Received: 17 March 2017 | Accepted: 4 August 2017

DOI: 10.1111/2041-210X.12888

RESEARCH ARTICLE

Methods in Ecology and Evolution BRITISH ECOLOGICAL SOCIETY

A non-invasive method for sampling the body odour of mammals

Brigitte M. Weiß[1,2] | Andrea Marcillo[3] | Marta Manser[4] | Ruben Holland[5] | Claudia Birkemeyer[3] | Anja Widdig[1,2,6]

[1]Behavioural Ecology Research Group, Institute of Biology, University of Leipzig, Leipzig, Germany; [2]Junior Research Group of Primate Kin Selection, Department of Primatology, Max-Planck-Institute for Evolutionary Anthropology, Leipzig, Germany; [3]Research Group of Mass Spectrometry, Institute of Analytical Chemistry, University of Leipzig, Leipzig, Germany; [4]Department of Evolutionary Biology and Environmental Studies, University of Zurich, Zurich, Switzerland; [5]Zoo Leipzig GmbH, Leipzig, Germany and [6]German Center for Integrative Biodiversity Research (iDiv), Leipzig, Germany

Correspondence
Brigitte M. Weiß
Email: brigitte.schloegl@uni-leipzig.de

Funding information
University of Leipzig; European Fund for Regional Structure Development, Grant/Award Number: 100195810

Handling Editor: Robert Freckleton

Abstract

1. Olfaction is a central aspect of mammalian communication, providing information about individual attributes such as identity, sex, group membership or genetic quality. Yet, the chemical underpinnings of olfactory cues remain little understood, one of the reasons being the difficulty in obtaining high quality samples for chemical analysis.
2. In this study, we adjusted and evaluated the use of thermal desorption (TD) tubes, commonly used in plant metabolomic and environmental studies, for non-invasive sampling of mammalian body odour. We obtained chemical profiles of meerkat (*Suricata suricatta*) body odour samples, using TD tubes analysed with gas chromatography–mass spectrometry.
3. TD tubes captured a wide range of volatile and semi-volatile organic compounds, including compounds likely originating from the target animals. Adjustment of sampling parameters (distance, volume, flow rate, interruption of sampling) to increase the feasibility for a non-invasive application yielded samples of adequate quality. However, to minimize the variability between samples, sampling parameters should be kept constant and samples should be collected when no conspecifics are close-by.
4. The method was sensitive enough to pick up population differences in the chemical profiles of two captive groups of meerkats, demonstrating its applicability to biological questions. With sufficiently habituated animals, the method is applicable non-invasively, allowing short- and long-term studies on a wide range of questions, including e.g. chemical signatures of kinship, diet, individual health or reproductive state.

KEYWORDS
body odour, chemical profile, GC-MS, odour sample, olfaction, thermal desorption

1 | INTRODUCTION

Chemical cues may be important mediators of animal social interactions such as species-, group- and kin-recognition, mate choice and reproduction (Wyatt, 2014). Accordingly, the chemical composition of animal scents has received increasing interest in research areas such as behavioural ecology (e.g. Charpentier, Boulet, & Drea, 2008; Stoffel et al., 2015; Webster, Hayes, & Pike, 2015) and conservation biology (Larsson, 2016). Chemical cues comprise volatile, semi- or non-volatile organic compounds that may emanate from the skin, excretions such as urine or various gland secretions. Volatile organic compounds (VOCs) will be of particular interest for olfactory communication, i.e.

via sniffing from a range of distances, while less volatile compounds are likely to be relevant only during close-range interactions (Drea et al., 2013). While studies of plant volatiles and, to some extent, insects, have a relatively long history (see overview in Dormont, Bessière, & Cohuet, 2013; Dormont, Bessière, McKey, & Cohuet, 2013), the composition of non-human vertebrate chemical cues has been explored little and particularly mammalian chemical communication cues remain poorly understood (Drea et al., 2013; Stoffel et al., 2015).

Challenges in studying mammalian chemical ecology arise from the complexity of their chemical profiles, which are characterized by small amounts, high variability and a large number of exogenous or unknown compounds (Charpentier, Barthes, Proffit, Bessière, & Grison, 2012; Dormont et al., 2013). In part, this challenge is overcome by the continuous improvement of analytical tools such as gas chromatography–mass spectrometry (GC–MS), which allow separating very small quantities of complex mixtures into individual compounds (Charpentier et al., 2012). Furthermore, collecting high-quality odour samples poses another challenge, as most mammals are highly mobile. Consequently, studies investigating the composition of mammalian chemical cues are still limited and have been performed almost exclusively on captive and temporarily captured animals (e.g. Charpentier et al., 2008; Kean, Müller, & Chadwick, 2011; Stoffel et al., 2015). For ecological or other questions, studies on captive or restrained animals may not be desirable and considerably limit possible study questions, particularly in the wild.

Various sampling regimes have been applied to capture human body odour (reviewed in Dormont et al., 2013), while the majority of sampling regimes for collecting the body odour of non-human mammals comprised the use of cotton swabs or other materials rubbed over the skin or fur (e.g. Stoffel et al., 2015), sampling of secretions directly from scent glands (e.g. Crewe, Burger, Roux, & Katsir, 1979) or a combination thereof (e.g. Charpentier et al., 2008; Delbarco-Trillo, Sacha, Dubay, & Drea, 2012; Safi & Kerth, 2003). However, cotton or similar materials as intermediate medium usually are not analytically clean and may introduce high and irregular levels of contaminations (Birkemeyer et al., 2016; Dormont et al., 2013). Although some sample types are suitable for solvent-free extraction such as solid phase microextraction (reviewed in Drea et al., 2013), cotton swabs are eluted with a solvent before chemical analysis and further appear to be more appropriate for collecting semi-volatile than volatile compounds (Birkemeyer et al., 2016). Sampling secretions directly from scent glands overcomes many of these issues but, obviously, limits studies to species that possess scent glands. Furthermore, gland secretions are frequently metabolized by bacterial communities, which also contribute to an animal's smell (Douglas & Dobson, 2013; Theis, Schmidt, & Holekamp, 2012). Depending on the study question, one might be interested in these metabolized products (as this is what is likely to be smelled by conspecifics) rather than the non-metabolized secretions.

An alternative method for sampling VOCs is the use of sorbent traps, i.e. tubes or similar containers filled with an adsorbent material that captures the compounds present in ambient air or fluids (see Harper, 2000). The traps can be directly inserted into the GC for thermal desorption and thus avoid the use of solvents for extraction (Dormont et al., 2013). Thermal desorption (TD) tubes have been applied widely in plant metabolomic studies (e.g. Fatouros, van Loon, Hordijk, Smid, & Dicke, 2005; Mattiacci et al., 2001; Pierre et al., 2011) and environmental monitoring (e.g. Rabaud, Ebeler, Ashbaugh, & Flocchini, 2002; Wu, Feng, Lo, Lin, & Lo, 2004) but not for animal body odour, particularly in vertebrates (but see Marneweck, Jürgens, & Shrader, 2017 for an application to dung odour). In contrast to rubbing a medium over the skin or fur or sampling secretions directly from scent glands, TD tubes do not necessarily require handling and capture of animals, as only the ambient air in the immediate vicinity of an animal is sampled. This makes the method an attractive alternative for studying mammalian (and other vertebrate) body odour. The aim of this study therefore was to adjust the use of TD tubes for sampling the body odour of unrestrained mammals. Specifically, we aimed to (1) investigate the impact of various sampling parameters on the resulting odour profiles in order to assess and optimize the method for non-invasive use in the field, and (2) to demonstrate the method's applicability to a behavioural ecological question by investigating population differences in meerkats (*Suricata suricatta*).

For a non-invasive application in the wild, the sampling method needs to be feasible under field conditions while maintaining an adequate sample quality, i.e. sufficient amounts of the target odour and keeping contamination from the environment low. For example, larger sampling distances will often increase feasibility, but are likely to result in lower levels of the target odour and higher levels of the environmental background. Similarly, from a feasibility aspect the sampling duration should be as short as possible and thus minimize the sampled air volume while maximizing how fast the air is pulled through the TD tube (i.e. flow rate). However, flow rates recommended in environmental monitoring lie between 10 and 200 ml/min (Woolfenden, 1997). Together with recommended volumes of 1–6 L for the commonly used adsorbent Tenax (Uhde, 1999), sampling would take 5 min to over an hour, which will be unrealistic in many field scenarios. To what extent volumes and flow rates can be adjusted to make mammalian studies more feasible therefore needs to be investigated. Other aspects to consider in the field include if sampling can be continued at a later time without noteworthy quality loss in case the target animal moves away during sampling, or if conspecifics in close proximity confound a target odour sample. In this study, we evaluated these aspects by collecting odour samples with TD tubes at different sampling distances, volumes and flow rates, samples divided into different numbers of subsamples and samples collected in the presence or absence of conspecifics in close proximity to the focal animal.

We conducted the study in two populations of captive, human-habituated meerkats moving freely around in their enclosures and not being handled for sampling. Meerkats live in territorial groups comprising a dominant breeding pair and subordinate helpers related to the dominant pair at various degrees (Clutton-Brock & Manser, 2016). They are known to discriminate between conspecific scents based on kinship (Leclaire, Nielsen, Thavarajah, Manser, & Clutton-Brock, 2013) and group membership (Mares, Young, Levesque, Harrison, & Clutton-Brock, 2011), and possess scent glands in an anal pouch, whose bacterial communities and corresponding odours vary with sex and group

membership (Leclaire, Jacob, Greene, Dubay, & Drea, 2017; Leclaire, Nielsen, & Drea, 2014). These attributes, in combination with the relatively controlled captive setting, create an attractive system for investigating the use of TD tubes for non-invasive sampling of mammalian body odour from both, a methodological and a biological perspective.

2 | MATERIALS AND METHODS

2.1 | Study animals

We studied two groups of meerkats, housed at Leipzig Zoo, Germany, and the University of Zurich, Switzerland, respectively. Both groups had access to an inside and outside area, between which the animals could move freely. Odour samples were collected in the inside compartments (Leipzig 8 m^2, Zurich 60 m^2), which had a sand substrate and were equipped with hides and heat lamps. All study animals were fed a mixed diet that predominantly consisted of fruits and insects.

We sampled all individuals of the Leipzig group, which consisted of 14 individuals (four adult females, five adult males and five juveniles) at the time of sample collection. The animals were individually identifiable by subcutaneous transponders that were read with a Minimax II (Datamars) reading device. The Zurich group comprised 15 adult individuals, of which five males and five females were sampled for this study. The individuals in the Zurich group were individually identifiable by means of dye marks. Prior to sampling the animals were habituated to the experimenter and the sampling procedure, until all focal animals allowed the experimenter to approach to within a few centimetres and no longer visibly reacted to manipulating and operating the sampling equipment.

2.2 | Odour sampling

We collected airborne volatile compounds, using adsorbent traps, i.e. TD tubes (stainless steel, Supelco) filled with two porous polymers (Tenax TA and XAD-2, Sigma Aldrich, see supporting information for details). Tubes were connected to an air pump (BiVOC-2, Holbach) with a 1 m plastic hose, operating at sound levels below 60 dB. The pump produced a constant air flow through the TD tube until a specified volume (0.5 or 1 L) had passed through. The air flow was set to the maximum (1.9 L/min without drag), which produced flow rates of 0.88 ± 0.26 L/min (*M* ± *SD*, range 0.34–1.5) depending on how densely the adsorbent was packed into the TD tubes. Before each use, TD tubes were cleaned in a thermal conditioner (TD Clean-Cube, Scientific Instruments Manufacturer) for 120 min under a constant stream of nitrogen. Immediately afterwards, tubes were closed with Swagelok brass caps on both sides, wrapped in aluminium foil and stored in airtight bags until use.

For sampling, B.M.W. held the tip of the TD tube to the anogenital region of the target animal at a distance of 1, 3 or 5 cm. The air flow was started as soon as the tube was in position and was maintained until the intended sample volume was reached or the animal moved away. In the latter case, the pump was immediately stopped, the volume already sampled was noted and the sampling was resumed later. Immediately after sampling, the tube was closed with brass caps at both ends. If the sampling was interrupted because the animal had moved away, it was resumed as soon as possible (90% of samples within 5 min., all within same day) and as often as necessary to obtain the intended sample volume. As a consequence, we ran the air pump between 1 and 7 times (*M* ± *SD*: 2.3 ± 1) per sample, with the TD tubes firmly closed between subsamples. For each sample, we recorded the distance to the target, sample volume, realized flow rate and number of divisions.

A total of 57 anogenital samples were collected from the Leipzig and 44 from the Zurich group. We further sampled the Zurich group in one of two group conditions: when there was no other animal within one body length of the focal animal or when the focal animal was in body contact to at least one other animal. As the latter typically involved one meerkat lying on top of another, we could not collect these samples from the anogenital region but sampled from the back instead. We collected two samples per individual and condition, i.e. a total of 40 back samples, from a distance of 1 cm. Finally, we collected three types of blank samples from both locations: three method blanks that were handled in the same manner as the meerkat samples except that no air was pulled through the tube, three outside blanks that captured 1 L of air in open space outside the meerkat compartments, and 10 inside blanks that captured 1 L of air inside the meerkat compartments when no animal was within 2 m of the experimenter.

All equipment was handled, using disposable laboratory gloves; the experimenter wore the same set of clothes and avoided the use of perfumed products or strongly smelling foods before and on sampling days. The air pump was cleaned with ethanol (70%) after each sampling day.

2.3 | Gas chromatography–mass spectrometry analysis and profiling

Samples were analysed on a Shimadzu TQ8040 GC-MS coupled to a thermal desorption unit (TD 20, Shimadzu, see supplementary methods for instrumental parameters and GC program); the scan range was set to a mass-to-charge ratio (*m/z*) from 30 to 300. The GC–MS data were processed in a semi-automated procedure. In brief, we first performed automated signal deconvolution and peak picking, using AMDIS (version 2.71, Stein, 1999), grouped all recurring peaks with similar retention times (RTs) into RT ranges and manually corrected these ranges by taking into account not only their RTs but also their specific *m/z* ratios. We applied the resulting compound library to the entire dataset, using the Shimadzu GCMS Browser software, which searched for each library entry based on both, its retention time *and* most characteristic *m/z* ratio. We excluded compounds consistently occurring in blanks or otherwise being determined as contaminants from further analysis (see supplementary methods for details). Overall, we discarded 231 of the library entries from further analysis and performed statistical analyses for the remaining 111 compounds.

2.4 | Statistical analysis

All analyses were conducted in R version 3.2.4 (R Core Team, 2016). In particular, we assessed four aspects of anogenital odour profiles in relation to variation in sampling parameters and population differences using different statistical approaches: (1) summed peak areas per sample as a proxy for sample *intensity*, (2) the number of detected compounds as a proxy for sample *complexity*, (3) the *similarity* between odour profiles and (4) the *composition* of the odour profiles. For samples collected from the back, we assessed (5) individual *repeatability* in relation to the presence of conspecifics.

To determine the effect of sampling parameters and the population on (1) sample *intensity* and (2) sample *complexity*, we performed Linear Mixed Models (LMMs), with (1) the summed peak area and (2) the number of compounds as the respective response variable. In both models, we fitted the sampling distance, volume, flow rate, number of divisions (the number of subsamples required to collect the specified sample volume) and the population (Leipzig or Zurich) as fixed effects test predictors. We fitted individual identity (ID) and sampling date as random effects as well as the random slopes of flow rate within ID. Both models fulfilled the assumptions of LMMs. Significance of the full models and the individual test predictors was assessed, using Likelihood Ratio Tests (LRT, Forstmeier & Schielzeth, 2011). Further details on model formulation and test assumptions are given in the supplementary methods.

To assess if sampling parameters and population affected (3) overall *similarities* between odour profiles, we conducted nonparametric Analysis of Similarity (ANOSIM) for categorical predictors (i.e. volume, population) and partial Mantel tests for continuous predictors (i.e. distance, flow rate, number of divisions) based on pairwise Bray–Curtis similarities (see supporting information for details).

For a detailed analysis of (4) odour profile *composition* while taking into account the multiple fixed and random effects predictors we used a generalized linear mixed model (GLMM) approach as described by Jamil, Ozinga, Kleyer, and ter Braak (2013), which vectorizes the multivariate data matrix and includes the matrix rows and columns (i.e. the samples and compounds) as random factors (Jamil et al., 2013). We fitted relative peak areas of each sample (n = 101) and compound (n = 111) as response and the sampling parameters (distance, volume, flow rate, n divisions) and population as fixed effects. The sample, compound, ID and sampling date were fitted as random effects. As the effects of interest in this model are the interactions between the predictors and compound, we further fitted the random slopes of all fixed effects predictors within compound (see supporting information for a detailed explanation). We tentatively identified the compounds with the steepest slopes by comparing their mass spectra with the best matches of the NIST 14 library. Test assumptions and significances were assessed as described in the supporting information.

Finally, we assessed the effect of the presence of conspecifics on (5) sample *repeatability* by comparing Bray–Curtis similarities between two back samples collected from an individual when no other animal was within body length to similarities between two back samples from the same individual while it had body contact with a conspecific, using paired t tests (see supporting information for details).

3 | RESULTS

The 101 analysed anogenital samples contained 71 ± 20 ($M \pm SD$, range 21–101) of the 111 compounds included in statistical analyses (see Figure 1 for example chromatograms of a meerkat and blank sample).

3.1 | Sampling distance

Larger sampling distances were associated with lower (1) sample *intensity* (i.e. summed peak areas), while (2) sample *complexity* (i.e. the number of compounds) was not affected by sampling distance (Table 1). (3) Bray–Curtis *similarities* between pairs of samples were not related to

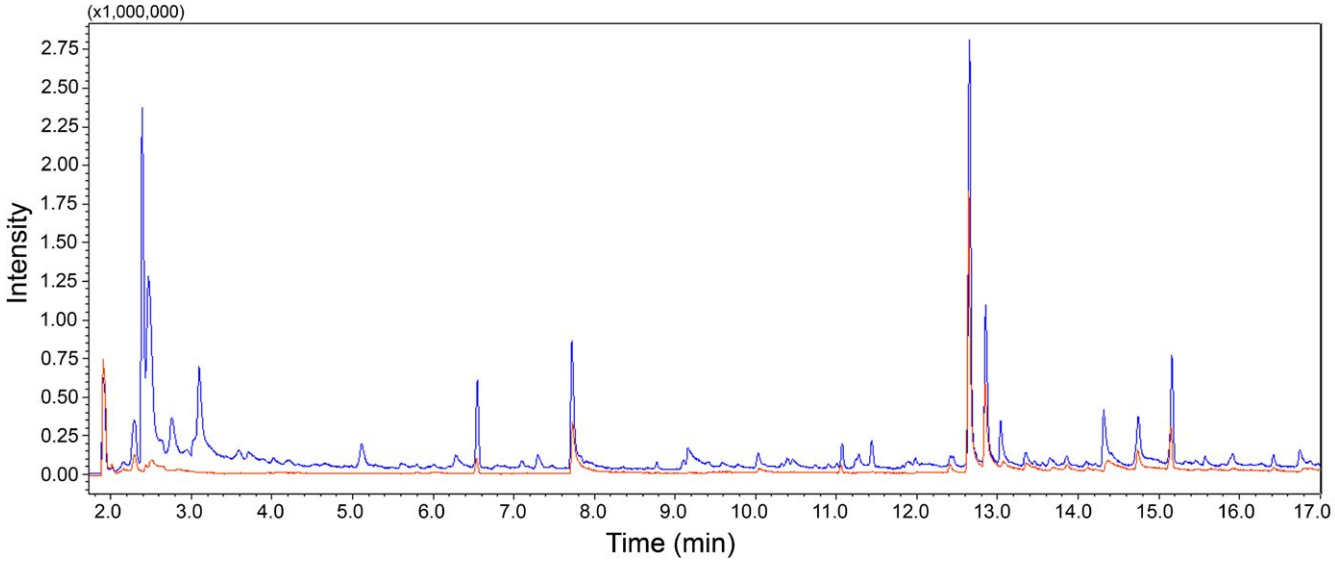

FIGURE 1 Example chromatograms of a meerkat anogenital sample (blue line) and a method blank (red line) over the range of volatile compounds

differences in sampling distance (partial Mantel test, $N = 101$, $r = -.036$, $p = .256$), but (4) the *composition* of odour profiles was affected by sampling distance, as the slope of distance within compound significantly explained variation in standardized peak areas (Table 2). In other words, sampling distance affected some compounds more than others (see Table S1), with the largest slope estimates pointing to the compounds affected most by distance. For most of the affected volatile compounds, the relative intensity increased with distance, while it decreased for the affected semi-volatiles (Table 3).

3.2 | Volume

Whether we sampled 0.5 or 1 L of air did not affect (1) *intensity* or (2) *complexity* (Table 1), (3) *similarity* (ANOSIM, $N = 101$, $r = -.005$, $p = .672$) or (4) *composition* of the samples (Table 2).

3.3 | Flow rate

(1) Sample *intensity* tended to be higher when flow rates were higher, while flow rate did not affect (2) sample *complexity* (Table 1). However, (3) *similarity* between pairs of samples was slightly lower if flow rates differed more between the samples (partial Mantel test, $N = 101$, $r = .110$, $p = .002$). Similarly, (4) sample *composition* was affected by variation in flow rates (Table 2), as indicated by the significant random slope component of flow rate within compound (Tables 2, 3 and Table S1).

TABLE 1 Results of the LMMs with (1) sample intensity (summed peak area) and (2) sample complexity (number of compounds) as response variables. ID and sampling date were included as random effects, the random slope of flow rate was fitted within ID and sampling date. Results of the fixed effects predictors of the complexity model not shown as the full-null model comparison was not significant

	Estimate	*SE*	$\chi 2$	*p*
Full-null model (intensity)			**18.349**	**.003**
Intercept	15.242	0.431		
Volume (1 L)	−0.170	0.225	0.557	.456
Flow rate	*0.637*	*0.331*	*3.453*	*.063*
n divisions	0.026	0.089	0.083	.773
Distance	**−0.211**	**0.075**	**7.418**	**.006**
Population (Zurich)	**1.477**	**0.458**	**7.877**	**.005**
Full null model (complexity)			4.977	.419

Significant predictors are indicated in bold, trends in italics.

TABLE 2 Results of the Likelihood Ratio Tests for the random slopes model investigating composition of odour profiles, with standardized peak area as response variable and the random slopes components of the respective predictors within the compound

	df	$\chi 2$	*p*
Full-null model	**9**	**954.170**	**<.001**
Volume	3	5.099	.165
Flow rate	**1**	**181.640**	**<.001**
n divisions	**1**	**35.242**	**<.001**
Distance	**1**	**18.015**	**<.001**
Population	**3**	**483.150**	**<.001**

Significant predictors are indicated in bold.

3.4 | Number of divisions

The number of divisions had no effect on (1) sample *intensity* or (2) sample *complexity* (Table 1), but (3) the more two samples differed in the number of divisions needed to collect them, the less *similar* these samples were to each other (partial Mantel test, $N = 101$, $r = .113$, $p = .035$). Along these lines, also (4) the sample *composition* was affected by the number of divisions (Table 2), with certain compounds being affected more than others (Table 3 and Table S1).

3.5 | Population differences

Samples from the Zurich meerkat group were (1) more *intense* than those from Leipzig but did not differ in (2) sample *complexity* (Table 1). Samples from the same population were (3) more *similar* to each other than samples collected from different populations (ANOSIM, $N = 101$, $r = .222$, $p = .001$, Figure 2). Similarly, (4) sample *composition* differed between the two populations (Table 2 and Table S1). We were able to putatively identify 7 out of the 10 compounds differing most between the two groups as substances likely deriving from the meerkats as well as their environment (Table 3 and Table S2).

3.6 | Presence of conspecifics

Conspecifics in close proximity to the focal animal tended to reduce (5) the *repeatability* of individual odour profiles collected from the animal's back, as indicated by lower similarity between samples of the same individual if the animal had body contact with others than if others were at least one body length away (paired *t* test: $t = -2.008$, $df = 9$, $p = .076$, Figure 3).

4 | DISCUSSION

By using TD tubes for non-invasive sampling of meerkat body odour, we were able to capture a wide range of volatile and semi-volatile compounds, including compounds that likely are of mammalian origin. TD tubes thus represent an effective method for sampling mammalian body odour that can be applied non-invasively and covers chemical compounds across a wide range of volatility. This makes the method widely applicable to various biological questions, as demonstrated here e.g. for the detection of population differences in meerkats. In order to achieve best possible results in the field, several sampling parameters need to be taken into account (discussed below).

TABLE 3 Very volatile (VVOC), volatile (VOC) and semi-volatile (SVOC) compounds affected most by sampling parameters and/or population. N gives the number of samples (out of 101) in which the compound was detected. Plus, minus and < indicate the direction of the effect; L, Leipzig; Z, Zurich. Entries in bold mark the compounds with the steepest slopes (2 *SD* or more above average). Similarities (from 0 to 100) describe the match between mass spectra and their respective matches in the NIST library

Class	nr.	*n*	Distance	Flow	*n* divisions	Population	Putative ID	Similarity
VVOC	5	78	**–**				Acetaldehyde[a]	91
VVOC	10	98	+	**–**			2-Methyl-2-propanol	94
VVOC	11	41				Z < L	Glycolaldehyde[a]	86
VVOC	12	67	+	–			1-Propanol[a]	86
VOC	13	53		+			Acetic acid[a]	97
VOC	14	82			**+**		Ethylacetate	96
VOC	15	73			+		Unknown	
VOC	18	83	**–**				Organic solvent	90
VOC	19	49				**L < Z**	Organic solvent	96
VOC	30	95				Z < L	Hexanal[a]	96
VOC	33	42				**L < Z**	3-Methoxy-1-butanol	98
VOC	36	31				Z < L	2-Pentylacetate	92
VOC	42	80		–			Heptanal[a]	89
VOC	48	77	+				Unidentified benzene	
VOC	58	57			+		Unidentified alkane	
VOC	61	92		–			3-Carene[b]	85
VOC	78	96	**+**				Adsorbent breakdown product	
VOC	81	98	**+**		**–**		Adsorbent breakdown product	
VOC	84	95	+				Nonanal[a]	92
VOC	94	89	+				Adsorbent impurity	
VOC	104	61		**–**	–		Unknown	
VOC	108	93	**+**				Adsorbent impurity	
VOC	123	44	–				3,3,4,4-Tetramethyl-2-pentanone[a]	89
VOC	125	86	+				Unidentified benzene	
VOC	151	50		**–**	–		Unidentified alcohol	
SVOC	195	59		**–**	**–**		Unidentified acetophenone	
SVOC	267	67	–		–		(n-)Hexadecanoic acid[a]	95
SVOC	279	40		+		Z < L	Unknown	
SVOC	292	97			–		Plasticizer	
SVOC	294	52			**+**		Unidentified cholesterol derivate[a]	
SVOC	295	73			**+**		Unidentified cholesterol derivate[a]	
SVOC	298	78			+		Unidentified cholesterol derivate[a]	
SVOC	299	63			**+**		Spirostan-23-ol	81
SVOC	302	48			+		Unknown	
SVOC	309	72	**–**				Unknown	
SVOC	313	80		+			Unidentified cholesterol derivate[a]	
SVOC	315	84		**+**			Unidentified cholesterol derivate[a]	

(Continues)

TABLE 3 (Continued)

Class	nr.	*n*	Distance	Flow	*n* divisions	Population	Putative ID	Similarity
SVOC	317	30				Z < L	Octacosane[b]	95
SVOC	318	75	–		–		Unknown	
SVOC	319	59				Z < L	Unidentified lactone[a]	
SVOC	324	76			+	L < Z	Unknown	
SVOC	328	81	–	+		Z < L	Cholesterol[a]	88
SVOC	330	53	–				5-Henicosyldihydrofuran-2(3 H)-one[a]	88
SVOC	331	69	–				Lathosterol[a]	88
SVOC	332	47				**Z < L**	Unknown	
SVOC	333	72			**–**	L < Z	Cholesta-3,5-dien-7-one[a]	87
SVOC	339	68	–				Lanosterol[a]	79

[a]Compounds previously described in mammals.
[b]Compounds described in mammals but likely originating from plants (see Table S2 for references).

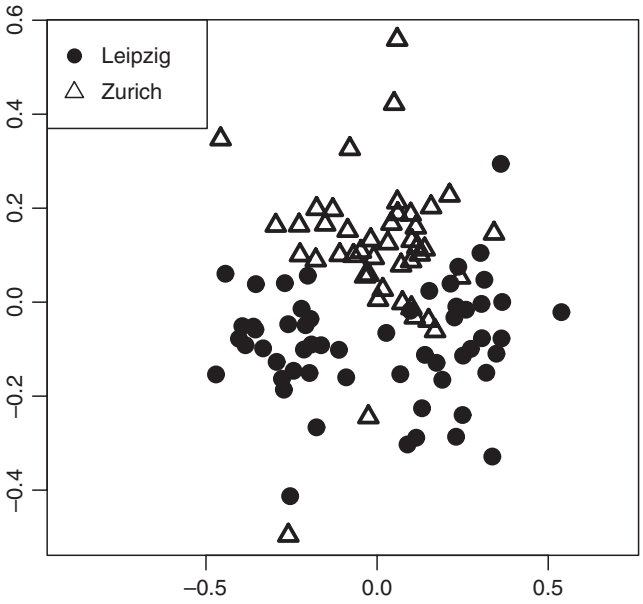

FIGURE 2 Two-dimensional non-metric multidimensional scaling plot of chemical profiles of two populations of meerkats based on Bray–Curtis dissimilarities. The axes are dimensionless, with symbols in close proximity indicating similar chemical profiles

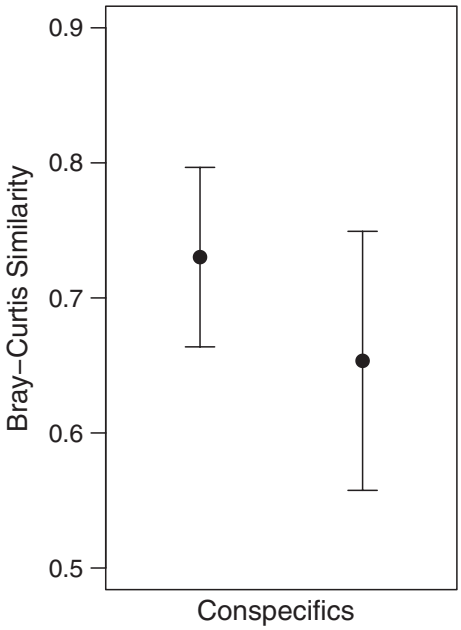

FIGURE 3 Pairwise similarities of odour profiles collected from the same individuals when sampled in the absence (no conspecifics within body length) or presence (body contact to one or more individuals) of conspecifics. Circles show mean pairwise similarities (*N* = 10 individuals), whiskers show standard deviations

4.1 | Distance

Samples were more intense when collected closer to the animal. This increased intensity was not attributable to more compounds being captured, as profile complexity was unaffected by distance. Although overall similarity between chemical profiles was similarly unaffected, model results suggested a certain shift in profile composition towards more volatile compounds as distance from the sampled animal increased. Rather than absolute intensities of VOCs increasing with distance, their relative increase likely resulted from an absolute decrease in the semi-volatile components, which, by definition, do not pass into and through the air as easily as VOCs and thus should be less likely picked up further from their source. Additionally or alternatively, chemical profiles could have contained relatively more of the volatile environmental background when samples were collected at a larger distance from the target. Putative identifications of the compounds most affected by distance, suggest a mix of both, as they comprised volatiles of potential mammalian origin as well as volatiles likely deriving from the environment (e.g. plant volatiles).

As sampling distance affects certain aspects of the chemical profiles, it should be kept as constant as possible. How close one needs to get will depend on several aspects: if the research focus includes semi-volatiles (e.g. when investigating close-range interactions) and/or if the study site is likely to comprise strong background odours (e.g.

flowering plants), we recommend sampling at very close distances (1 cm), while otherwise distances of several centimetres should produce good results. Many animals in the field may not be approachable so closely by an experimenter, but in larger animals, distances for volatile sampling can probably be prolonged to some extent. Furthermore, animals may be more tolerant towards TD tubes than towards humans close to them. For example, in a pilot study on chemical communication in wild crested macaques (*Macaca nigra*), human observers used an extendable pole to bring TD tubes to within 5–20 cm of the animals, while they themselves stayed at a distance of 2–5 m (A. Widdig, unpublished). Preliminary assessment of chromatograms from macaques and blank control samples suggests that macaque body odour could be successfully sampled in this manner.

4.2 | Volume

In studies applying TD tubes for environmental monitoring or plant metabolomics, sampled air volumes usually were 2–6 L or more (e.g. Pierre et al., 2011; Rabaud et al., 2002; Woolfenden, 1997), requiring too much time for sampling to be feasible for mammals in many field situations. However, Salthammer and Uhde (2009) describe adequate sensitivities being achieved also with volumes as low as 0.5 or 2 L. In line with the latter study, we did not detect differences in intensity, complexity, similarity or composition between samples of 0.5 or 1 L, suggesting that volumes of 0.5 L suffice and sampling time can thus be reduced to a total of *c.* 20–40 s (depending on flow rate).

4.3 | Flow rate

In this study, we observed considerable variation in flow rates when operating the pump at maximal capacity, ranging from values similar to those recommended previously in other research areas (Woolfenden, 1997) to about 5 times higher (i.e. *c.* 0.3–1.5 L/min). However, Woolfenden (1997) described variation in flow rates of up to 300 ml/min as having negligible effects on the retention of compounds, and also higher flow rates were considered as appropriate (for sampling durations up to 10 min, Woolfenden, 1997) or noted as even slightly increasing sensitivity (Harper, 2000). In this study, higher flow rates indeed tended to produce more intense samples, although compounds were affected differently by the variation in flow rate. In particular, relative amounts increased for some of the compounds but decreased for others, whereby the relative amounts of semi-volatile compounds affected most by flow rate almost all increased with increasing flow (Table 3). Possibly, the higher suction associated with a faster flow rate facilitated the movement of semi-volatiles through the air, thereby shifting profile composition slightly towards certain SVOCs. Such a shift could also explain why samples collected with more similar flow rates resulted in more similar odour profiles. Accordingly, also for selecting a suitable flow rate, researchers should take into account whether their research focus includes SVOCs or not. Furthermore, variation in flow rates introduced by different flow resistance should be assessed before data collection in order to keep the flow relatively constant, either by ensuring homogenously packed tubes and/or by setting the flow lower than the one the slowest tube achieves at maximum capacity of the pump.

4.4 | Number of divisions

While overall sample intensity and the number of detected compounds were unaffected by dividing a sample, similarities between samples as well as composition of the chemical profiles were related to sample divisions. Possible reasons for the relative increase observed in some compounds include higher levels of contamination resulting from repeated handling of the TD tubes when several subsamples were collected. Alternatively, interrupting the sampling for several minutes could have allowed the air around the animal to get more saturated again with compounds that spread or are emitted slowly. On the other hand, the relative reduction in other compounds could be due to divisions creating a temporally more heterogeneous sample in which transient compounds in the ambient air are less emphasized. Overall, data suggest that as long as individuals are identifiable and can be found again later, sampling appears to be divisible, if the number of divisions is kept relatively constant. Depending on the question, it might even be desirable to split the sample up not just for feasibility purposes but to reduce temporal fluctuations and/or capture more of compounds that are slowly replenished.

4.5 | Presence of conspecifics

Samples from the same individual tended to be less repeatable if the animal had body contact with another individual during sampling, implying that some of the body odour of conspecifics in close proximity is picked up too and confounds the target odour. To reduce the amount of unwanted background noise, sampling a focal animal while in the close presence of conspecifics should thus be avoided, whereby the exact definition of "close presence" will depend on characteristics such as body size or "smelliness" of the species under investigation.

4.6 | Population differences

Odour profiles from the two study populations differed with respect to sample intensity, similarity and composition. Sources for these differences could be environmental volatiles sampled directly or emanating from the fur of the meerkats, or actual differences in the body odour of the meerkats. Putative identifications of the compounds differing most between the groups comprised substances previously found in mammals, such as hexanal and cholesterol (Burger, 2005; De Lacy Costello et al., 2014), compounds likely deriving from fruits, and one organic solvent (likely a contamination from cleaning, Table 3 and Table S2). This suggests that the group differences stemmed from different housing conditions and feeding regimes, but also from the meerkats themselves. Hence, these results correspond to earlier findings on group-specific meerkat scents, as meerkats were found to distinguish scent marks of intruders from those of group members (Mares et al., 2011).

Furthermore, bacterial communities of adult meerkat scent secretions and social odours differed between groups, which was mainly attributed to group members sharing the same socio-ecological environment (Leclaire et al., 2014, 2017). Accordingly, our results also provide evidence that sampling with TD tubes is suitable for investigating biological questions on mammalian chemical communication.

4.7 | Lessons for practical application of TD sampling

Except for the sample volume, sampling parameters affected sample intensity and/or the exact composition to some extent; yet we obtained useful body odour samples of meerkats across the entire tested parameter space. Accordingly, the method should be applicable across a range of field conditions and associated sampling regimes. However, given that body odour is complex and inherently variable, removing any sources of unnecessary variation is preferable. Within the limits of sampling feasibility, we therefore recommend bringing TD tubes to the closest distance that can reliably be maintained throughout the study, and to sample only when no one else is too close (rule of thumb: one body length). With the adsorbent combination used in this study, a sample volume of 0.5 L suffices; if necessary (or desirable) sampling can be further split into various parts. The number of divisions as well as the flow rate should, however, be kept relatively constant. If variation in these parameters cannot be avoided, the statistical design of the study should control for their potential effects when investigating biological aspects.

5 | CONCLUSIONS

Putative compound identifications indicate that sampling with TD tubes captures meerkat body odour along with environmental compounds present in the ambient air and/or on the animals. Such environmental components may be part of the research question or otherwise may contribute to specific odours, as in the case of group differences in meerkats and other mammals (e.g. Safi & Kerth, 2003; Stoffel et al., 2015). Regardless of the sampling method used, establishment of a species-specific compound library should eventually allow researchers to identify compounds and make informed decisions about which compounds to include in their statistical analyses or not. Overall, TD tubes appear to be a promising tool for field studies on mammalian chemical communication that capture the airborne body odour of animals, and as such the cues and signals relevant for olfactory communication. Particularly when sampling at close distances, also less volatile compounds that may act through direct contact between individuals can be captured. When having sufficiently habituated animals, the method is applicable non-invasively and individuals can be sampled repeatedly across multiple contexts, allowing studies on a wide range of questions, including e.g. chemical signatures of kinship, diet, individual health or genetic quality.

ACKNOWLEDGEMENTS

We thank L. Siebert–Lang for assistance in sample collection and the animal keepers at the Zoo Leipzig for their support. L. Kulik kindly provided R scripts for data assembly and a customized ANOSIM. M. Kücklich and S. Jänig provided valuable input for chemical profiling and compound identification. This project was funded by the University of Leipzig and the European Fund for Regional Structure Development, EFRE ("Europe funds Saxony"), grant no. 100195810 to A.W.

AUTHORS' CONTRIBUTIONS

B.M.W., C.B. and A.W. conceived the ideas and designed methodology; B.M.W. collected the data and M.M. supervised data collection in Zurich; B.M.W. and A.M.L. analysed the data; B.M.W. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

DATA ACCESSIBILITY

Processed chemical profiles and sample information are available from the Dryad Digital Repository https://doi.org/10.5061/dryad.2m39d (Weiß et al., 2017).

ORCID

Brigitte M. Weiß http://orcid.org/0000-0001-6316-5636

REFERENCES

Birkemeyer, C., Thomsen, R., Jänig, S., Kücklich, M., Slama, A., Weiß, B. M., & Widdig, A. (2016). The sampling of body odour of primates: Cotton swabs sample semi-volatiles rather than volatiles. *Chemical Senses*, *41*, 525–535.

Burger, B. V. (2005). Mammalian semiochemicals. *Topics in Current Chemistry*, *240*, 231–278.

Charpentier, M. J. E., Barthes, N., Proffit, M., Bessière, J.-M., & Grison, C. (2012). Critical thinking in the chemical ecology of mammalian communication: Roadmap for future studies (C. Fox, Ed.). *Functional Ecology*, *26*, 769–774.

Charpentier, M. J. E., Boulet, M., & Drea, C. M. (2008). Smelling right: The scent of male lemurs advertises genetic quality and relatedness. *Molecular Ecology*, *17*, 3225–3233.

Clutton-Brock, T., & Manser, M. (2016). Meerkats: Coopaerative breeding in the Kalahari. In W. D. Koenig, & J. L. Dickinson (Eds.), *Cooperative breeding in vertebrates: Studies of ecology, evolution, and behavior* (pp. 294–317). Cambridge, UK: Cambridge University Press.

Crewe, R. M., Burger, B. V., Roux, M. L., & Katsir, Z. (1979). Chemical constituents of the chest gland secretion of the thick-tailed galago (*Galago crassicaudatus*). *Journal of Chemical Ecology*, *5*, 861–868.

delBarco-Trillo, J., Sacha, C. R., Dubay, G. R., & Drea, C. M. (2012). Eulemur, me lemur: The evolution of scent-signal complexity in a primate clade. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, *367*, 1909–1922.

De Lacy Costello, B., Amann, A., Al-Kateb, H., Flynn, C., Filipiak, W., Khalid, T., … Ratcliffe, N. M. (2014). A review of the volatiles from the healthy human body. *Journal of Breath Research*, *8*, 014001.

Dormont, L., Bessière, J.-M., & Cohuet, A. (2013). Human skin volatiles: A review. *Journal of Chemical Ecology*, *39*, 569–578.

Dormont, L., Bessière, J.-M., McKey, D., & Cohuet, A. (2013). New methods for field collection of human skin volatiles and perspectives for their application in the chemical ecology of human–pathogen–vector interactions. *Journal of Experimental Biology*, *216*, 2783–2788.

Douglas, A. E., & Dobson, A. J. (2013). New synthesis: Animal communication mediated by microbes: Fact or fantasy? *Journal of Chemical Ecology*, *39*, 1149.

Drea, C. M., Boulet, M., Delbarco-Trillo, J., Greene, L. K., Sacha, C. R., Goodwin, T. E., & Dubay, G. R. (2013). The "secret" in secretions: Methodological considerations in deciphering primate olfactory communication. *American Journal of Primatology*, *75*, 621–642.

Fatouros, N. E., van Loon, J. J. A., Hordijk, K. A., Smid, H. M., & Dicke, M. (2005). Herbivore-induced plant volatiles mediate in-flight host discrimination by parasitoids. *Journal of Chemical Ecology*, *31*, 2033–2047.

Forstmeier, W., & Schielzeth, H. (2011). Cryptic multiple hypotheses testing in linear models: Overestimated effect sizes and the winner's curse. *Behavioral Ecology and Sociobiology*, *65*, 47–55.

Harper, M. (2000). Sorbent trapping of volatile organic compounds from air. *Journal of Chromatography A*, *885*, 129–151.

Jamil, T., Ozinga, W. A., Kleyer, M., & ter Braak, C. J. F. (2013). Selecting traits that explain species–environment relationships: A generalized linear mixed model approach. *Journal of Vegetation Science*, *24*, 988–1000.

Kean, E. F., Müller, C. T., & Chadwick, E. A. (2011). Otter scent signals age, sex, and reproductive status. *Chemical Senses*, *36*, 555–564.

Larsson, M. C. (2016). Pheromones and other semiochemicals for monitoring rare and endangered species. *Journal of Chemical Ecology*, *42*, 853–868.

Leclaire, S., Jacob, S., Greene, L. K., Dubay, G. R., & Drea, C. M. (2017). Social odours covary with bacterial community in the anal secretions of wild meerkats. *Scientific Reports*, *7*, 3240.

Leclaire, S., Nielsen, J. F., & Drea, C. M. (2014). Bacterial communities in meerkat anal scent secretions vary with host sex, age, and group membership. *Behavioral Ecology*, *25*, 996–1004.

Leclaire, S., Nielsen, J. F., Thavarajah, N. K., Manser, M., & Clutton-Brock, T. H. (2013). Odour-based kin discrimination in the cooperatively breeding meerkat. *Biology Letters*, *9*, 20121054.

Mares, R., Young, A. J., Levesque, D. L., Harrison, N., & Clutton-Brock, T. H. (2011). Responses to intruder scents in the cooperatively breeding meerkat: Sex and social status differences and temporal variation. *Behavioral Ecology*, *22*, 594–600.

Marneweck, C., Jürgens, A., & Shrader, A. M. (2017). Dung odours signal sex, age, territorial and oestrous state in white rhinos. *Proceedings of the Royal Society. B*, *284*, 20162376.

Mattiacci, L., Rocca, B. A., Scascighini, N., D'Alessandro, M., Hern, A., & Dorn, S. (2001). Systemically induced plant volatiles emitted at the time of "danger". *Journal of Chemical Ecology*, *27*, 2233–2252.

Pierre, P. S., Jansen, J. J., Hordijk, C. A., van Dam, N. M., Cortesero, A.-M., & Dugravot, S. (2011). Differences in volatile profiles of turnip plants subjected to single and dual herbivory above- and belowground. *Journal of Chemical Ecology*, *37*, 368–377.

R Core Team. (2016). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.

Rabaud, N. E., Ebeler, S. E., Ashbaugh, L. L., & Flocchini, R. G. (2002). The application of thermal desorption GC/MS with simultaneous olfactory evaluation for the characterization and quantification of odor compounds from a dairy. *Journal of Agricultural and Food Chemistry*, *50*, 5139–5145.

Safi, K., & Kerth, G. (2003). Secretions of the interaural gland contain information about individuality and colony membership in the Bechstein's bat. *Animal Behaviour*, *65*, 363–369.

Salthammer, T., & Uhde, E. (2009). *Organic indoor air pollutants: Occurrence, measurement, evaluation*. Weinheim, Germany: Wiley-VCH.

Stein, S. E. (1999). An integrated method for spectrum extraction and compound identification from gas chromatography/mass spectrometry data. *Journal of the American Society for Mass Spectrometry*, *10*, 770–781.

Stoffel, M. A., Caspers, B. A., Forcada, J., Giannakara, A., Baier, M., Eberhart-Phillips, L., … Hoffman, J. I. (2015). Chemical fingerprints encode mother–offspring similarity, colony membership, relatedness, and genetic quality in fur seals. *Proceedings of the National Academy of Sciences*, *112*, E5005–E5012.

Theis, K. R., Schmidt, T. M., & Holekamp, K. E. (2012). Evidence for a bacterial mechanism for group-specific social odors among hyenas. *Scientific Reports*, *2*, 1–8.

Uhde, E. (1999). Application of solid sorbents for the sampling of volatile organic compounds in indoor air. In T. Salthammer, & E. Uhde (Eds.), *Organic indoor air pollutants: Occurrence-measurement-evaluation* (pp. 3–18). Weinheim, Germany: Wiley-VCH.

Webster, B., Hayes, W., & Pike, T. W. (2015). Avian egg odour encodes information on embryo sex, fertility and development. *PLoS ONE*, *10*, 1–10.

Weiß, B. M., Marcillo, A., Manser, M., Holland, R., Birkemeyer, C., & Widdig, A. (2017). Data from: A non-invasive method for sampling the body odour of mammals. *Dryad Digital Repository*, https://doi.org/10.5061/dryad.2m39d

Woolfenden, E. (1997). Monitoring VOCs in air using sorbent tubes followed by thermal desorption-capillary GC analysis: Summary of data and practical guidelines. *Journal of the Air & Waste Management Association*, *47*, 20–36.

Wu, C.-H., Feng, C.-T., Lo, Y.-S., Lin, T.-Y., & Lo, J.-G. (2004). Determination of volatile organic compounds in workplace air by multisorbent adsorption/thermal desorption-GC/MS. *Chemosphere*, *56*, 71–80.

Wyatt, T. D. (2014). *Pheromones and animal behavior: Chemical signals and signatures*. Cambridge, UK: Cambridge University Press.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

How to cite this article: Weiß BM, Marcillo A, Manser M, Holland R, Birkemeyer C, Widdig A. A non-invasive method for sampling the body odour of mammals. *Methods Ecol Evol*. 2018;9:420–429. https://doi.org/10.1111/2041-210X.12888