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Relation between HLA genes, human skin volatiles and attractiveness of humans to malaria mosquitoes

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ABSTRACT

Chemical cues are considered to be the most important cues for mosquitoes to find their hosts and humans can be ranked for attractiveness to mosquitoes based on the chemical cues they emit. Human leukocyte antigen (HLA) genes are considered to be involved in the regulation of human body odor and may therefore affect human attractiveness to mosquitoes, and hence, affect the force of malaria transmission. In the present study the correlations between HLA profiles, human skin volatiles and human attractiveness to the malaria mosquito *Anopheles gambiae* Giles *sensu stricto* were examined.

Skin emanations of 48 volunteers were collected by rubbing a foot over glass beads. Previously the attractiveness of these emanations to *An. gambiae* was determined. In this study, the chemical composition of these emanations was determined by gas chromatography–mass spectroscopy (GC–MS) and blood samples of all volunteers were taken for HLA analysis. Hierarchical cluster analysis (HCA), partial least squares discriminant analysis (PLS-DA), Fisher's exact test and random forest regression were used to test for correlations between individuals classified as either highly or poorly attractive to mosquitoes and their HLA profile and volatile composition.

HLA profiling suggests that people carrying HLA gene Cw*07 are more attractive to mosquitoes. GC–MS revealed that limonene, 2-phenylethanol and 2-ethyl-1-hexanol were associated with individuals that were poorly attractive to *An. gambiae* and lactic acid, 2-methylbutanoic acid, tetradecanoic acid and octanal with individuals that were highly attractive. Such compounds offer potential for disruption of mosquito behavior in malaria intervention programs.

1. Introduction

Female mosquitoes use physical and chemical cues to find their hosts. Long distance attraction is mainly determined by olfactory stimuli, whereas at shorter distance mosquitoes find their hosts by integrating information from olfactory, visual, temperature and humidity receptors (Gillies and Wilkes, 1969; Takken, 1991). Chemical cues are especially important for nocturnal mosquito species and play a role in the differential attractiveness of humans to mosquitoes as has been shown in several studies (Bernier et al., 2002; Brady et al., 1997; Brouwer, 1960; Knols et al., 1995; Lindsay et al., 1993; Logan et al., 2008; Mayer and James, 1969; Mukabana et al., 2002; Qiu et al., 2006; Schreck et al., 1990). Humans can be ranked for attractiveness to mosquitoes by testing the emanations from their total body (Knols et al., 1995; Lindsay et al., 1993; Mukabana et al., 2002) or by testing emanations from parts of the body (Logan et al., 2008; Mayer and James, 1969; Smart and Brown, 1957; Verhulst et al., 2011b).

Several factors have been shown to influence the differential attractiveness of humans to mosquitoes including skin temperature and humidity (Gilbert et al., 1966; Smart and Brown, 1957), human body mass (Port et al., 1980; Spencer, 1967) and surface area (Spencer, 1967). There is at least one report of a correlation

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between sex and attractiveness to mosquitoes (Muirhead-Thomson, 1951), although more recent studies did not confirm this (Carnevale et al., 1978; Kirk et al., 2000; Logan et al., 2008; Qiu et al., 2006). To eliminate the effect of physical parameters like skin temperature or humidity, skin emanations can be collected on glass beads which are tested for their attractiveness to mosquitoes (Bernier et al., 1999; Qiu et al., 2006; Schreck et al., 1990).

A genetic background for human attractiveness to mosquitoes was investigated among 197 monozygotic and 326 dizygotic twin pairs (Kirk et al., 2000). The study indicated that the frequency of being bitten was strongly influenced by genetic background. The volatile blends released by twins can be matched by human sniffers (Roberts et al., 2005; Wallace, 1977) and gas-chromatography (GC) analysis (Kuhn and Natsch, 2009; Sommerville, 1994). The genetic background of human body odor is determined, at least partly, by the human leukocyte antigen (HLA) genes of the major histocompatibility complex (MHC) (Penn and Potts, 1998a; Roberts et al., 2008; Wedekind and Furi, 1997; Wedekind and Penn, 2000) that serve functions in the human immune system.

The differential attractiveness of humans, as determined by their skin emanations, remains relatively stable over time (Qiu et al., 2006). This, together with the genetic background of the human odor profile (Kuhn and Natsch, 2009; Roberts et al., 2005; Sommerville, 1994) and genetic background of the number of mosquito bites received by humans (Kirk et al., 2000), supports the hypothesis that genetic factors, in particular HLA genes, are involved in determining differential attractiveness of humans to mosquitoes (Logan, 2008; Verhulst et al., 2010). How genes might influence body odor was summarized by Penn and Potts (1998a). They hypothesized that MHC molecules bind allele-specific subsets of peptides, which are volatilized by the activity of the commensal microorganisms. This hypothesis was confirmed at least partly by an *in vitro* experiment in which HLA peptides determined the production of 3-methylbutanal by skin bacteria (Savelev et al., 2008).

Skin microbiota play a crucial role in the production of human body odor, probably by converting HLA specific peptides to volatile compounds (Penn and Potts, 1998a). Without skin bacteria human sweat is odorless (Shelley et al., 1953) and there is a strong correlation between human body odor and the presence of specific microorganisms (Ara et al., 2006; Leyden et al., 1981; Rennie et al., 1990, 1991; Taylor et al., 2003). Recently we showed that the human skin bacterial composition determines an individual's attractiveness to the malaria mosquito *Anopheles gambiae* Giles *sensu stricto* (henceforth termed *An. gambiae*) (Verhulst et al., 2011b). Skin emanations were collected on glass beads from 48 individuals and tested for their attractiveness to *An. gambiae* in an olfactometer. The relative attractiveness of the individuals to *An. gambiae* showed significant differences and nine out of the 48 individuals could be ranked as highly attractive (HA) and seven other individuals as poorly attractive (PA) (Verhulst et al., 2011b). Skin emanations of individuals classified as HA caught 2.14 more mosquitoes on average than skin emanations of PA individuals. The attractiveness to mosquitoes of these two groups was correlated with skin bacterial diversity and composition (Verhulst et al., 2011b). In the present study we examined the correlations between the attractiveness of these two groups of individuals (determined previously and described in Verhulst et al. 2011b) with their HLA profile and the chemical composition of their skin emanations.

2. Materials and methods

2.1. Attractiveness of individuals to malaria mosquitoes

Olfactometer tests to determine the attractiveness of 48 individuals to malaria mosquitoes and the results of these tests were published before (Verhulst et al., 2011b). Briefly, the attractiveness of 48 adult males aged between 20 and 64 years to *An. gambiae* was examined. Volunteers were requested to refrain from drinking alcohol (Lefèvre et al., 2010; Shirai et al., 2002), eating garlic, onions or spicy food, not to take a shower, not to use perfumed cosmetics and to wear socks provided by the research team 24 h before the collection of skin emanations.

A dual-choice olfactometer consisting of a Perspex flight chamber of 1.60 × 0.66 × 0.43 m (Pates et al., 2001) was used to determine the attractiveness of the volunteers to female *An. gambiae* (Verhulst et al., 2011b). Skin emanations from each individual were collected by rubbing six glass beads (15 mm in diameter, contained in a Teflon holder) for 10 min against the underside of the left foot. The glass beads that had been in contact with the feet of each individual were placed in one of the trapping devices of the olfactometer, into which clean air from an air sample bag was pumped. Gaseous ammonia (136 ppm), which is moderately attractive to *An. gambiae*, was pumped into the other trapping device (Smallegange et al., 2005; Verhulst et al., 2011b), which contained six clean glass beads. In each trial, 30 female mosquitoes were released in the flight chamber. During 15 min, the mosquitoes were allowed to fly upwind and to enter one of the trapping devices. Odor samples of each volunteer were taken and tested for their attractiveness to *An. gambiae* six times in total; twice in a row on three different mornings (Qiu et al., 2006; Verhulst et al., 2011b).

2.2. Collection of skin emanations on glass beads for GC–MS

Glass beads have the advantage that emanations collected on them can be removed by thermodesorption for analysis by gas chromatography–mass spectroscopy (GC–MS), avoiding the need of solvent extraction (Bernier et al., 1999; Qiu et al., 2006; Schreck et al., 1990). Therefore, on three different mornings, directly after two subsequent olfactometer experiments, skin emanations from the left foot of each volunteer were collected on small glass beads (4 mm in diameter, Witeg, Germany).

For this purpose, one hundred beads were divided over seven ridges in a Teflon tray (Fig. 1). Volunteers were asked to rub the sole of their left foot (just behind the toes) over the beads for 10 min. The beads coated with human skin emanations were then transferred to five steel cartridges. The Teflon tray was cleaned

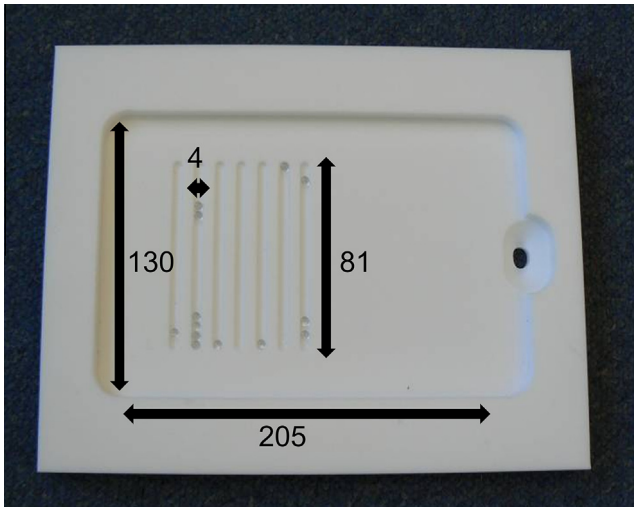

Fig. 1. Sampling skin emanations for gas chromatography–mass spectroscopy (GC–MS). The foot was rubbed from left to right over 100 small glass beads (a few are shown on the photo) laying in small ridges in a Teflon tray. Distances in millimeters.

between experiments with 70% ethanol (Merck, Germany) and quick-dried with a heat gun (Ferm B.V., The Netherlands).

2.3. GC–MS analysis of skin emanations from glass beads

The cartridges with glass marbles with skin emanations were placed in an autosampler (Ultra 50:50 TD, Markes International Ltd., UK). Emanations on the beads were analyzed by using thermodesorption followed by GC–MS. The system consisted of a thermal desorption autosampler, an electrically-cooled trap for focusing (Unity, Markes International Ltd., UK) and a flow controller (Air server, Markes International Ltd., UK) for thermal desorption injection into a Trace GC Ultra (Thermo Scientific, USA) coupled to a quadruple mass detector (DSQ, Thermo Scientific, USA).

The cartridges were dry-purged for one minute with helium (5.0 grade) at 30 °C to remove residual moisture and oxygen. Cartridges were desorbed at 150 °C for 10 min and the volatiles were focused on an electronically cooled sorbent trap (general purpose hydrophobic, Markes, UK) at −10 °C. Analytes were transferred to the analytical column by ballistic heating of the cold trap to 250 °C for 3 min and splitting of the helium carrier gas resulting in an injection of 1/6 of the total amount.

Analytes were separated on a RTX-5 ms GC column (30 m × 0.25 mm ID, 1.0 μm film thickness, Restek, USA) using helium as carrier gas at a constant flow rate of 1.0 mL/min. The GC temperature was programmed at 45 °C for 3 min followed by a ramp of 8 °C/min to 280 °C and a 2 min hold at 280 °C. The transfer line between the GC and MS was kept at 275 °C. The column effluent was ionized by electron impact at 70 eV and mass-spectra were recorded in positive mode from 35–300 *m/z* with a scan speed of five scans/s and an ion source temperature of 250 °C.

2.4. Identification and abundance of compounds in skin emanations

Volatile profiles from skin emanations collected on glass beads of HA and PA individuals were screened for compounds that were either: (1) Identified in the headspace of skin bacteria attractive to *An. gambiae* (Verhulst et al., 2009), (2) Identified as attractive to *An. gambiae* in previous experiments (Smallegange et al., 2009), or (3) Associated with individuals less attractive to the yellow fever mosquito *Aedes aegypti* L. (Logan et al., 2008).

Compounds were identified by comparing their mass spectra and retention times with those of authentic reference compounds (purchased at Sigma and Fluka, Germany). Relative quantification of the compounds was done based on characteristic mass ions (Table 1) for each compound using the software package Xcalibur (Version 2.07, Thermo Scientific, USA). For each component, expected retention time, characteristic mass (Table 1), and integration settings were inserted in a processing setup. Next, a sequence of the chromatograms from the skin emanations of the individuals that significantly differed in attractiveness (HA and PA) was batch processed with the processing setup. Identified peaks were evaluated in the Quan browser and identifications and peak areas manually adjusted where necessary. The abundances of each compound in the chromatograms determined by integration of the peak area was exported to Excel for further statistical analysis.

2.5. HLA analysis

From each volunteer 5–10 mL of blood was collected for DNA isolation in EDTA tubes (Vacutainer, BD, USA) to prevent clotting. Genomic DNA was isolated using a commercial semi-automated beads-based assay (Chemagen, Germany).

HLA-A, -B and -Cw typing was done with the reverse line hybridization strip assay (RELI™ SSO, Invitrogen, USA). HLA-DRB and -DQB typing was performed with a reversed approach of the PCR/SSOP technique described previously (Verduyn et al., 1993). Briefly, using Biotin-labeled generic primers the polymorphic regions of the HLA genes were amplified by PCR. After amplification the PCR fragments were hybridized under critical conditions to HLA specific probes. Signals to discriminate for positive and negative probe reaction were achieved by adding horseradish peroxidase streptavidin followed by a luminogen (Amersham ECL Kit, GE Healthcare Biosciences Pittsburgh, USA).

With computer assisted analysis software (SCORE, Nellcor, USA) the probe hybridization patterns were interpreted to HLA types (Helmberg et al., 1998).

2.6. Ethical clearance

The Dutch Medical Ethical Review committee (METC, Project number ABR NL16928.081.07 amended in 2007) approved the study, and written informed consent was acquired from all subjects prior to participation.

2.7. Statistical analysis

Abundances of identified volatiles in the chromatograms of the HA and PA individuals were analyzed using a supervised and unsupervised method. Hierarchical cluster analysis (HCA, Ward Linkage, squared Euclidean distance , SPSS 20.0.0) was used as an unsupervised method with the objective to partition the individuals into subgroups (or clusters) based on the abundance (log) of the volatiles analysed, without any prior knowledge about the attractiveness of the individual to *An. gambiae* (Ho et al., 2002; Ward, 1963). Partial least squares discriminant analysis (PLS-DA) was used as a supervised method using the software program SIMCA-P 12.0 (Umetrics, Sweden) (Bruinsma et al., 2010; Eriksson et al., 2006). The objective of PLS-DA is to find a model that separates classes of observations on their X-variables. Because PLS-DA is a supervised technique class memberships of the observations are predefined (Eriksson et al., 2006). Therefore, the response was a vector y containing the values 1 and 0 as dummy variables for the PA and HA individuals, respectively. The X-matrix contained the integrated peak areas, which were log transformed, centered and scaled to unit variance. The number of significant PLS components was determined by cross-validation (Eriksson et al., 2006).

A two-tailed Fisher's Exact test was used to test for possible correlations between individuals classified as HA or PA and their HLA profiles. *P*-values were corrected for multiple comparisons applying the Bonferroni method. Odds ratios were calculated according to Woolf Haldane's test (Svejgaard and Ryder, 1994).

To test for possible correlations between HLA genes and the peak areas of selected compounds in the gas chromatograms of the skin emanation samples (Table 1), random forest regression was performed (Breiman, 2001). A random forest regression was done with 500 regression trees and 22 variables tried at each split and performed with the Random Forest package (Liaw and Wiener, 2002) in the R programming environment (http://www.R-project.org).

3. Results

3.1. GC–MS analysis of skin emanations from glass beads

Fifteen volatiles detected in previous studies could be identified in the chromatograms of the skin emanations of the 48 individuals that had been collected on glass beads (Table 1) (Logan et al., 2008;

Table 1
Characteristics of compounds detected in the chromatograms of the skin emanations collected from the foot.

Compound	KRI	CAS Nr.	Origin	Mass
3-Methylbutanal	655	590-86-3	Verhulst et al. (2009)	58
2-Methylbutanal	664	96-17-3	Verhulst et al. (2009)	57
3-Hydroxy-2-butanone	743	513-86-0	Verhulst et al. (2009)	88
3-Methylbutanoic acid	851	503-74-2	Verhulst et al. (2009)	60
2-Methylbutanoic acid	858	116-53-0	Verhulst et al. (2009)	74
6-Methyl-5-hepten-2-one	988	409-02-9	Logan et al. (2008)	108
Octanal	1005	124-13-0	Logan et al. (2008)	84
Limonene	1027	138-68-3	Logan et al. (2008)	93
2-Ethyl-1-hexanol	1029	104-76-7	Logan et al. (2008)	83
Lactic acid	1072	79-33-4	Smallegange et al. (2009)	45
Nonanal	1104	124-19-6	Logan et al. (2008)	98
2-Phenylethanol	1117	60-12-8	Verhulst et al. (2009)	91
Decanal	1202	112-31-2	Logan et al. (2008)	112
Geranyl acetone	1445	689-67-8	Logan et al. (2008)	151
Tetradecanoic acid	1771	544-63-8	Smallegange et al. (2009)	185

Column 'Origin' refers to the criteria based on which each compound was selected. The abundance in the samples was determined by selecting a characteristic mass (see materials and methods section). Synthetic references were injected for each compound.

Smallegange et al., 2009; Verhulst et al., 2009). Hierarchical cluster analysis could only partly separate the PA and HA group based on the peak areas of the selected compounds (Supplementary Material figure S1). The PLS-DA did not significantly differentiate the PA and HA group. To get an indication which compounds associated best with the PA or HA group, the first two PLS components were calculated ($R^2X = 0.367$, $R^2Y = 0.613$, $Q^2 = -0.210$; Fig. 2 and 3) and clearly separated the HA from the PA individuals (Fig. 2).

The loading plot shows that more compounds were associated with HA individuals than PA individuals. Limonene, 2-phenylethanol and 2-ethyl-1-hexanol correlate best with individuals that were poorly attractive to *An. gambiae* and lactic acid, 2-methylbutanoic acid, tetradecanoic acid and octanal with individuals that were highly attractive (Fig. 3).

3.2. HLA profiles

When the HLA profiles of the HA and PA group were compared, HLA antigen Cw*07 occurred significantly more often in the HA group than in the PA group (Fisher's Exact test, $P = 0.0073$). Antigen Cw*07 was detected in all volunteers in the HA group and in only two out of seven in the PA group. After correction for testing multiple alleles, the *P*-value was 0.064 (Bonferroni corrected; Fig. 4, Supplementary Material Table S1)."

Testing the correlation between HLA profiles and peak areas of compounds in the chromatograms of the skin emanations with the random forest method showed that the abundance of tetradecanoic acid could be explained by the HLA profile of the volunteers, but the explained variance was only 4.5% .

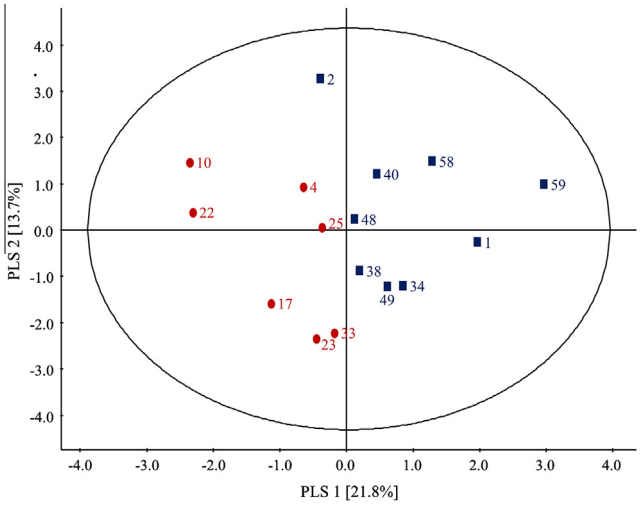

Fig. 2. Score plot of volatile patterns of Poorly Attractive (PA) and Highly Attractive (HA) individuals. Projection to latent structures-discriminant analysis (PLS-DA) of the volatile pattern of PA (circles) and HA (squares) individuals. Percentage variation explained for each PLS axis is given in parentheses. The ellipse defines the Hotelling's T^2 confidence region (95%).

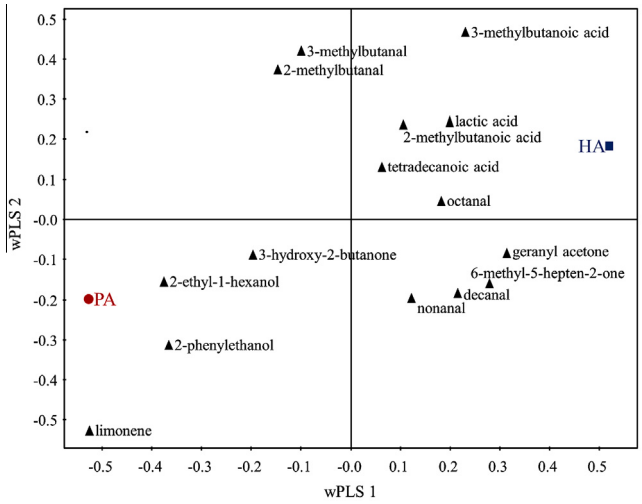

Fig. 3. Loading plot of volatile patterns of Poorly Attractive (PA) and Highly Attractive (HA) individuals. Projection to latent structures-discriminant analysis (PLS-DA) as based on the amounts (log) of 15 volatile compounds from the skin emanations of PA and HA individuals. Volatiles closer to the PA or HA individuals in the plot are more correlated to either group of individuals.

4. Discussion

MHC genes influence the body odor of mice (Beauchamp et al., 1988; Ninomiya and Brown, 1995; Penn and Potts, 1998b; Yamazaki et al., 1976) and consequently several studies have suggested a relation between the HLA genes located in the MHC region of humans and human body odor (Savelev et al., 2008; Wedekind and Furi, 1997; Wedekind and Penn, 2000). The present study provides evidence for a positive correlation between carrying HLA gene Cw*07 and high attractiveness of human skin emanations to anthropophilic mosquitoes, with $P = 0.0073$ before and $P = 0.064$ after correction for multiple comparisons (Bonferroni-corrected;

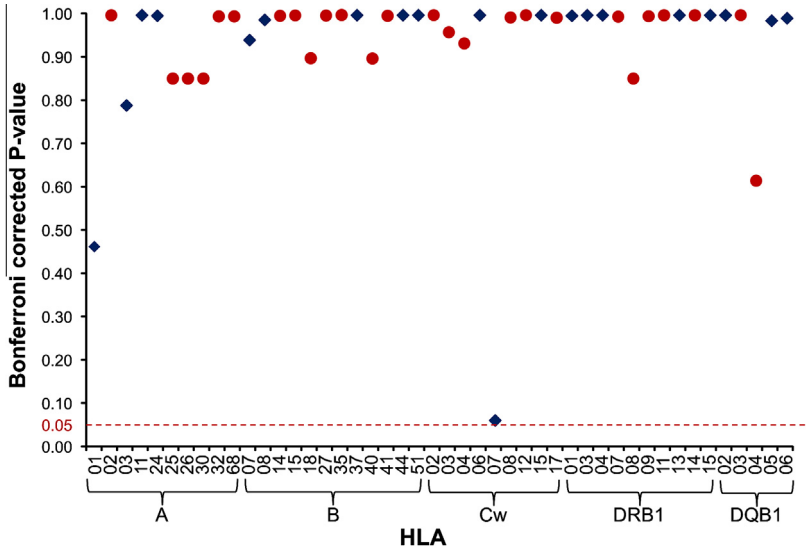

Fig. 4. Correlations between individuals mosquito attractiveness and their HLA profiles. Bonferroni corrected *P*-values for a two-tailed Fisher's Exact test to determine the correlations between individuals classified as Highly Attractive (HA) or Poorly Attractive (PA) and their HLA profiles. Diamond's indicate alleles that were more often associated with HA individuals and circles indicate alleles that were more often associated with PA individuals. Dashed line indicates $P = 0.05$.

Fig. 4, Supplementary Material Table S1). HLA-Cw alleles play a role in the modulation of natural killer (NK) cell alloreactivity (Colonna et al., 1993), that constitutes the first line of innate immunity. Should a person's attractiveness to the malaria mosquito *An. gambiae* be affected by HLA gene Cw*07, then this will have an impact on the number of bites received and therefore, possibly, the intensity of *Plasmodium* transmission. Based on the current results, it is hypothesized that the presence of Cw*07 would result in the production of skin volatiles that attract mosquitoes and therefore result in more bites. This hypothesis seems to be confirmed by comparing the allelic frequencies from malaria endemic areas with other parts of the world. The frequency of Cw*07 is lower in Africa (0.20) compared to western Europe and North America (0.34), although the number of studied individuals from Africa is limited (Gonzalez-Galarza et al., 2011). Future field studies should confirm whether the frequency of HLA gene Cw*07 is significantly lower in malaria endemic areas and whether individuals carrying this gene are bitten more often. Determining the skin microbial composition of these individuals could also reveal a possible link between skin microbial profile and HLA composition (Penn and Potts, 1998a). This link was not investigated in the current study because of the limited number of individuals compared to the high number of HLA's and bacterial diversity on the skin (Verhulst et al., 2011b).

The abundances of the selected compounds determined by GC–MS analysis did not correlate with the HLA profiles of the individuals. Based on the finding in this study that the presence of HLA gene Cw*07 and skin bacterial composition correlates with the attractiveness of humans to mosquitoes (Verhulst et al., 2011b), it was hypothesized that the human volatile composition and/or abundance could be linked to either human attractiveness to mosquitoes, the HLA profile or skin bacterial composition. However, of only 15 compounds identified in the skin emanations the abundance could successfully be determined. Other compounds that were screened for and showed behavioral significance in previous studies could not be identified, possibly because of the different sampling methods used in these studies (Logan et al., 2008; Smallegange et al., 2009; Verhulst et al., 2009). More than 300 compounds can be identified in human skin emanations collected on glass beads (Bernier et al., 1999, 2000) and it is plausible that other compounds than the 15 quantified compounds in the current study correlate with HLA profile or skin bacterial composition. Automatic alignment and comparison of chromatograms would be essential for the analysis of more than 300 compounds in the chromatograms. This requires chromatograms with low background noise and injection of samples in a relatively short time to prevent shifts in retention time. The latter, however, cannot be realized in mosquito behavioral studies with large groups of individuals in which only a few individuals can be tested for mosquito attractiveness each experimental day.

The screening for compounds in the chromatograms obtained from the foot emanations of individuals that differed in attractiveness (HA and PA) did not result in compounds that were significantly correlated to either group, possibly because of the limited number of individuals included in the analysis. To get an indication which compounds associated best with the PA or HA group, the first two PLS components were calculated. This showed that three compounds (limonene, 2-phenylethanol and 2-ethyl-1-hexanol) were more abundant in the samples taken from PA individuals (Fig. 3). Because limonene and 2-ethyl-1-hexanol are commonly used in perfumes, the duration for which behavioral restrictions were imposed upon the volunteers regarding washing and the use of soap may not have been sufficient to remove all residues of this compound. PA individuals may have used perfumes with limonene and 2-ethyl-1-hexanol that were not used by HA individuals. Limonene has insecticidal properties (Ibrahim et al., 2001; Kassir et al., 1989), is a common constituent of essential oils that repel mosquitoes (Debboun et al., 2007; Omolo et al., 2004) and inhibits volatile production by human skin microbiota (Ara et al., 2006). 2-Phenylethanol was previously identified in the headspace of skin bacteria (Verhulst et al., 2009) and inhibited the attraction of *An. gambiae* to a blend consisting of ammonia, lactic acid and tetradecanoic acid (Verhulst et al., 2011a). Together with the results obtained in this study, this suggests that 2-phenylethanol may lower a person's attractiveness and may be used as a mosquito repellent in push–pull systems (Cook et al., 2007).

Higher amounts of L-lactic acid, 2-methylbutanoic acid, tetradecanoic acid and octanal were associated with individuals that were highly attractive to *An. gambiae*. Lactic acid and tetradecanoic acid are two main constituents of a blend that has shown to be highly attractive to *An. gambiae* under both laboratory and field conditions (Mukabana et al., 2012; Smallegange et al., 2009). Octanal is an attractant to *An. gambiae* when combined with other

synthetic compounds (Smallegange et al., 2012). 2-Methylbutanoic acid has shown various results depending on the concentration and setup used (Verhulst et al., 2011a). Tetradecanoic acid could be explained by the HLA profile of the volunteers, but the explained variance was only 4.5%.

The results presented in the current study provide a better understanding of the traits affecting the production of volatiles released from the human skin that determine a person's attractiveness to mosquitoes. HLA profiling suggested that HLA composition influences the attractiveness of humans to mosquitoes, possibly by affecting the production of tetradecanoic acid. If individuals with a specific HLA profile are less attractive to mosquitoes and therefore are bitten less often, they will have a lower chance of becoming infected with *Plasmodium* parasites and consequently have a higher survival probability. We identified volatiles that may be associated with persons that are highly attractive and volatiles that may be associated with persons that are less attractive to *An. gambiae* mosquitoes; the latter compounds are of interest for the development of repellents and the former may contribute to the development of attractant blends to apply in lure and kill strategies or to be used to monitor malaria mosquito populations (Mukabana et al., 2012).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.meegid.2013.05.009.

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