Title: Chemical composition of scent-gland secretions in an Old World monkey (*Mandrillus sphinx*): influence of sex, male status, and individual identity.

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ABSTRACT

Primates are traditionally considered to be microsmatic, with decreased reliance on olfactory senses in comparison to other sensory modalities such as vision. This is particularly the case for Old World monkeys and apes (catarrhines). However, various lines of evidence suggest that chemical communication may be important in these species, including the presence of a sternal scent-gland in the mandrill. We investigated the volatile components of mandrill odour using gas chromatography-mass spectrometry. We identified a total of 97 volatile components in 88 swabs of the sternal gland secretion and 95 samples of sternal gland hair saturated with scent-gland secretion collected from 27 males and 18 females. We compared odour profiles with features of the signaller using principle components and discriminant function analyses, and found that volatile profiles convey both variable (age, dominance rank in males) and fixed (sex, possibly individual identity) information about the signaller. The combination of an odour profile that signals sex, age and rank with increased motivation to scent-mark and increased production of secretion in high-ranking males leads to a potent signal of the presence of a dominant, adult male with high testosterone levels. This may be particularly relevant in the dense Central African rain-forest which mandrills inhabit. By contrast, we were unable to differentiate between either female cycle stage or female rank based on odour profiles, which accords with behavioural studies suggesting that odour signals are not as important in female mandrills as they are in males. The similarity of our findings to those found in other mammals, and in primates that are more distantly related to humans, suggests a broader role for odour in primate communication than is currently recognised.

KEYWORDS: pheromones; gas-chromatography mass-spectrometry; dominance rank; signalling; olfaction; communication; microsmatic

INTRODUCTION

Mammalian social systems depend on signals that communicate information between individuals (Bradbury & Vehrencamp 1998). These signals often comprise complex chemosignals, which can communicate information ranging from identity (species, sex, group and individual) to current status (social, reproductive and health) to conspecifics (Brennan & Kendrick 2006; Thom & Hurst 2004; Wyatt 2003). Such olfactory signalling has important influences on a diversity of behaviours that are critical for reproductive success, including kin recognition (Mateo 2006; Porter & Moore 1981; Sun & Muller-Schwarze 1997), mate choice (Penn & Potts 1998), and intra-sexual competition (Gosling & Roberts 2001).

Olfactory cues mediate kin recognition in a variety of species (Wyatt 2003). The ability to recognise kin is fundamental to kin-biased social behaviour (kin selection, Hamilton 1964). It also minimises the risks associated with mating between close relatives, which would otherwise reduce heterozygosity, and permit the expression of deleterious recessive alleles in offspring, decreasing fitness (inbreeding depression, Crnokrak & Roff 1999; Keller & Waller 2002).

In addition to conveying information concerning relatedness, odour may also inform mate choice by acting as an honest signal of condition. Scent-marking is costly, both in energetic terms and in the risk of attracting predators and potential competitors (Gosling & Roberts 2001). This is consistent with the 'handicap' principle of sexual selection: If traits are condition-dependent, then only high quality individuals should be

able to express them fully, and the opposite sex should prefer to mate with such individuals to obtain resources or genetic benefits for their offspring (Andersson 1994; Zahavi 1975). Furthermore, olfactory signals are often more labile than morphological traits, and the components of scent signals are under the control of numerous endogenous physiological and exogenous factors including hormones. Their chemical composition may, therefore, reflect the current biological state of the marker, including social, health and nutritional status to potential mates more reliably than less dynamic modes of signalling (Penn & Potts 1998).

Finally, scent glands, scent-marking behaviour and chemical signals are often more exaggerated in males than in females (Blaustein 1981), and odour signals may function in male-male competition, signalling dominance status to potential rivals. For example, the odours of male mice contain androgen-dependent volatile compounds that reflect social dominance (Gosling & Roberts 2001). The physiological consequences of encountering the scent-marks of a dominant individual include reproductive suppression in both males and females (Barrett et al. 1990; Carter & Roberts 1997). In contrast to other means of signalling dominance, for example via visual traits, scent-marking also permits both the signaller and the receiver to avoid potential costly escalated aggression by transmitting information in the absence of the owner.

Chemical communication in primates

Olfaction is far less well understood in primates than in other mammals and our knowledge of chemical communication in primates lags behind our understanding of both visual and auditory communication (Heymann 2006). This may be because primates are traditionally regarded as microsmatic, and thought to rely on other

sensory modalities, such as vision, rather than olfaction (Dominy & Lucas 2001; Zhang & Webb 2003). However, various studies suggest that the role of olfaction in the regulation of primate behavior has been underestimated. For example, experiments have shown that olfactory sensitivity in squirrel monkeys is as good as, or better than, that of rats or dogs for some substances (Laska et al. 2000). Further, odour signals are known to advertise reproductive state, dominance rank and individual identity in strepsirrhines (ring-tailed lemurs, Palagi & Dapporto 2006; Scordato & Drea 2007) and callitrichids (marmosets and tamarins, Belcher et al. 1986; Epple et al. 1993; Smith et al. 1997; Ziegler et al. 1993), and sex, age and family membership in owl monkeys (MacDonald et al. 2007). There is also evidence that odour profiles may reflect individual genotype and genetic similarity in ring-tailed lemurs (Charpentier et al. 2008; Knapp et al. 2006). Finally, olfactory cues may also mediate reproductive suppression of subordinate individuals by dominants in marmosets (Barrett et al. 1990) and mouse lemurs (Izard 1990; Schilling et al. 1984)

While some research has been carried on olfactory communication in strephsirrhines and New World primates, very little information exists for Old World monkeys and apes (catarrhines). This is not surprising, as catarrhines are considered to be the most microsmatic primates. They have significantly higher proportion of olfactory receptor pseudogenes than other primates (Gilad et al. 2004), and the vomeronasal organ (VNO), which binds pheromones, is traditionally thought to be absent or vestigial in these species (reviews in Dulac & Torello 2003; Monti-Bloch et al. 1998). Moreover, TRPC2, a gene that is essential for VNO function in the mouse, is a pseudogene in humans (Liman & Innan 2003). However, various lines of evidence suggest that it would be premature to conclude that chemical communication is of no importance to catarrhines. First, scent-glands are known to occur in various Old World

primate species, including gibbons (Geissman & Hulftegger 1994) and the genus *Mandrillus* (Hill 1970). Second, intriguing experimental evidence has shown that humans can discriminate between kin and non-kin via odour alone (Porter & Moore 1981), and are able to detect individual differences in MHC genotype via olfactory cues (Jacob et al. 2002; Wedekind & Füri 1997; Wedekind et al. 1995). Third, while approximately 50% of olfactory receptor genes in hominoids (apes) are pseudogenes (vs. 0% in mice), only approximately 27% are pseudogenes in Old World monkeys (Rouquier et al. 2000). Fourth, the existence, homology and potential function of the VNO in humans and other Old World monkey species has been the focus of controversy (e.g. Smith et al. 2001a and references therein). Although it appears doubtful that Old World primates possess a VNO that is functional as a pheromone receptor (review in Dulac & Torello 2003), studies in mice have shown that non-volatile immune chemicals function as olfactory cues in the mammalian main olfactory epithelium, suggesting a general role for chemical communication even in vertebrates that lack a functional VNO (Spehr et al. 2006). Furthermore, there is ample evidence suggesting that a functional VNO is not necessary for semiochemical communication and that highly volatile chemicals received by the main olfactory epithelium function as chemical messages (e.g. Wysocki et al. 2004), Taken together, this evidence suggests that odour may play a larger role in the regulation of catarrhine behaviour than is currently recognised.

Chemical communication in mandrills

We report the first detailed chemical analyses of scent gland secretions for a non-human catarrhine primate, the mandrill (*Mandrillus sphinx*). Mandrills are found in the dense rainforests of Gabon, Congo, mainland Equatorial Guinea and southern Cameroon to the

south of the Sanaga river (Grubb 1973), and are a particularly interesting model for assessing the importance of chemical communication in Old World primates for several reasons. First, unlike most Old World monkeys, both male and female mandrills possess a sternal gland (Hill 1970), which produces a glandular secretion that they rub vigorously against tree trunks and vertical branches (Feistner 1991). These sternal glands are visible as a patch of modified hairs on the chest and are more active in males than in females, with maximum activity in alpha males, in which the hairs are dark and wet with glandular secretion (Setchell & Dixson 2001a; b). Scent-glands are active throughout the year (Setchell & Dixson 2001c), males scent-mark more than females do and dominant males scent-mark more than subordinate males do (Feistner 1991).

Second, in contrast to other primate species in which chemical signalling has been studied, which live in small multimale-multifemale groups in which females are dominant over males (ring-tailed lemurs and sifaka) or are monogamous/polyandrous with high intra-sexual competition between females (callitrichids), mandrills live in large multimale-multifemale groups in which males dominate females. Females form stable matrilines within these groups, while male group membership is more variable (Abernethy et al. 2002; Setchell & Dixson 2001a). Male-male competition is intense, and mandrills have a polygynous mating system, with high reproductive skew in favour of the alpha male (Charpentier et al. 2005; Setchell et al. 2005). As a consequence, mandrills are extremely sexually dimorphic: males are more than three times the body mass of females (Setchell et al. 2001) and possess large canine teeth (Setchell & Dixson 2002), and a suite of sexually selected traits, including bright red, blue and violet skin coloration (Setchell & Dixson 2001a; b; Setchell et al. 2001) and loud vocalisations. The evolution of such extreme, multi-modal signalling may be related to the large, fluid groups in which mandrills live, and their deep rainforest environment (Setchell et al. in

press-a; Setchell & Kappeler 2003). It has also been suggested that odour signals may function in the suppression of secondary sexual development of subordinate males by dominants (Setchell & Dixson 2001a).

Third, we have shown recently that mandrills reproduce preferentially with individuals that are genetically dissimilar to themselves at the major histocompatibility complex (MHC) (Setchell et al. in press-b). While the striking visual secondary sexual traits possessed by male mandrills may convey information regarding mate 'quality' (Hamilton & Zuk 1982; Zahavi 1975), including dominance rank (Setchell & Dixson 2001a; b), they cannot signal genetic compatibility with members of the opposite sex, as this is contingent on the chooser's own genotype. However, if relatives have similar odour profiles, or if genetic similarity in unrelated animals is reflected in similar odour profiles, then olfaction may play a role in the assessment of mate compatibility, as demonstrated for both rodents and humans (review in Penn 2002).

Finally, a recent study suggests that mandrills are able to discriminate paternal kin from non-kin, despite their polygynandrous mating system (Charpentier et al. 2007). The mechanism underlying this behaviour is unknown, but phenotype matching based on odour is one possibility (Widdig et al. 2001). As with mate choice based on genetic dissimilarity, if odour plays a role in kin selection, then this requires that individual mandrills have a unique chemical signature.

We investigated the volatile components of mandrill sternal gland secretions using gas chromatography-mass spectrometry (GC-MS) and compared mandrill scent gland secretions with features of the signaller. Based on current knowledge of mandrill behaviour and ecology, and olfactory communication in other primate species, we predicted that scent-gland secretions would encode information concerning sex, and that male secretions would reflect dominance rank, and the presence of receptive

females, when male-male competition is most intense. We also examined whether odour profiles signal individual identity, as reported for ring-tailed lemurs (Palagi & Dapporto 2006) and common marmosets (*Callithrix jacchus*) (Smith et al. 2001b).

METHODS

The naturalistic breeding colony at the Centre International de Recherches Médicales, in Franceville (CIRMF), in Gabon, has provided an invaluable resource for studies of mandrill behaviour and reproduction. The colony was established in 1983–1984 when 15 unrelated animals (7 males, 8 females) were released into a 6.5 ha forest enclosure (E1). A second semi-free-ranging group was established in 1994 in a smaller enclosure (E2, 3.5 ha) by transferring 17 mandrills (including 6 adult females and 4 adult males) from the first enclosure. All subsequent increases in the group have been due to natural reproduction of the founder animals, countered by deaths and occasional removals. The mandrills forage freely and receive daily supplements of monkey chow, fruit and vegetables. Water is always available from a stream, which runs through both enclosures. Group size and composition during the study are detailed in **Table 1**, and correspond to smaller groups observed in the wild (Hoshino et al. 1984; Rogers et al. 1996).

Daily observations

We noted the status of females daily as cycling (females in any stage of the menstrual cycle, during which females show conspicuous perineal swellings, Dixson 1998), pregnant (assigned post hoc from the birth of an infant, beginning with the final

detumescence of the perineal skin), lactating (the period following the birth of an infant to the resumption of cycling), or other (not pregnant, lactating or cycling). We calculated dominance rank separately for males and females using dyadic interaction matrices, including all interactions where one individual avoided or fled when another individual approached. Female dominance ranks were stable during the study period, male ranks changed periodically, but the identity of the top-ranking (alpha) male was always unambiguous (Setchell et al. 2008). Finally, we scored the occurrence of mate-guarding on a daily basis to determine days on which males were attracted to and actively competing for access to receptive females. Mate-guarding is an easily observed, unambiguous behaviour where a male maintains close spatial proximity to a female and monitors her continuously (Setchell et al. 2005).

Odour samples

Primate Centre staff captured most of the mandrills in March and October 2004 and March 2005, for a routine veterinary control and as part of a larger study of sexual selection in mandrills. We collected odour samples directly from anaesthetised individuals during these capture, with additional opportunistic sampling when animals were captured by primate centre staff for other reasons. We obtained odour samples from males aged 6.2-17.3 yr (n = 27, mean 10.7 yr), and females aged 6.5-26.4 yr (n = 19, mean 14.8 yr). We term males 'adolescent' until the age of 9 yr, when they attain adult body mass, crown-rump length and full expression of secondary sexual traits (Setchell et al. 2006), and 'adult' thereafter. All females sampled were multiparous and adult size.

We collected odour samples in two ways. First, we rubbed a sterile cotton swab against the sternal gland 10 times vertically and 10 times horizontally, using steady pressure. We also exposed control swabs to the air in the primate centre during sampling, to identify any volatile compounds in the air that did not derive from the mandrills. Second, we also collected hairs from the sternal gland area, because we observed that these hairs were often wet with secretion even if the sternal gland was not active – possibly due to the effects of capture and anaesthesia. We collected approximately 60g of hair, which we cut with sterilised scissors, and also collected paired samples of hairs from a non-scent gland area (the epigastric area) for 24 (25 %) of the hair samples. We transferred the swabs, hair samples and control swabs to separate sterile vials, froze them in liquid nitrogen immediately, and stored them at -80°C. In total we obtained 88 swab samples and 95 samples of sternal gland hair (details in **Table 2**). We were unable to collect equal numbers of replicates from all individuals because we could not guarantee to capture and sample an individual mandrill during each capture period.

Odour analyses

We carried out laboratory analyses of odour in the Mass Spectrometry Center, Florence University, Italy. We subjected swab samples to dynamic headspace extraction (DHS) followed by gas chromatography-mass spectrometry (GC-MS) analysis, because they comprised only a very low amount of odour secretion and DHS provides a high concentration factor for volatiles. We placed swab samples into 10 ml screw capped vials, closed by teflon-faced rubber septa and seals (Supelco, Bellefonte, PA, USA). We passed purified nitrogen (50 ml min^{-1}) through the system for 20 min at 50°C and

adsorbed the entrained volatiles on an adsorbent cartridge trap filled with XLTenax Tm (Gerstel GmbH & Co.KG, Mülheim an der Ruhr, Germany), maintained at 20°C within a Gerstel DHS device. The volatile compounds were subsequently thermally desorbed and transferred to the GC system using a thermal desorption unit (Gerstel GmbH & Co.KG, Mülheim an der Ruhr, Germany). We carried out desorption at 300°C for 10 min under a helium flow (30 ml min^{-1}) and cryofocused the analytes in a programmable temperature vaporizer injector (Gerstel CIS 4) maintained at −40°C with liquid carbon dioxide. We injected the volatile components into the GC capillary column by heating, the CIS 4 injector to 300°C at 720°C min^{-1}. We carried out blank analyses using an empty 10 ml vial (Supelco, Bellefonte, PA, USA) to assess possible environmental contamination. We purged the adsorbent traps at 300°C for 10 min after each analysis using the thermal desorption unit (TDU) apparatus to avoid any possible carry-over effects.

We subjected hair samples to solid phase microextraction (SPME) and GC-MS. We placed hair samples into 10 ml screw capped vials, and closed the vials with teflon-faced rubber septa and seals (Supelco, Bellefonte, PA, USA). We introduced a 65 µm pdms/dvb SPME syringe needle through the vial septum and exposed the fibre to the headspace above the sample in the vial for 20 min at 40°C. We assessed possible environmental contamination via blank analyses using an empty 10 ml vial (Supelco, Bellefonte, PA, USA) following the same procedure as for the samples, and purged the fibre in the injector, with the split ratio at 100:1 for 25 min after each analysis to avoid any possible carry-over effects.

We analysed the adsorbed volatile analytes of both types of sample using a 5975C mass spectrometer (Agilent Technologies, Santa Clara, CA, USA) EI, 70 eV, coupled directly to a 7890A gas chromatograph (Agilent Technologies, Santa Clara, CA,

USA) equipped with a fused silica HP 5-MS capillary column (Agilent Technologies, Santa Clara, CA, USA) 30 m x 0.25 mm crossbonded 5%-phenyl-95%-dimethylpolysiloxane, film thickness 0.25 μm. We maintained the injector and transfer line temperatures at 270°C and 280°C, respectively. We made injections in splitless mode with a constant flow of helium carrier gas of 1.5 ml min^{-1}. We started the oven temperature program at 45°C for 2 min, then raised it by 4°C min^{-1} to 170°C, by 7°C min^{-1} to 300°C, and finally by 20°C min^{-1} to a final temperature of 320°C.

We standardised peak retention times using an internal standard (alpha pinene). We identified the eluted compounds by comparing the experimental spectra with those of the NIST mass spectral database, Version 5.0 (Agilent Technologies, Santa Clara, CA, USA). We determined the relative amounts of compounds by integrating the areas of the corresponding peaks in the Total Ion Current (TIC) profile and calculated percentages with respect to the total area. We retained peaks that comprised at least 0.05% of the total area of the chromatogram to avoid problems associated with unreliable quantification at very low relative amounts, although this may mean that we missed trace chemicals (Smith et al. 2001b). This use of relative, rather than total abundance of the compounds that comprise mandrill odour profiles controls for any differences in the amount of secretion produced. We analysed all samples in a short period of time to minimise inter-assay variability. We used control swabs to identify compounds that did not derive from the animals and remove these from the swab results.

Data analysis

We used principal component analysis (PCA) to reduce the compounds we identified to a smaller number of uncorrelated principal components that explained most of the

variance. We retained principal components with eigenvalues > 1 and used these as covariates in discriminant function analysis (DFA), grouping samples using the following variables:

- Hair type: sternal gland vs. epigastric (hair samples only).
- Sex of the individual sampled.
- Male age: adolescent vs. adult.
- Male rank: alpha vs. not alpha, and high (rank 1-3), mid (4-7) or low (8-13) (we chose categories to equalize the number of samples falling into each class).
- Male competition for females: occurrence of mate-guarding on the day the sample was collected (yes/no).
- Female cycle stage: cycling (undergoing menstrual cycles), lactating, pregnant or quiescent (none of the previous categories). Unfortunately we obtained too few samples to include specific stage of the menstrual cycle (e.g. follicular vs. luteal).
- Female rank: high (top 25%), mid (25-75%) and low (bottom 25%).
- Identity of the individual sampled.

DFA generates a discriminant function (or a set of discriminant functions, where there are more than two groups) based on linear combinations of the predictor variables that provide the best discrimination between the groups. We tested the statistical significance of group differences using Wilks' λ and χ^2. Where results are significant, we plot functions as mean +/- SE for single functions, and as scatter plots of the first two functions where there was more than one function. We also report classification statistics as the number of cases correctly and incorrectly assigned to each of the groups based on the discriminant analysis. Use of the same samples as for the calculation of the discriminant functions (due to low overall sample size) may lead to

over-estimates of accuracy, so we also report results of 'leave-one-out' cross-validation analyses to address this issue.

Our dataset included repeat samples for some individuals, which gives rise to problems of pseudo-replication if these non-independent data points are treated as independent replicates and increases the risk of Type I error. To circumvent this issue, we followed up significant analyses for sex and male age using a subset of the data including one sample for each individual, selected at random. This reduced the sample size to 27 males and 18 females, as well as removing variation within individuals, which may be considerable. Other significant results (male dominance rank and the mate-guarding variable) varied within an individual, meaning that pseudo-replication would lead to less variation between states, rather than more, biasing our analyses towards a non-significant result.

We conducted all statistical analyses in SPSS 15.0 for Windows.

RESULTS

Swab samples

We identified a total of 19 distinct peaks in the control swabs which were also present in all swab samples. These included siloxane derivatives and silanols, originating from the GC capillary column, phalates and alcohols, and additional peaks that could not be identified. Removing these compounds from the swab sample results yielded a total of 47 distinct peaks in 88 swab samples of mandrill sternal gland secretions that were not present in the controls. These compounds included a series of hydrocarbons and organic aliphatic acid esters, aldehydes and ketones (tentative identifications are listed

in **Table 3**, typical chromatograms are shown in **Figure 1**). Ten compounds were present in all 88 samples, the modal representation was 100%, and 53% of compounds were present in >90% of samples. When we explored the dataset we found and removed two obvious outliers (one female and one male, with scores that were 9 SD and 7 SD greater than the mean, respectively). This was likely due to both samples having very low total amount of secretion, because the total area was very low in both samples.

PCA reduced the chemical composition of odour samples to 15 principal components, explaining a total 79.3% of the variance. The chemical profiles of males and females were not significantly different when all males were included in the analyses, but we found a significant difference between the two sexes when we examined only adult individuals (**Table 4**, **Fig. 2A**), with 20/28 females, and 33/37 males classified correctly. This was not due to pseudo-replication: the two sexes were also significantly different when we used only one sample per individual, with good classification accuracy (**Table 4**).

Chemical profiles of adult and adolescent males were significantly different (**Table 4**, **Fig. 3A**), with 92% of adult males (34/37), but only 70% of adolescent males (14/20) classified correctly. Adult and adolescent males were also significantly different when we restricted analysis to one sample per individual, and classification accuracy was high (**Table 4**). We found no significant difference between chemical profiles of alpha and non-alpha males, but splitting males into high, mid and low ranking yielded two functions that explained 58.4% and 41.5% of the variance, and significantly differentiated between male ranks, although classification analysis was poor (**Table 4**). High-ranking males were classified as high or mid, mid-ranking males as mid or low, and low-ranking males were 68% correctly classified (**Table 5**). High-ranking males fell into two clusters, one clearly separated from other males, and one that overlapped with

mid-ranking males, while mid and low-ranking males showed some overlap (**Fig. 4A**). The separate high-ranking males were not all alpha males, nor were they all samples taken during periods when mate-guarding occurred. Using adult males only, DFA also differentiated significantly between male ranks (**Table 4**), with two functions that explained 68.0% and 32.0% of the variance. Classification was better in this case, with 87% of high, 94% of mid, and 67% of low correct. We also found a significant influence of mate-guarding on male odour (**Table 4**, **Fig. 5**), with 9/13 mate-guarding samples correct and 41/44 no-mate-guarding samples correct.

In females, we found no significant difference in chemical profiles among cycle stages or female ranks (**Table 4**).

Finally, DFA based on individual identity revealed three discriminant functions that differentiated significantly between individuals when combined (**Table 4**). Of these, Function 1 explained 39.4% of the variance, Function 2 explained 16.1% (0.90), and Function 3 explained 12.5% (0.88). **Figure 6** illustrates the degree of separation using individuals represented by >1 sample. However, classification was relatively poor.

Hair samples

We identified a total of 59 distinct peaks in the volatile chemical composition of hair samples from mandrill sternal glands (95 samples). As for the swab samples, these compounds included a series of organic aliphatic acid esters and hydrocarbons, as well as aldehydes and ketones (tentative identifications in **Table 6**). Twelve compounds (20%) were present in all samples, the mode representation was 100%, and 33 (56%) were present in >90% of samples. Nine of the compounds identified in hair were also

found in the swab samples, and all but five of the 59 compounds were also found in epigastric hair samples.

PCA of the identified compounds yielded 18 principal components, explaining a total 76.8% of the variance. The chemical profiles of sternal gland hairs were significantly different from those of epigastric hair (DFA: $\lambda = 0.60$, $\chi^2_{18} = 55.12$, $p < 0.001$; note that this analysis does not account for the paired nature of the samples); all further analyses concern only sternal gland hairs.

Chemical profiles of males and females were significantly different, with good classification (**Table 4**, **Fig. 2B**). However, this may have been due to pseudo-replication, because when we restricted analysis to one sample per individual, differentiation based on sex was no longer significant (**Table 4**). Chemical profiles of adult males were significantly different from those of females, with good classification, but again, differentiation was no longer significant when we restricted the dataset to one sample per individual (**Table 4**).

Chemical profiles of adolescent and adult males were significantly different (**Fig. 3B**), with 33/39 adults and 19/24 adolescents correctly classified. However, when we restricted analysis to one sample per individual, the differentiation was no longer significant (**Table 4**), although only one sample was incorrectly classified for each group (11/12 adolescents, 13/14 adults). Chemical profiles of alpha and non-alpha males were significantly different (**Table 4, Fig. 4B**), with perfect classification accuracy for alpha males (8 of 8 samples), and 95% for non-alpha males (49/52 correct). However, chemical profiles for different male rank classes were not significantly different, either for all males or for adult males only, and chemical profiles did not differ between days when mate-guarding did and did not occur (**Table 4**).

We found no significant difference between chemical profiles with female cycle

stage or rank (**Table 4**).

Finally, DFA of volatile profiles from hair samples based on individual identity revealed 11 functions, explaining a total of 97.2% of the variance. Together these functions differentiated significantly between individuals, although classification was poor (**Table 4**).

DISCUSSION

We identified a total of 97 volatile components in the chemical profile of swabs of the sternal gland secretion, sternal gland hair and epigastric hair from mandrills. Many of the compounds identified were volatile hydrocarbons that have also been identified in GC-MS odour profiles for other mammals, including primates. For example, 4-methyl phenol and generic lactones have been identified in odour-secretions of *Callithrix jacchus* (Smith et al. 2001b), generic hydrocarbons (Hayes et al. 2004) and pentadecane have been found in *Lemur catta* (Knapp et al. 2006), and hexanoic acid has also been identified in *Lemur catta* (Knapp et al. 2006) and *Aotus nancymaae* (MacDonald et al. 2007). As in lemurs (Scordato et al. 2007), some compounds were relatively high–molecular weight hydrocarbons, including squalene, which may act as a fixative that slows the release of more volatile compounds, as suggested for 2-phenoxyethanol in rabbits (Hayes et al. 2003) and major urinary proteins in mice (Hurst et al. 1998).

Only nine compounds were present in both swab and hair samples from the sternal gland. This relatively low degree of overlap may be due to the different chemical methods that we used for the two samples, which reduces our ability to compare the results directly. However, the two types of sample may also differ in composition because both include different substances that do not derive directly from the scent-

gland. Swab samples may include epidermal compounds, while the chemical components of sternal hair samples overlapped to a large extent with those for hair from elsewhere on the body (epigastric hair), although odour profiles for hair from the two sites were significantly different. Sternal gland hair may also accumulate scent gland secretion over time, while the swab samples measure recent scent-gland activity. Nevertheless, both swabs and hair samples measure potential odour signals that are transferred to the substrate during scent-marking, because both skin and hair are rubbed against the tree when mandrills scent-mark. Furthermore, both may contribute to an individual's body odour, transmitting information to conspecifics during social interactions.

Hair odour (and possibly sternal gland odour) may include bacterial breakdown products in addition to compounds produced by the host organism. Indeed, many of the volatile fatty acids that we identified are produced by bacteria, over which the host may have little control, other than providing a substrate and warm incubation conditions. However, selective bacterial colonisation, dependent on genotype, has been proposed as a underling mechanism for individual odourtypes (Schellinck & Brown 1992). This suggests that such compounds may vary systematically among individuals, and contribute to differences in odour profiles, rather than obscuring them.

As in other primate species (lemurs, Hayes et al. 2006; Palagi & Dapporto 2006; marmosets, Smith et al. 2001b), a high percentage of chemicals were shared among profiles. In combination with the significant differences we found between odour profiles, this suggests that variation in mandrill chemical signals may depend more on the relative concentration of compounds (quantitative variation), and on complex interactions between components, than on the simple presence or absence of specific chemicals (qualitative variation). This accords with 'chemical signature' theories of

odour signaling, in which the overall properties of a complex mixture of chemicals are greater than the sum of the effects of its constituent parts (Schaefer et al. 2001; Singer et al. 1997). Such a view is supported by behavioural bio-assays. For example, behavioural responses to chemically complex, natural odourants in beavers (*Castor canadensis*) are stronger than to any single individual component of the signal, or even than to synthetic mixtures of components (e.g. Mueller-Schwarze 1992; Schulte et al. 1994). Electrophysiological studies potentially explain this phenomenon, by showing that the response of individual olfactory neurons to chemical mixtures cannot be predicted by simply summing the effects of the individual compounds (Duchamp-Viret et al. 2003), and that mixtures stimulate neurons in the olfactory cortex that are not stimulated by their individual component odorants (Zou & Buck 2006).

We were able to differentiate between males and females based on the volatile profiles of swab profiles when we considered only adult individuals, but not when we included adolescent males. Volatile profiles of hair samples allowed us to differentiate the sexes, but when we restricted the dataset to one sample per individual the differentiation was no longer significant, although classification remained good. These results suggest that volatile profiles contained some information concerning sex in mandrills, as in other mammals (Wyatt 2003), including ring-tailed lemurs (Hayes et al. 2004; Scordato et al. 2007) and owl monkeys (MacDonald et al. 2007), but not sifakas (Hayes et al. 2004; 2006). The lack of a consistent pattern of differentiation between the sexes may be explained by the odour profiles of young and low-ranking males resembling those of females. This is supported by the differences in odour profiles that we found with male age and status.

In males, swab samples differentiated between adolescents and adults. The same was true for hair samples, although the differentiation was non-significant when we

used only one sample per individual. The difference between adolescent and adult males may be relevant to other mandrills, because a fully adult male presents more of threat to other males than a male that is still maturing, while a female may prefer to associate with, and reproduce with, a fully adult male, who has demonstrated his ability to survive to adulthood. Similarly, young male elephants produce a very different odour profile in their temporal gland secretion during musth than that produced by mature males (Rasmussen et al. 2002), and males appear to base their interactions on this odour different, with younger males avoiding the scent of mature males, while mature males ignore that of young males (Rasmussen et al. 2002). The lack of a perfect discrimination between adolescent and adult male mandrills is likely to be due to the artificial nature of this distinction – males vary in the pace of their development, so some males will be fully developed at 9 yr, but others may still be maturing (Setchell et al. 2006).

Our results concerning male rank differed slightly between the two types of sample, but our overall finding was that volatile profiles do contain information concerning male rank. Swab profiles differentiated between rank classes, and some high-ranking males clearly fell into a class of their own. Hair samples differentiated between alpha and non-alpha males, with perfect classification for alpha males, and 95% for non-alpha males. These results are similar to those for other mammals, in which odour profiles of dominant and subordinate males also differ, including European rabbits (Hayes et al. 2003) and mice (review in Gosling & Roberts 2001). However, they differ from those for other primates: the odour profiles of ring-tailed lemurs do not differ with rank (Scordato et al. 2007), and although saddleback tamarins are able to discriminate between scent-marks by unfamiliar dominant and subordinate males (Belcher et al. 1986), it is not clear whether this is due to the chemical profile of the

scent-mark, or to differences in the amount of scent applied by the male (Scordato et al. 2007). In mandrills, information concerning dominance rank is highly relevant to conspecifics, because a high-ranking male represents a dangerous rival to other males, and an attractive mate to females. In the deep forest environment, where males are not necessarily permanently associated with the social group of females (Abernethy et al. 2002; Setchell & Dixson 2001a), odour may provide an important, long-lasting signal of the presence and status of a male.

We also detected an influence of male-male competition and the presence of receptive females on male odour profiles, with swab profiles showing a significant influence of mate-guarding, although hair samples did not. This may relate to the fact that swab samples represent the most recent sternal gland activity – i.e. when mate-guarding is actually occurring – whereas hairs may represent a longer time-period of secretion, possibly including secretion that pre-dated the mate-guarding. Similar influences of the breeding season on odour profiles have been reported for ring-tailed lemurs (Scordato et al. 2007) and sifaka (Hayes et al. 2006).

Together, our results for male age, status and mate-guarding suggest that volatile profiles are influenced by endocrine status in male mandrills. Testosterone in mandrills is higher in adult than adolescent males (Setchell & Dixson 2002), higher in dominant males (Setchell & Dixson 2001a), and increases in the presence of receptive females (Setchell et al. 2008). However, testosterone is not perfectly related to male rank, and also increases in periods of rank instability (Setchell et al. 2008). If odour profiles accurately reflect testosterone levels, as in male mice (Gosling & Roberts 2001), rather than rank itself, which seems likely, then the imperfect relationship between rank and testosterone may explain why we did not find a difference between alpha and non-alpha male swab profiles, or a relationship between hair profiles and rank-class in males.

Our use of relative, rather than total abundance of the compounds that comprise mandrill odour profiles controls for any differences in the amount of secretion produced. However, differences in odour profiles according to sex, age, and male status in mandrills are also accentuated by behaviour and the quantity of secretion produced. Male mandrills scent-mark more than females, adult males mark more than younger males, and dominant males mark the most (Feistner 1991). Males also have far more active scent-glands than females, adult males have more active glands than younger males, and dominant males are the most active of all (Setchell & Dixson 2001a; b). The combination of an odour profile that signals sex, age and rank, increased motivation to mark in high-ranking males (so much so that high-ranking males often have grazed chests which occasionally get infected), and increased production of secretion, leads to a potent signal of the presence of a dominant, adult male with high testosterone levels in the forest. Such signals may help to mediate male interactions, and avoid confrontation and physical aggression between rival males, in addition to potentially attracting females. Thus odour may act in a similar fashion to the bright red coloration that male mandrills also display, which signals dominance (Setchell & Dixson 2002), mediates male interactions (Setchell & Wickings 2005), and is attractive to females (Setchell 2005). Unlike visual signals, odour has the additional advantage of continuing to inform conspecifics in the absence of the signaller (Gosling & Roberts 2001), while signal degradation provides information about the timing of scent-mark deposition. Finally, scent-marking also permits both the signaller and the receiver to avoid potential costly escalated aggression by transmitting information in the absence of the owner.

In females, we were unable to differentiate between either cycle stage or female rank based on either swab or sternal gland hair samples. However, our results for cycle stage should be regarded as preliminary, as we were unable to address changes across

the menstrual cycle. Odour profiles vary with season in female ring-tailed lemurs (Scordato et al. 2007), and sifaka (lumping the two sexes, Hayes et al. 2006) and it remains possible that female mandrill odour also advertises receptivity. The lack of a relationship between odour profile and rank in mandrills is not surprising, however, because although dominant females may mark more often, female mandrills rarely scent-mark (Feistner 1991 and JMS pers. obs.), suggesting that odour is not as important in female signalling as it is in males. This is not surprising, since rank is stable in female mandrills, unlike in males, meaning that an up-to-date signal of status is un-necessary.

Finally, we found a significant signal of individual identity in the volatile profiles of both swab and hair samples, based on group differences, although classification was rather poor in both cases. These results should be regarded as preliminary, because they are based on few replicates for each individual. Nevertheless, they suggest that odour may encode information about signaller identity in mandrills, as demonstrated for other mammals (Thom & Hurst 2004; Wyatt 2003), including lemurs (Palagi & Dapporto 2006; Scordato et al. 2007), and marmosets (Smith 2006; Smith et al. 2001b). Experiments have also demonstrated that lemurs (Palagi & Dapporto 2006), various species of New World monkeys (Epple et al. 1979; Epple et al. 1988; Laska & Hudson 1995; Smith 2006), and humans (Porter & Moore 1981) are able to distinguish between the scents of individual conspecifics. Our results for mandrills fill a phylogenetic gap between humans and more distantly related primate species, and suggest that Old World primates are not as microsmatic as previously assumed. The possibility that stable individual volatile profiles may occur in mandrills also suggests that, like lemurs (Charpentier et al. 2008), they may be able to advertise information about their genotype, facilitating mate choice for genetically dissimilar individuals (Setchell et al. in

press-b), inbreeding avoidance (Charpentier et al. 2005), and behavioural bias towards paternal as well as maternal kin (Charpentier et al. 2007). We are currently investigating relationships between odour profiles and MHC genotype, and between genetic relatedness and odour similarity in mandrills.

In conclusion, our findings suggest that mandrill volatile profiles convey both variable (age, dominance status in males) and fixed (sex, possibly individual identity) information about the signaller. The similarity of our findings to those found in primates that are more distantly related to humans suggests a broader role for odour in primate communication than is currently recognised, in line with other evidence reviewed in the introduction. Future studies should address the question of whether odour signals individual identity using more replicates for each individual, and whether odour profiles communicate health status, as in mice, where females are able to discriminate between the odours of infected versus non-infected males (Kavaliers & Colwell 1992; Zala et al. 2004) or quality, as in humans, where women prefer the scent of symmetrical men (Thornhill et al. 2003). Future work should also examine information perceived by the recipient, for example via habituation/dishabituation tests (e.g. Mateo 2006; Palagi & Dapporto 2006) or paired choice experiments (Scordato & Drea 2007; Smith 2006). Finally, we focussed on the volatile components of mandrill odour. However, chemical signals are mixtures of both volatile and non-volatile compounds, and high molecular weight (non-volatile) compounds may also be required for perception of the full biological information contained in a scent signal (Alborne 1984; Belcher et al. 1990; Hurst et al. 1998). For example, volatiles are thought to be the long-distance, airborne, 'broadcast' component of a scent signal in mice, important for drawing receivers' attention to the location of scent marks, and to any changes in the odoursphere, such as scent from a new individual or a change in the status of a familiar individual. By

contrast, once a scent-mark has been located and investigated, highly polymorphic involatile components ('major urinary proteins') provide a reliable short-range signal of ownership (Hurst et al. 2001; Nevison et al. 2003).

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FIGURE LEGENDS

Figure 1: Example TiCs of swab samples from the sternal gland of a male (A) and a female (B) mandrill.

Figure 2: Discriminant function differentiating volatile profiles of male and female mandrills, based on (A) swab samples, (B) hair samples.

Figure 3. Discriminant function differentiating volatile profiles of adolescent and adult males, based on (A) swab samples, (B) hair samples.

Figure 4. Discriminant function differentiating volatile profiles of males based on rank: A. rank class, based on swab samples, B. alpha vs. not alpha, based on hair samples.

Figure 5. Discriminant function differentiating volatile profiles of males on days when mate-guarding occurred and days when no mate-guarding occurred, based on swab samples.

Figure 6. Discriminant function differentiating volatile profiles from different individual mandrills, based on swab samples.

Table 1: Composition of study groups in March 2004

Enclosure	Infants and juveniles		Females of breeding age	Adolescent males	Adult males	Total
	male	female				
1	18	27	15	7	8	75
2	12	24	15	11	6	68

Table 2: Details of samples obtained

Sample type	Sex	Number of samples					
		1	2	3	4	5	total
swab	male	11	7	7	4	0	59
	female	10	7	2	0	0	29
hair	male	10	6	5	4	2	63
	female	9	8	1	1	0	32

Table 2: Volatile compounds present in swab samples of mandrill sternal gland secretions identified tentatively using the NIST 2005 mass spectral database, listed in order of retention time

Molecular weight	Compound
116	Butanoic acid, 3-methyl-, methyl ester
88	Propanoic acid, 2-methyl-
130	Butanoic acid, 3-methyl-, ethyl ester
130	Pentanoic acid, ethyl ester
116	Hexanoic acid
114	2(3H)-Furanone, 5-ethyldihydro-
106	Pentanedinitrile, 2-methylene-
108	**Phenol, 4-methyl-**
170	cis-Linaloloxide
170	Linalool oxide trans
156	**Undecane**
114	2H-Pyran-2-one, tetrahydro-6-methyl-
150	Benzoic acid, ethyl ester
128	**Naphthalene**
184	Undecane, 3,6-dimethyl-
134	Benzaldehyde, 3,4-dimethyl-
184	Dodecane, 6-methyl-
164	**Benzeneacetic acid, ethyl ester**
184	Dodecane, 4-methyl-
146	Naphthalene, 1,2,3,4-tetrahydro-6-methyl-
142	**Naphthalene, 2-methyl-**
198	Dodecane, 4,6-dimethyl-
212	Pentadecane
142	Naphthalene, 2-ethyl-
282	Nonadecane, 9-methyl-
156	Naphthalene, 1,5-dimethyl-
156	Naphthalene, 1,4-dimethyl-
196	12-Methyl-oxa-cyclododec-6-en-2-one
220	**Butylated hydroxytoluene**
194	Benzoic acid, 4-ethoxy-, ethyl ester
162	1,4,8-Dodecatriene, (E,E,E)-
234	3,5-di-tert-Butyl-4-hydroxybenzaldehyde
254	Octadecane
252	**Oxacycloheptadec-8-en-2-one**
270	Pentadecanoic acid, 14-methyl-, methyl ester
324	1,1'-Biphenyl, 2,3',4,4',5-pentachloro-
296	**9-Octadecenoic acid, methyl ester, (E)-**
298	**Octadecanoic acid, methyl ester**
312	Hexadecanoic acid, butyl ester
324	1,1'-Biphenyl, 2,3,4,4',6-pentachloro-
390	1,2-Benzenedicarboxylic acid, diisooctyl ester
-	Hydrocarbon "A"[1]
410	Squalene
-	Hydrocarbon "B"[1]
-	Hydrocarbon "C"[1]

-	Hydrocarbon “D”[1]
-	Hydrocarbon “E”[1]

Compounds in **bold font** were found in both swab and hair samples
[1] compounds that are hydrocarbons but we were unable to identify precisely by comparing the experimental spectra with those of the NIST mass spectral database

Table 3: Results of discriminant function analysis comparing odour profiles of different groups of mandrill sternal gland samples

Sample	Test	Dataset	λ	χ^2	df	p	% correct	% cross-validation
Swab	Males vs. females	all data	0.82	15	15.41	0.422		
	Adult males vs. females	all data	0.63	15	25.76	0.041	81.5	69.2
		one sample per ID	0.26	15	32.64	0.005	97.1	84.1
	Adult vs. adolescent males	all data	0.55	15	27.99	0.022	84.2	63.2
		one sample per ID	0.17	15	29	0.016	100.0	84.6
	Alpha vs. non-alpha males	all data	0.8	15	10.16	0.810		
	Male rank-class	all data	0.33	30	49.72	0.013	61.8	41.8
		adults only	0.11	30	56.74	0.002	88.9	72.2
	Mate-guarding in males	all data	0.55	15	28.61	0.018	87.7	73.7
	Female cycle stage	all data	0.06	30	38.71	0.132		
	Female rank	all data	0.14	30	35.18	0.236		
	Individual identity	all data	0.00	660	896.14	<0.001	68.4	
Hair	Males vs. females	all data	0.45	18	85.20	<0.001	87.4	82.4
		one sample per ID	0.47	18	26.66	0.086		
	Adult males vs. females	all data	0.38	18	58.76	<0.001	89.5	89.3
		one sample per ID	0.29	18	27.21	0.075		
	Adult vs. adolescent males	all data	0.56	18	30.25	0.035	80.5	64.9
		one sample per ID	0.17	18	26.87	0.082		
	Alpha vs. non-alpha males	all data	0.52	18	32.17	0.021	93.2	84.9
	Male rank-class	all data	0.36	36	49.73	0.064		
		adults only		18	21.07	0.276		
	Mate-guarding in males	all data	0.46	18	20.06	0.329		
	Female cycle stage	all data	0.09	54	47.62	0.717		
	Female rank	all data	0.2	36	33.37	0.599		
	Individual identity	all data	0	810	0.00	<0.001	62.0	

We report classification results only for significant analyses. Cross-validation could not be performed for individual identity because some individuals contributed only one sample to the dataset

Table 4: Count (%) of correct assignments of swab volatile profiles by male rank

		Predicted Group			Total
		high	mid	low	
Actual group	high	7 (46.7)	8 (53.3)	0 (0.0)	15
	mid	0 (0.0)	14 (66.7)	7 (33.3)	21
	low	2 (10.5)	4 (21.1)	13 (68.4)	19

Table 5: Volatile compounds present in hair samples from mandrill sternal gland secretions identified tentatively using the NIST 2005 mass spectral database, listed in order of retention time

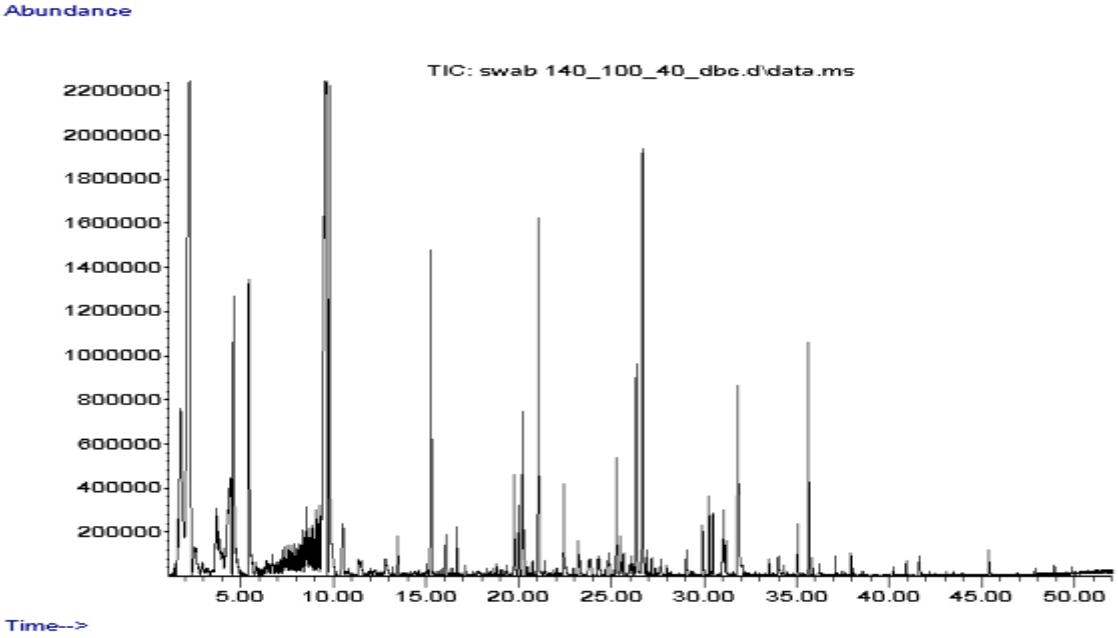
Molecular weight	Compound
76	Carbon disulfide
102	Propanoic acid, 2-methyl-, methyl ester
102	Butanoic acid, methyl ester
116	Propanoic acid, 2-methyl-, ethyl ester
116	Butanoic acid, 3-methyl-, methyl ester
116	Pentanoic acid, methyl ester
130	Butanoic acid, 3-methyl-, ethyl ester
102	Butanoic acid, 3-methyl-
114	Heptanal
151	Oxime-, methoxy-phenyl-
130	Hexanoic acid, methyl ester
144	Hexanoic acid, ethyl ester
128	Octanal
198	2,3,4,5,6-Pentafluorobenzylalcohol
144	Heptanoic acid, methyl ester
136	D-Limonene
130	1-Hexanol, 2-ethyl-
142	Cyclohexanecarboxylic acid, methyl ester
108	**Phenol, 4-methyl**
136	Benzoic acid, methyl ester
156	**Undecane**
142	Nonanal
158	Octanoic acid, methyl ester
342	Fluoren-9-ol, 3,6-dimethoxy-9-(2-phenylethynyl)-
150	Benzeneacetic acid, methyl ester
128	**Naphthalene**
172	Octanoic acid, ethyl ester
170	Dodecane
170	3-Nonenoic acid, methyl ester[1]
172	Nonanoic acid, methyl ester
164	**Benzeneacetic acid, ethyl ester**
164	Benzenepropanoic acid, methyl ester
142	**Naphtalene, 2-methyl-**
186	Decanoic acid, methyl ester
200	Decanoic acid, ethyl ester
200	Undecanoic acid, methyl ester
202	Octanedioic acid, dimethyl ester
220	**Butylated hydroxytoluene[1]**
220	1,9-Cyclohexadecadiene
218	1s,4R,7R,11R-1,3,4,7-Tetramethyltricyclo[5.3.1.0(4,11)]undec-2-en-8-one
220	**Butylated hydroxytoluene[1]**
214	Dodecanoic acid, methyl ester

216	Nonanedioic acid, dimethyl ester
226	Hexadecane
216	Sebacic acid monomethyl ester
242	Methyl tetradecanoate
256	Methyl 9-methyltetradecanoate
256	Tetradecanoic acid, 12-methyl-, methyl ester
256	Pentadecanoic acid, methyl ester
252	**Oxacycloheptadec-8-en-2-one**
268	9-Hexadecenoic acid, methyl ester (Z)
270	Hexadecanoic acid, methyl ester
294	9,12-Octadecadienoic acid (Z, Z), methyl ester
296	9-Octadecenoic acid, methyl ester
296	13-Octadecenoic acid, methyl ester
296	9-Octadecenoic acid (Z), methyl ester
296	11-Octadecenoic acid, methyl ester, (Z)
298	**Octadecanoic acid, methyl ester**
228	Phenol, 4,4' -(1-methylethylidene)bis-

Compounds in **bold font** were found in both swab and hair samples
[1] compounds that refer to two isomers of the same compound (butylated hydroxytoluene)

Figure 1: Example TiCs of swab samples from the sternal gland of a male (A) and a female (B) mandrill

A



B

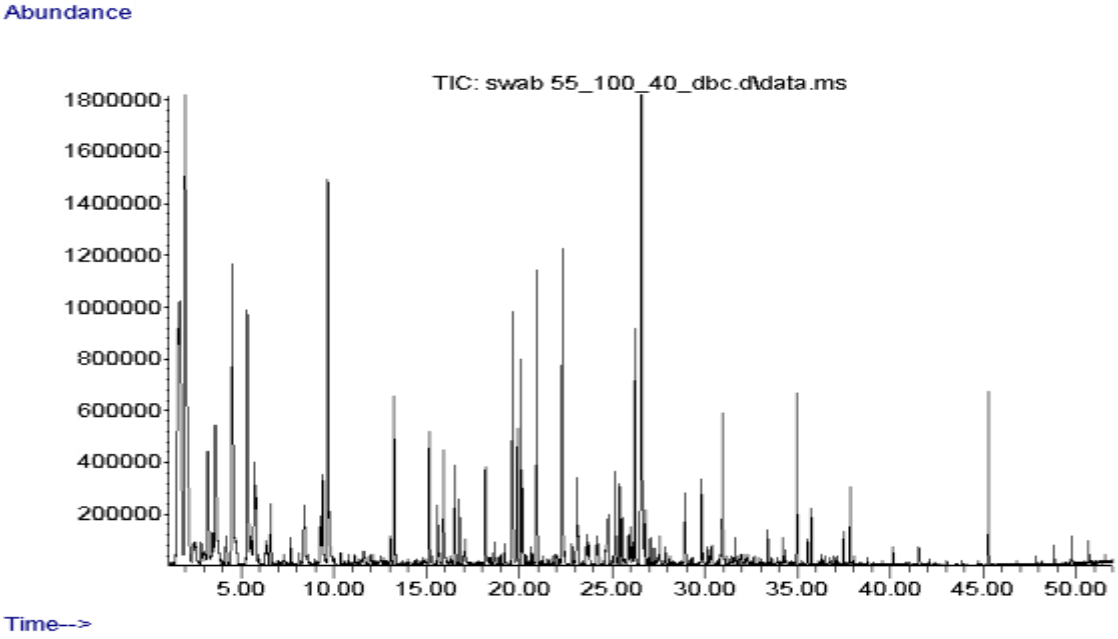
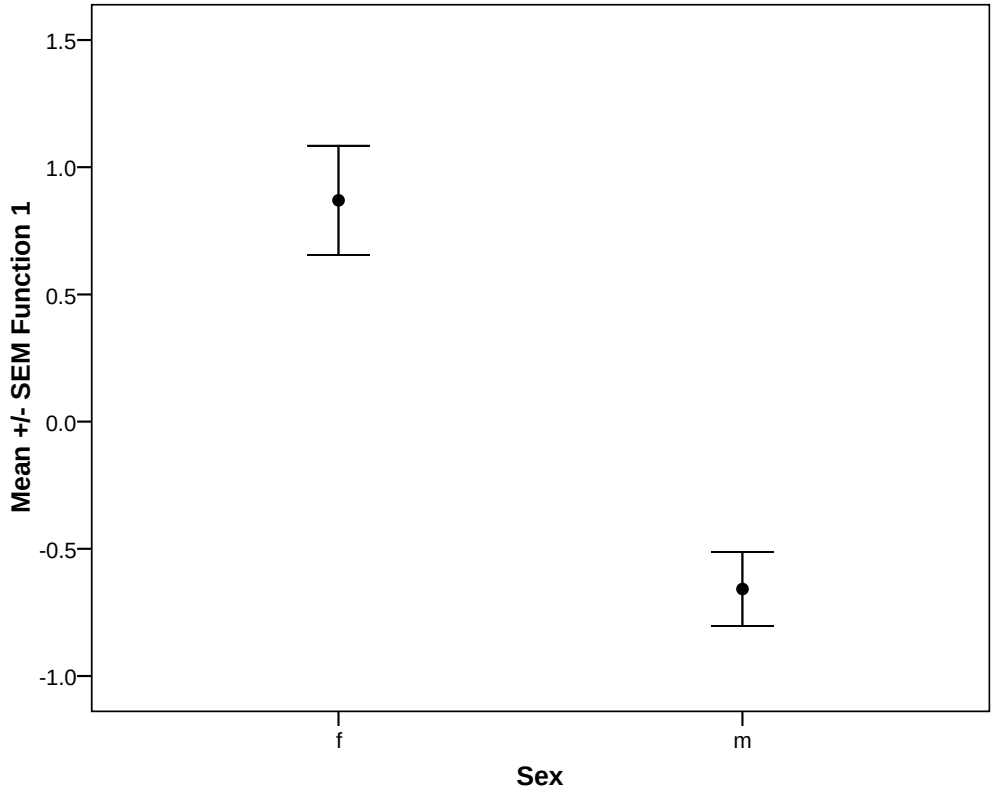


Figure 2: Discriminant function differentiating volatile profiles of male and female mandrills, based on (A) swab samples, (B) hair samples.

A.



B.

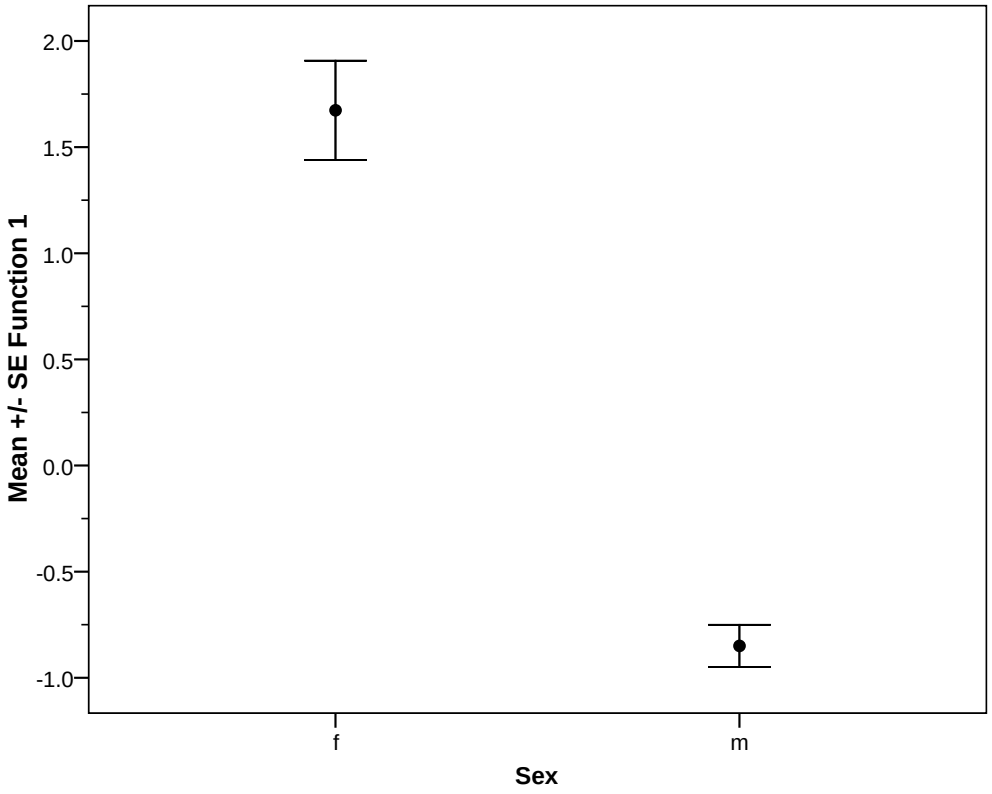
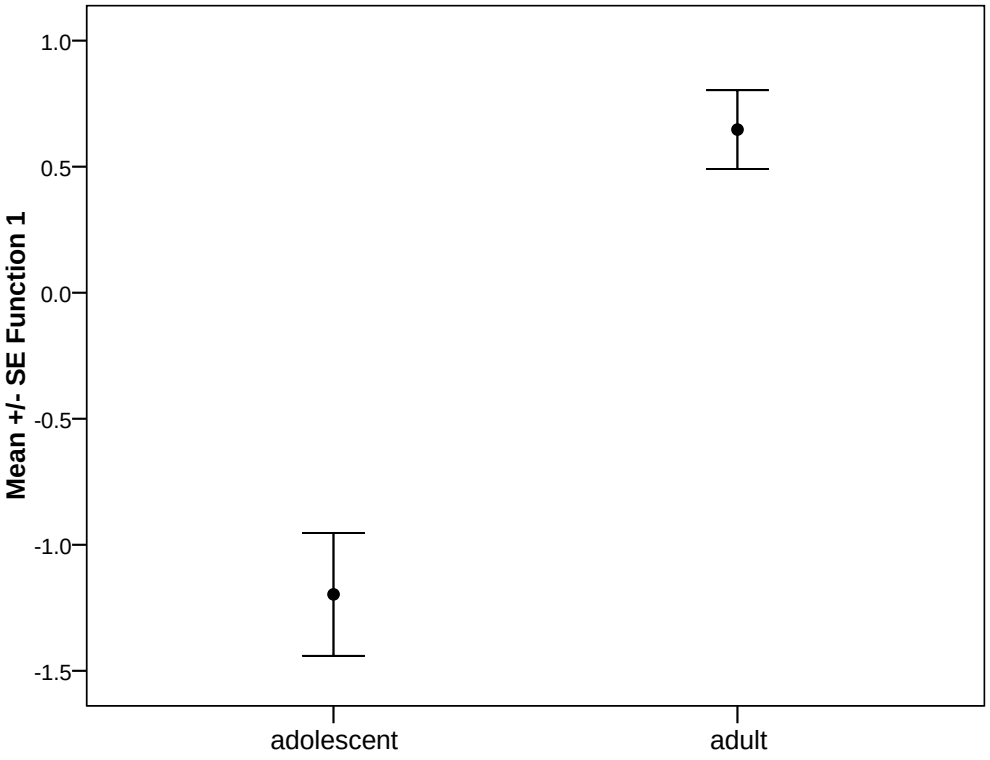

2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
Mean +/- SE Function 1
f
m
Sex

Figure 3. Discriminant function differentiating volatile profiles of adolescent and adult males, based on (A) swab samples, (B) hair samples

A.



B.

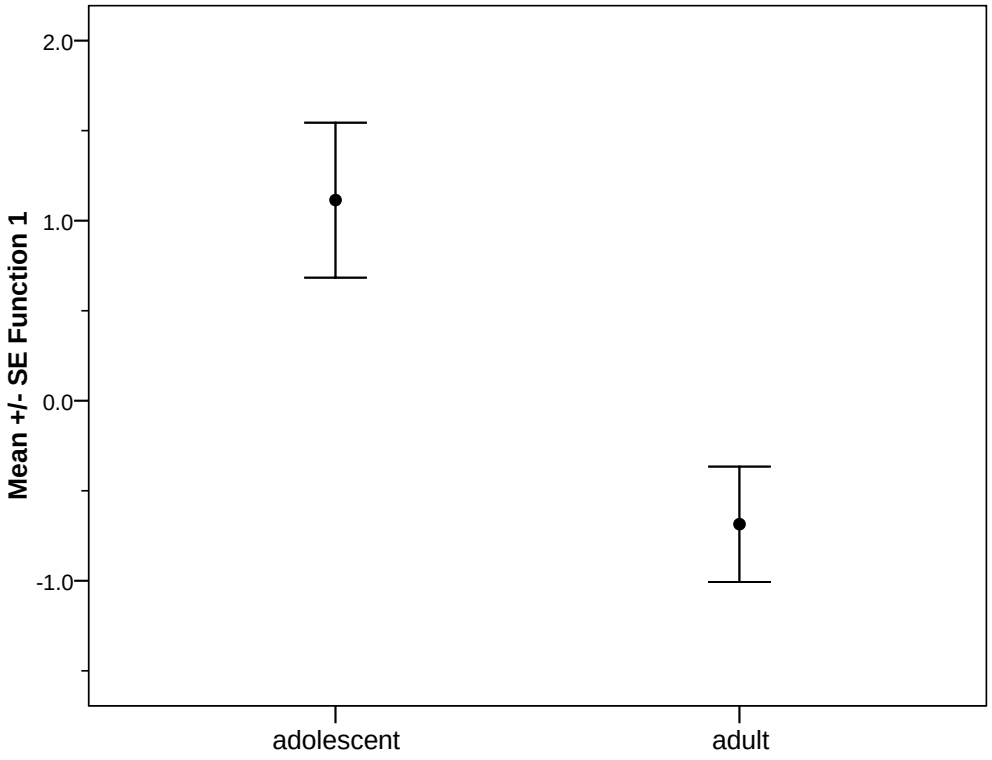
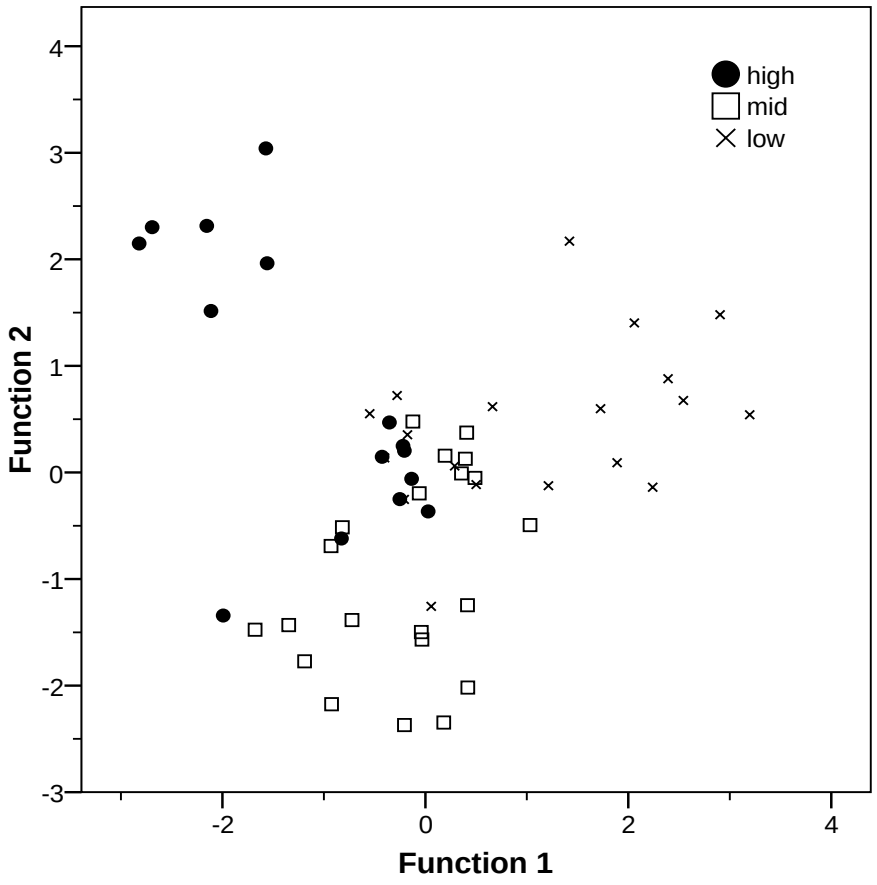

Mean +/- SE Function 1
2.0
1.0
0.0
-1.0
adolescent
adult

Figure 4. Discriminant function differentiating volatile profiles of males based on rank:
A. Scatter plots of the first two functions for rank class, based on swab samples,
B. Mean +/- SE for the single discriminant function alpha vs. not alpha, based on hair samples.

A.



B.

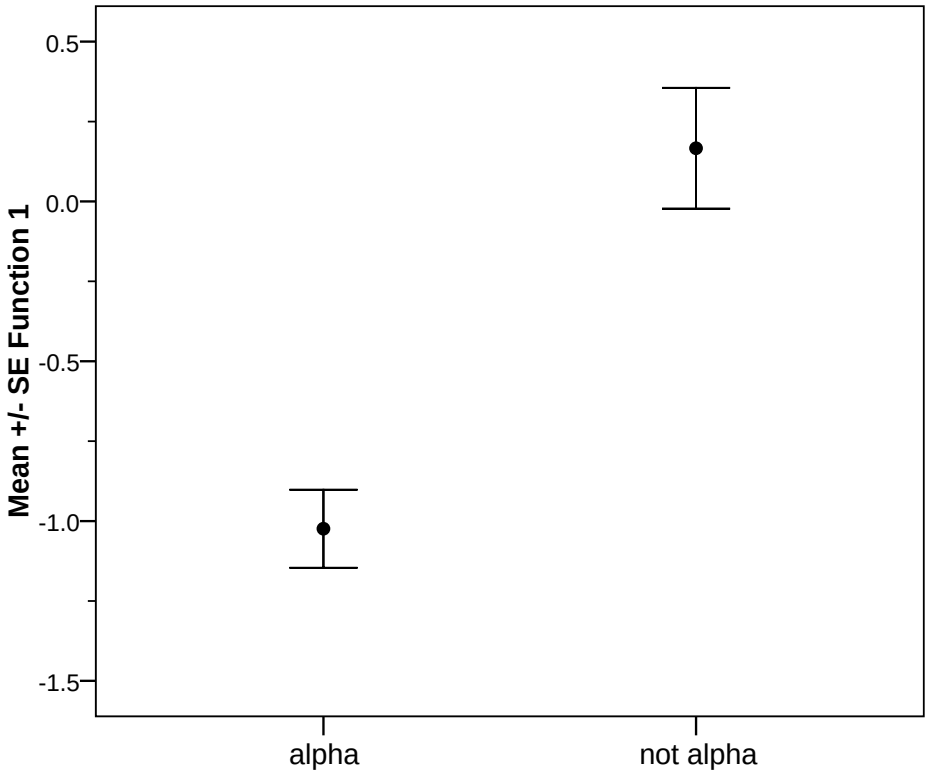

0.5
0.0
-0.5
-1.0
-1.5
Mean +/- SE Function 1
alpha
not alpha

Fig. 5. Discriminant function differentiating volatile profiles of males on days when mate-guarding occurred and days when no mate-guarding occurred, based on swab samples

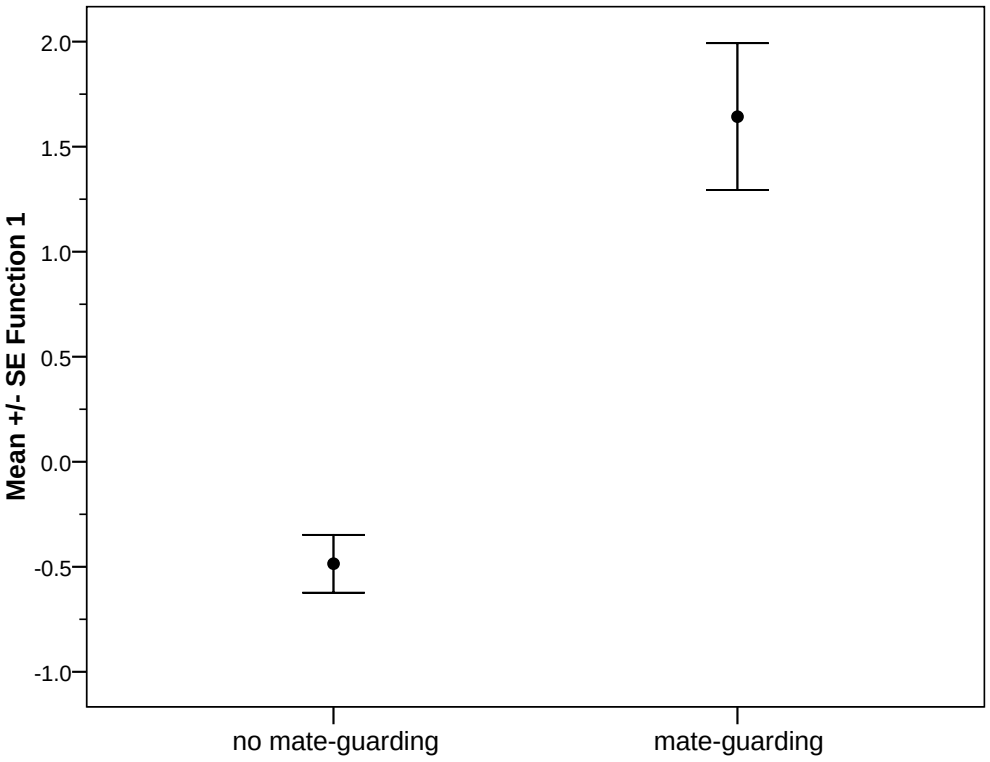
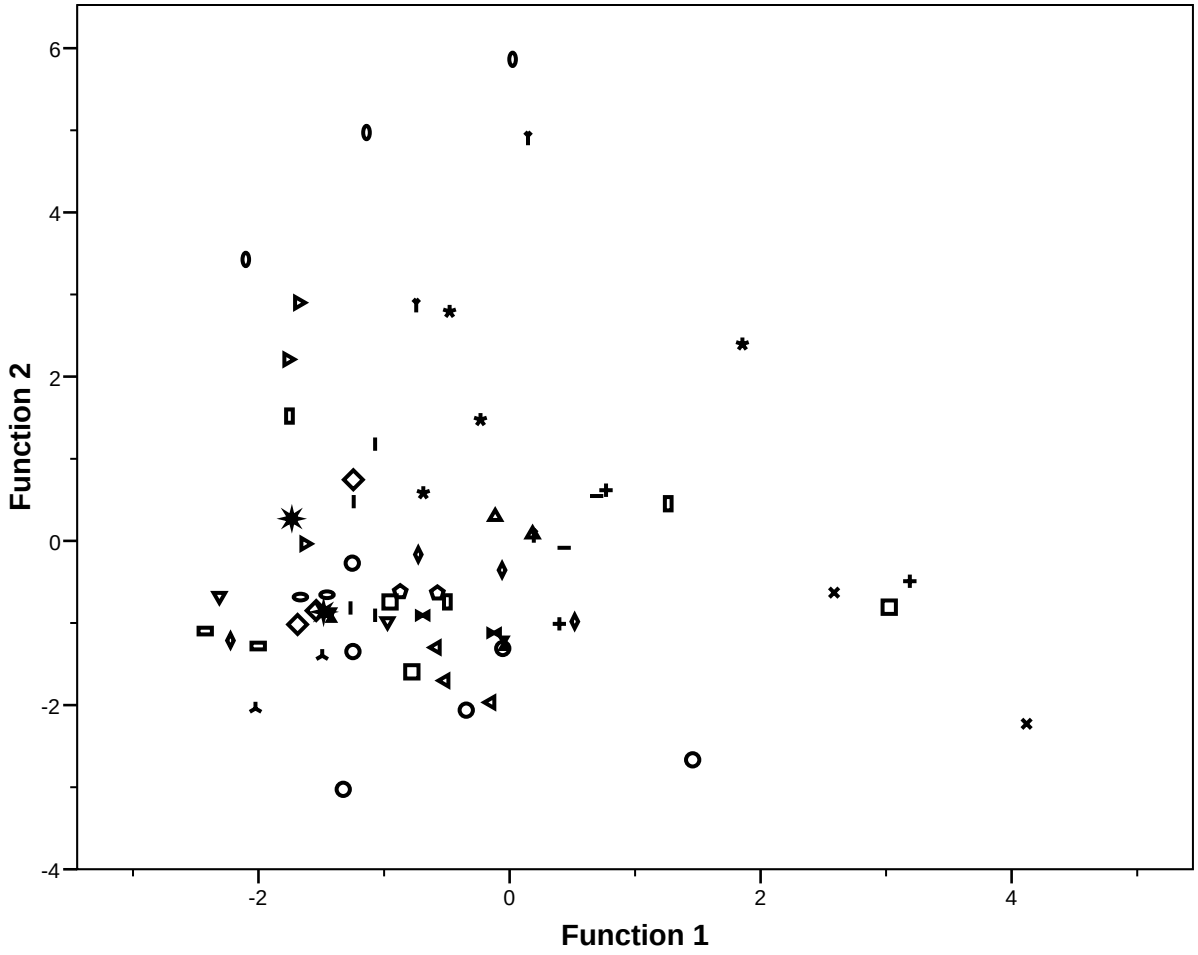


Figure 6. Discriminant function differentiating volatile profiles from different individual mandrills, based on swab samples



Each symbol represents a different individual. For simplicity plot shows only individuals contributing >1 sample.