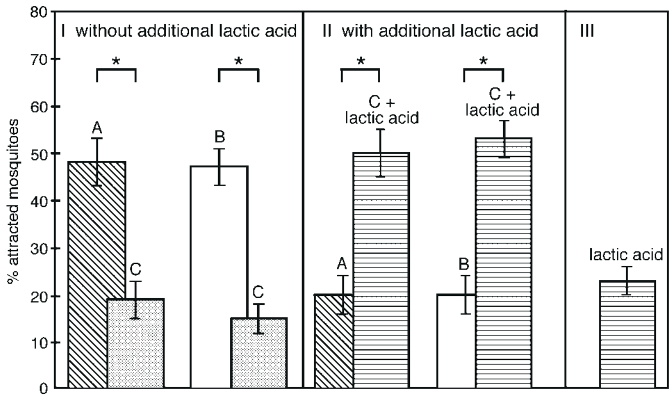

Figure 3 Comparison of odour samples obtained from three different humans (A–C) **(I)**, with additional lactic acid added (3.1 μg/min) **(II)** and lactic acid alone tested against air **(III)**. Values are means ± SE of 16 repeated bioassays with 20 (± 3) mosquitoes, respectively. *, significantly different ($P < 0.05$).

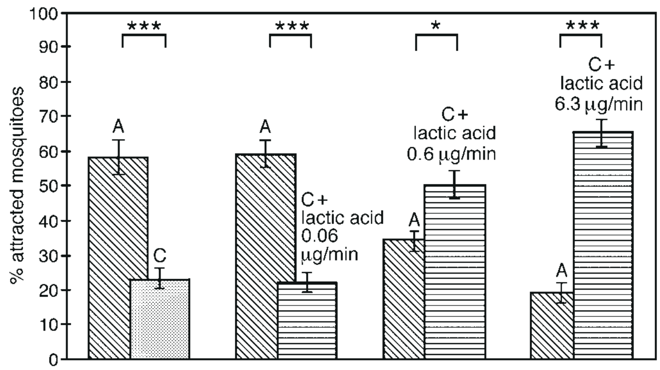

Figure 4 Effect of the dose of lactic acid added on the attractiveness of the less attractive odour sample C when compared with A. Values are means ± SE of 16 repeated bioassays with 20 (± 3) mosquitoes, respectively. Significance levels: ***, $P < 0.001$; *, $P < 0.05$.

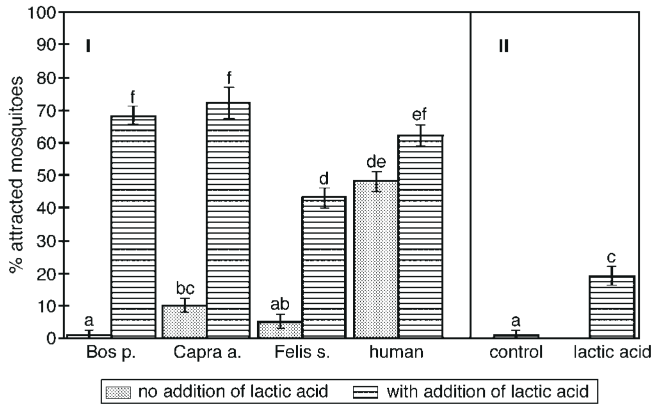

Figure 5 Attractiveness of four different mammalian odour samples with and without lactic acid added (3.1 μg/min released) **(I)** and control and lactic acid alone (3.1 μg/min) **(II)**. Tested odour stimuli were: Bos p., *Bos primigenius* f. *taurus*; Capra a., *Capra aegagrus* f. *hircus*; Felis s., *Felis silvestris* f. *catus*; human person C. Odour sources, extracts of skin rubbings with cotton; control, extracts of cotton without skin rubbing; lactic acid, lactic acid + extract of cotton without skin rubbing. All stimuli were tested against air. Values are means ± SE of 12 repeated measurements with 20 (± 3) mosquitoes, respectively. Means with no letters in common are significantly different ($P < 0.05$).

iveness of human and animal odour samples (Figure 5). Human odour samples were attractive, with about half of the yellow fever mosquitoes responding to them. Animal skin odour samples were either only marginally attractive or not attractive at all, the level of attractiveness ranging from 1 (calf) to 10% (goat). The relative amounts of lactate in the skin extracts of different species were determined. Whereas the human extract produced from rubbings of subject C, in the same way as the animal extracts, contained a high amount of lactate (803 μg/ml ethanol extract), no lactate was found in the samples of the other mammals (sensitivity of the test 15 μg/ml extract). Lactic acid alone was only slightly attractive as a stimulus, with 19% of the mosquitoes responding to it. However, animal odours combined with lactic acid were highly attractive: 70% of the mosquitoes were attracted to the calf or goat odour when lactic acid was added, indicating a marked synergistic effect.

Discussion

Inter-individual variations in the attractiveness of human skin odours

The wind tunnel tests demonstrated a pronounced response of *A. aegypti* to human skin odours, already documented in the literature (Mayer and James, 1969; Bar-Zeev *et al.*, 1977; Schreck *et al.*, 1981, 1990; Eiras and Jepson, 1994; Geier *et al.*, 1996, 1999a; Kline, 1998; Geier and Boeckh, 1999; Steib, 2000). Two-choice tests with odour samples from human hands revealed statistically significant differences in the level of attraction among individuals. Inter-individual variations in the attractiveness of human hosts have been reported earlier for mosquitoes (Brouwer, 1960; Lindsay *et al.*, 1993; Knols *et al.*, 1995a,b) as well as for other haematophagous Diptera, such as black flies (Schofield and Sutcliffe, 1996, 1997) and sand flies (Hamilton and Romsoondar, 1994). So far, in most studies with *A. aegypti* attractiveness was assessed either by comparing human individuals who were sitting in a room (Canyon *et al.*, 1998) or by exposing forearms or hands to the mosquitoes (Rahm, 1956, 1958; Freyvogel, 1961; Khan *et al.*, 1965; Maibach *et al.*, 1966). Only Schreck *et al.* presented skin residues on glass beads to exclude the influence of other host-related factors such as size of the host, visual stimuli or body temperature (Schreck *et al.*, 1990). Here we also used skin rubbings on glass as odour sources in order to bioassay under standardized conditions. In contrast to Schreck *et al.*, who used successive trials (Schreck *et al.*, 1990), we tested the odour samples directly against each other. The two-choice tests revealed consistent inter-individual ranking according to their attractiveness. So far it has not been understood which components of body odour are responsible for inter-host differences of attractiveness. In our study we increased the amount of lactic acid in skin odour samples by adding this compound. The results suggest that for *A. aegypti* the amount of lactic acid in the host odour contributes to host preferences in a dose-dependent manner. Lactic acid is produced in the eccrine sweat glands by glycolysis and secreted onto the skin with eccrine sweating (Gordon *et al*., 1971; Sato, 1977). According to Smith *et al*. lactic acid evaporates from a human hand at a rate of 0.4–2.2 µg/min (Smith *et al*., 1970). In our results a dose of 0.6 µg/min, an amount that is within the range determined by Smith *et al*. (Smith *et al*., 1970), was required to influence the mosquitoes' preferences significantly. When combined with this amount of lactic acid even the previously least attractive human odour samples were preferred to the originally most attractive ones.

The effect of lactic acid on the attractiveness of mammalian odour samples

In contrast to human odour samples, skin odours from animals were not or only slightly attractive to yellow fever mosquitoes. This might be the reason behind the rather anthropophilic behaviour of this species (Chow *et al*., 1993; Scott *et al*., 1993). Our enzymatic test revealed that the high quantity of lactate found in the human skin residue is not present in the animal odour samples. This could explain the low degree of attractiveness of the tested animal samples. The very weak response of *A. aegypti* to odours without lactic acid is consistent with the results determined by Geier *et al*., who removed lactate from human samples and found that none of the other components which contribute to the attractiveness of human skin are effective without addition of lactic acid (Geier *et al*., 1996). The large quantities of lactate on the skin, excreted by eccrine sweat glands (Sato, 1977), can be regarded as characteristic for humans and could be a factor that, in conjunction with other host odour components, guides anthropophilic mosquitoes to their hosts. Interestingly, when lactic acid was added to animal odours the attractiveness increased enormously, from 7.2-fold up to 68-fold. Thus, lactic acid acts synergistically with other components of human skin odour (Geier *et al*., 1996) as well as with components of body odour of animals, but the high degree of attractiveness of animal odours combined with lactic acid demonstrates that the other components which are required for host finding are not necessarily human specific.

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