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Original Investigation

Scent signals individual identity and country of origin in otters

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ABSTRACT

Signalling individual identity conveys fitness benefits to signaller and receiver, for example by enabling the avoidance of breeding with kin. Chemical analysis indicates that scent marks are used to communicate individual identity in several mammalian species, but prior to the current study there has been no detailed assessment of individuality in otter scent marks despite their widespread use to survey population distributions. Repeated spraint samples were collected from captive Eurasian otters, *Lutra lutra*, and analyzed using solid phase microextraction and gas chromatography mass spectrometry. Permutational Multivariate Analysis of Variance (PerMANOVA) was chosen over ordination techniques because it uses all of the scent profile rather than a subset of the data. Spraint scent was significantly associated with the identity of the individual otter that deposited it, and the country of origin. Scent similarity between otters at the same location may be explained by genetic similarity. Within-individual variation in scent profiles was also observed which we hypothesize could be explained by hormonal fluctuations. Future research should aim to explain this within-individual variation further and explore other odour signals of individual identity in otters (for example non-volatile compounds).

Introduction

The traits of signalling identity and the ability to recognize individual conspecifics have evolved because of the associated fitness benefits for both signaller and receiver (Tibbetts and Dale 2007). For example, individual recognition may allow individuals to invest resources and care only in their own offspring (reviewed by Lévy et al. 2004), to avoid potentially costly interactions with stronger opponents (Gosling 1982), or to aid the identification of mates and avoid inbreeding (Hurst 2009). Some of these behaviours, however, may be possible without individual identification (for example inbreeding may be avoided by kin/non-kin cues rather than individual identity). In mammals, individual identity may be communicated through vocal or visual cues, but scent is the most common modality (Brown and Mcdonald 1985; Wyatt 2003; Thom and Hurst 2004; Brennan and Kendrick 2006). Thom and Hurst (2004) identified three key characteristics of individual scent cues: independence from background variation, a high degree of diversity, and temporal stability. Variation in major histocompatibility complex (MHC) is commonly thought to be the mechanism for individually distinct scent cues, but this was recently found not to be the case for mice, calling into question the assumption of a vertebrate-wide mechanism (Cheetham et al. 2007). Extrapolation from model organisms should therefore be treated with caution.

Two methods are commonly applied to the study of individual recognition: behaviour and chemical analysis. Both were criticized in the past – chemical analysis because it is an indirect measure of individual recognition, and behavioural experiments because they frequently test familiarity of scents rather than true individual recognition (Halpin, 1986). Although improvements have been made, Thom and Hurst (2004) more recently reviewed the field and found many of Halpin's concerns had not been addressed, and in particular more focus is needed on function. Despite the criticism of chemical analysis, for species that are difficult to manipulate in captivity (for practical or legislative reasons) or to observe in the wild, chemical analysis of scent marks offers a more viable option than behavioural experiments.

Since early chemical investigations into individual scent signatures (for example black tailed deer; Müller-schwarze, 1971), many species have been investigated. Methods of early investigations (for example the visual comparison of chromatograms) were limited compared to the complexity of analytical and statistical methods available today. Chemical evidence for individually distinct volatile scent signatures now exists for many mammalian orders, including ungulates (Müller-schwarze, 1971), rodents (Sun and Müller-schwarze, 1998), carnivores (Hagey and Macdonald 2003; Burgener et al. 2009), primates (Scordato et al. 2007; Smith et al. 2001; Setchell et al. 2010), bats (Safi and Kerth 2003) and lagomorphs (Goodrich and Mykytowy 1972). Differences between

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individuals' scent exist even for species such as ourselves, for whom scent communication is assumed to be unimportant (Penn et al. 2007), and human scent bar-coding was recently suggested as a forensic tool (Curran et al. 2010).

Mustelids use scent marks for intra-specific communication and have been the subject of semiochemical research for over 140 years, probably as a result of the aggressively malodourous nature of their scent marks (Burger 2005). Within the mustelid family, individual scent signatures have been reported, by visual examination of chromatograms, in *Mustela ermine* (Erlinge et al. 1982), *M. vison* (Brinck et al. 1978) and *Meles meles* (Buesching et al. 2002a), or by examination of individual chromatogram peaks in *Mustela evermanni* and *M. sibirica* (Zhang et al. 2003). Stronger evidence comes from multivariate statistical analysis of scent marks from *Meles meles* (Buesching et al. 2002b) and *Mustela furo* (Zhang et al. 2005). Despite otter species representing 13 of the 58 extant species in the mustelid family, there has been little research to determine the function of scent-marking behaviour. There is behavioural evidence for scent discrimination of species, sex and social status in *Lontra canadensis*, (Rostain et al. 2004) and chemical evidence of scent signatures for sex, age and reproductive status in *Lutra lutra* (Kean et al. 2011) but there is no convincing evidence, behavioural or chemical, for the discrimination of individual identity in any otter species.

Here we focus on the Eurasian otter, *Lutra lutra*, which have two anal sacs to deposit secretions with faeces; collectively known as spraint. Their mainly solitary nature and large home range makes the odour of spraint an ideal form of intraspecific communication (Erlinge 1967; Gorman et al. 1978; Kruuk 2006; Trowbridge 1983). The Eurasian otter is a species of conservation concern, protected under the European Habitats Directive, not commonly kept in captivity and is elusive in the wild; all of which limit investigation of individual scent signatures through behavioural experiments. Early attempts to examine individual differences in Eurasian otter odour suggest individual differences (Gorman et al. 1978; Trowbridge 1983) but small sample sizes ($n=2$ and $n=3$) limit conclusions. The present study uses multiple samples from individual otters in captivity to address the question whether otters have an individually distinct signature in the volatile organic compounds (VOCs) of anal gland secretions.

Material and methods

Sample collection

Spraints were collected from 17 otters in captivity from six different centres, in the UK, Germany and Spain. Although ideally all subjects would be from the same location, this was not possible as Eurasian otters are kept infrequently in captivity, and in small numbers due to their solitary nature. For otters housed in pairs, spraints were collected only after observation to ensure known identity of the depositor; some otters were housed individually rendering this unnecessary. Between one and five samples were collected from each otter, with a minimum interval of one day and maximum interval of 354 days between samples (Table 1). Samples were collected and stored in plastic zip lock bags (one centre in Germany) or sterile plastic tubes (all other centres), and stored at −20 °C for a minimum of six weeks and a maximum of 19 months before analysis. Preliminary experiments found no effect of storage time over eight years (Kean 2012).

Chemical analysis

VOCs eluting from the spraint samples were sampled and analyzed using solid-phase Microextraction (SPME) and gas chromatography mass spectrometry (GC-MS). The order of sample analysis was randomized. Samples were defrosted and transferred to 10 ml SMPE glass vials (Supelco). Sample vials were placed in a water bath at 30 °C to ensure a consistent temperature during sampling. A StableFlex Divinylbenzene/Carboxen/PDMS (DVB/CAR/PDMS) 50/30 μm bonded fibre (Supelco) was exposed to the headspace above each sample for 45 min. Preliminary testing of exposure times of up to 1 h indicated 45 min to be sufficient to reach an equilibrium. Fibres were conditioned according to manufacturer's recommendations and reconditioned for 10 min in a GC injection port at 260 °C between each sample (or for 30 min if the fibre had not been used for several hours). An analysis of the fibre not exposed to a sample was conducted at least every sixth sample to detect non-sample compounds and any contamination or deterioration of the fibre, and fibres were replaced when damaged.

Following exposure, fibres were immediately injected manually into a GC-MS (Agilent 6890N/5973N) and desorped for 2 min

Table 1
Captive Eurasian otter spraint collected to investigate individuality in odour by analysis of volatile organic compounds (VOCs) using solid phase microextraction (SPME) and gas chromatography mass spectrometry (GCMS).

Location	Otter	Age (years)	Sex	Sampling period (days)	Sample *n*
RSPCA Eastwinch, UK	19161	<1	Male	9	5
RSPCA Westhatch, UK	16996	<1	?	1	1
British Wildlife Centre, UK	Minnie	12	Female	115	3
	Lilly	3	Female	1	2
	Oscar	12	Male	10	2
	Stirling	11	Male	18	2
	Thistle	7	Female	112	2
Newforest Wildlife, UK	Alpha	17	Male	354	2
	Sirius	1	Male	129	3
	Grace	2	Female	129	2
UK total					**24**
Otter Zentrum, Germany	Desiree	4	Female	68	3
	Evi	<1	Female	68	3
	Henri	2	Male	62	3
	Naima	7	Female	38	3
	Teufel	5	Male	64	3
Germany total					**15**
Terra Natura Murcia, Spain	Vagui	4	Female	8	4
	Cuca	3	Female	12	4
Spain total					**8**