Volatile faecal components related to sex and age in domestic cats (Felis catus)

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ABSTRACT
Volatile components of faeces from 55 domestic entire cats (36 males and 19 females) aged 1–12 were investigated using gas chromatograph–mass spectrometer headspace techniques. Twenty-four volatile organic compounds were found in the faecal samples. There was no significant difference in complexity, in terms of the number of compounds detected, between males (mode: 23, median: 23, quartile range: 0.6) and females (23, 21, 2.0). On the other hand, males (15.0, 13.0, 8.9) had a significantly (P < .05) higher concentration (mg/kg) of 1-butanol than females (11.0, 7.1, 3.1). In male cats, the concentrations of six compounds were significantly correlated with age: 1-propanol (r = 0.409, P < .05), methyl propionate (r = 0.464, P < .01), propionic acid (r = 0.443, P < .01), ethyl propionate (r = 0.333, P < .05), phenol (r = 0.370, P < .05) and indole (r = 0.467, P < .01). Similar correlations were not found for the females. These results suggest that domestic cats get information about the sex and age of males via the volatile organic compounds of faeces.

Introduction
There are both basic and applied scientific reasons to investigate the volatile components of faeces from domestic cats. The former relates to scent marking, which is observed as the most ubiquitous form of chemical signalling in mammals (as reviewed by Soso et al. 2014). As evidence of this, it has been demonstrated that domestic cats can distinguish faeces of familiar individuals from those of unfamiliar conspecifics (Nakabayashi et al. 2012). Furthermore, it is reported that heavier males, which probably are socially dominant, frequently bury their faeces at sites closer to the centre of their home range (Ishida and Shimizu 1998). However, the actual odour components of faeces that have the function of scent marking have remained undetermined. The latter relates to the annoyance that free-roaming cats cause to local residents. A survey of attitudes of local residents towards stray cats has clarified that the ‘bad smell of the feces and urine’ is the most annoying thing (Uetake et al. 2014). This becomes a problem in urban areas in particular. In order to consider countermeasures for this problem, basic information or data about faecal odour of domestic cats are necessary.

There are several studies of the chemical compounds of urine from Felidae animals. In the lion (Panthera leo), 55 compounds were found in the urine samples, and 7 of those were identified as species-specific. In addition, a sex difference in higher concentrations of two compounds was found. Specifically, males have a higher concentration of 2-butanone in their urine, and females have a higher concentration of acetone (Andersen and Vulpius 1999). While cauxin is excreted in large amounts in the domestic cat (Felis catus), bobcat (Lynx rufus), and lynx (Lynx lynx), the sulphur-containing volatile compounds 3-mercapto-3-methyl-1-butanol, 3-mercapto-3-methylbutyl-formate, 3-methyl-3-methylthio-1-butanol, and 3-methyl-3-(2-methyl-disulfanyl)-1-butanol were identified as cat-specific odorants and candidates for cauxin-dependent feline nine derivatives from the headspace gas of cat urine (Miyazaki et al. 2008).

In order to provide a basis for considering the above-mentioned scientific significance, we investigated profiles and characteristics of the volatile components of faeces from domestic cats. When we set the study hypotheses, previous studies on urine from Felidae animals were referred to, and we attempted to answer the questions: (1) Are there sex differences in the complexity and concentrations of faecal volatile compounds of the cat? (2) Do the concentrations of faecal volatile compounds change with age?

Materials and methods

Subject animals
One- to 12-year-old domestic short-hair cats were provided by an institute of laboratory animals, the Narita Bio Center, Kitayama Labes Co., Ltd., Japan, as subject animals. The subject animals consisted of 36 entire male cats aged 1–12 (median 4) and 19 entire female cats aged 1–10 (median 4). They were all healthy and had a regular intake of cat foods. Their mean (±SD) body weights were 3.9 ± 0.6 and 3.2 ± 0.5 kg, respectively. Subject cats were kept individually in compartments, and reproduction in any medium, provided the original work is properly cited.
two-level wire cages (width 450 × depth 600 × height 700 mm) indoors. They were reared under the same environmental conditions (light for 12 h from 7.00 and dark for 12 h from 19.00; room temperature 23 ± 2°C). In order to balance the potential effect of diet that correlates with gut microbiota composition (Claesson et al. 2012) and obtain standardized results, they were randomly provided with 1 of 10 commercial cat foods (Table 1) in the morning (9.00–12.00 hours). The amount of cat food provided was based on body weight. They could access water freely. All rearing management and faeces collection were conducted in accordance with approval from the Experimental Animal Welfare Committee of Kitayama Labes Co., Ltd.

Collection of faeces and gas chromatography-mass spectrometry

Faecal samples were collected in the morning (9.00 hours) from each cage. Individual faecal samples were placed in a polypropylene bag and frozen at −80°C until analysis. The faecal samples were conveyed in a frozen state by a parcel-delivery service and analysed at Niigata Environment Hygiene Central Laboratory Co.

The headspace gas chromatograph–mass spectrometer (GC/MS) used was an Agilent model 6890N gas chromatograph coupled to a Tekmar model HT3 headspace auto sampler and a JEOL model JMS-K9 mass spectrometer. 1.5 g of faecal samples were sealed up to a vial and heated at 80°C for 30 min. Using the GC/MS instrument, gas generated by heating was analysed to determine volatile organic compounds in the following measurement conditions:

**Headspace gas sampling**
- Mode: LOOP
- Sample loop size: 1 mL
- Platen/sample temperature: 80°C
- Mixing time: 30 min
- Stabilize time: 5 min
- Valve oven temperature: 160°C
- Loop fill pressure: 5 psi
- Injection time: 0.5 min

**GC/MS analyses**
- Flow rate: 1.0 mL/min
- Carrier gas: Helium
- Transfer line temperature: 160°C

**Table 1.** Nutrient composition of commercial cat foods provided to subject cats.

| Nutrient        | Mean ± SD  | Minimum | Maximum |
|-----------------|------------|---------|---------|
| Water (%)       | 9.3 ± 1.7  | 6.0     | 12.0    |
| Crude protein (%)| 29.4 ± 2.0 | 26.0    | 32.0    |
| Crude fat (%)   | 10.9 ± 3.1 | 8.0     | 18.5    |
| Crude fibre (%) | 3.6 ± 0.8  | 2.5     | 5.0     |
| Ash (%)         | 8.6 ± 0.7  | 7.0     | 9.0     |
| Energy (Kcal/100 g) | 357.8 ± 21.1 | 340.0   | 416.0   |
| Calcium (%)     | 1.0 ± 0.1  | 0.8     | 1.1     |
| Phosphorus (%)  | 0.9 ± 0.2  | 0.7     | 1.1     |
| Magnesium (%)   | 0.1 ± 0.0  | 0.1     | 0.1     |

Notes: They contained chicken or pork or beef meal, chicken or pork or beef powder, corn, wheat or barley or rice, corn gluten meal, chicken oil, canola oil, beet pulp, tuna meal, bonito meal, pork or seafood extract, vegetable powder, egg or milk powder, taurine, methionine, vitamins, and minerals.

Mass spectrometer data were acquired as a total ion current (TIC) chromatogram. Peaks of the TIC chromatogram were defined by their volatility-based retention times, and each peak represented one substance. The volatile compound represented by each peak was identified by searching the NIST Mass Spectral Database (NIST 02) for a compound with a similar mass spectrum. Then the concentration of each volatile compound in the cat faeces sample was determined by using the prepared analytical curve that the relative peak area of the internal standard of each compound was controlled. The minimum limit of determination was 0.1 mg/kg for all compounds.

**Statistical analyses**

All statistical analyses were performed using Stata 13 software (version 3, 2011; OMS Publishing Inc., Tokyo), an add-in to MS Excel. Mann–Whitney’s U test was performed to compare the sex difference in the numbers and concentrations of volatile organic compounds in cat faeces detected by GC/MS. Spearman’s correlation coefficient by rank test (rs) was used to study the relationship between age and the concentration of volatile organic compounds in cat faeces. For significance, a P value of <0.05 was selected.

**Results**

A typical TIC chromatogram is shown in Figure 1. Twenty-four volatile organic compounds were detected in cat faeces by a search of the compound database: 1-propanol, acetic acid, 2-butanone, ethyl acetate, methyl propionate, isovaleraldehyde, 1-butanol, 2-methylbutanal, propionic acid, ethyl propionate, isobutyric acid, butyric acid, ethyl butyrate, isovaleric acid, valeric acid, ethyl valeric acid, caproic acid, propyl valerate, phenol, benzaldehyde, p-cresol, 2-methyl-4-decanone, indole, and skatole. There was no significant difference in the number of compounds detected between males (mode: 23, median: 23, quartile range: 0.6) and females (23, 21, 2.0). Of the 24 compounds, only 1-butanol differed significantly by sex. Males (mode: 15.0, median: 13.0, quartile range: 8.9) had a significantly (P < .05) higher concentration (mg/kg) of 1-butanol than females (11.0, 7.1, 3.1) (Figure 2). On the other hand, no significant difference in the concentrations of six compounds were significantly correlated with age: 1-propanol (rs = 0.409, P < .05), methyl propionate (rs = 0.464, P < .01), propionic acid (rs = 0.443, P < .01), ethyl propionate (rs = 0.333, P < .05), phenol (rs = 0.370, P < .05), and indole (rs = 0.467, P < .01) (Figure 3). On the other hand, no significant correlations with age were found for any compound in the females.
Discussion

While the number of volatile organic compounds in cat faeces detected by GC/MS was not different between males and females, the concentration of 1-butanol differed significantly with males having higher concentration than females. This result suggests that 1-butanol may provide information about the sex of the individual that left the faeces (Gorman and Trowbridge 1989). Higher concentrations of 2-butanone has been obtained in the urine of male lions (Andersen and Vulpius 1999). However, 2-butanone can be synthesized from 1-butanol, therefore the two results are not mutually contradictory. As for complexity of volatile compounds in faeces, our results accord with the results of a prior study on the spotted hyena (Burgener et al. 2009). Burgener et al. also reported that there was no difference in complexity, in terms of the number of different compounds, between male and female anal scent gland secretions.

In the current study, we found that several compounds were related to age in male, but not in female cats. The domestic cat and the spotted hyena have a similar social structure that mainly contains non-dispersing adult females and their offspring (Doi 1997; East and Hofer 2001). The study of hyenas mentioned above (Burgener et al. 2009) also found that female scent profiles do not change with age when dominance status and clan membership are kept constant. In addition, it is reported that the feline faecal microbiome is predominantly determined by 30 weeks of age when diet and environment are controlled for (Deusch et al. 2015). If faecal microbiome does not change, the produced volatile faecal compounds will not change. All cats used in our study were kept individually in the same rearing conditions and ate the same food. Thus, it is thought that results for females obtained in our study agree with the results of these two prior studies (Burgener et al. 2009; Deusch et al. 2015).

On the other hand, the concentration of several volatile compounds increased with age in male cats, even though they lived in the same environment and ate the same food. This means that the age-dependent changes in the concentrations of volatile compounds directly reflect the effect of age and sex.

Figure 1. A total ion chromatogram of GC/MS from cat faeces. Twenty-four volatile organic compounds were detected in cat faeces: peaks A–X were distinguished as 1-propanol, acetic acid, 2-butanone, ethyl acetate, methyl propionate, isovaleraldehyde, 1-butanol, 2-methylbutanal, propionic acid, ethyl propionate, isobutyric acid, butyric acid, ethyl butyrate, isovaleric acid, valeric acid, ethyl valeric acid, caproic acid, propyl valerate, phenol, benzaldehyde, p-cresol, 2-methyl-4-decanone, indole, and skatole, respectively.

Figure 2. Sex difference in concentrations of 1-butanol in cat faeces. Plots represent the median (horizontal lines), 25th and 75th quartiles (boxes), and the minimum and maximum concentrations (Whiskers).
Sexual dimorphism is ‘the difference in appearance (size, shape, coloration), physiology, and behavior between males and females’ (Immelmann and Beer 1989). In cats, the dimorphism is not obvious and only seen in the differences in body size and weight. Cats are polyandrous, so males may remain non-competitive in copulation with females (Crowell-Davis et al. 2004). Even so, it is true that senior dominant male cats sire the highest number of kittens (Natoli et al. 2007). In conjunction with this, it is demonstrated that dominance is related to scent marking with unburied faeces by feral male cats (Ishida and Shimizu 1998). It is also observed that male cats react by spraying urine more often than females (Natoli 1985). While female cats live in a high-density matrilineal colony where life resources including food and safe hiding spots are abundant (Doi 1997), male cats are solitary and need to be territorial to increase mating success. Therefore, the importance of scent marking to male cats is relatively high compared to female cats. Consequently, the sex difference and age correlation found in this study in the concentration of odour components of faeces suggest the potential of a kind of sexual dimorphism that is related to ostentatious display of social dominance in male cats.

In order to obtain generalized results, we adopted a randomized feeding design using 10 commercial cat foods. However, intestinal microbiota composition can be associated with diet (Claesson et al. 2012). Therefore, further investigation using more a variety of diet is necessary for the generalization of these results obtained in this study.

Figure 3. Correlation between age and concentrations of six compounds in cat faeces. rs: Spearman’s rank correlation coefficient.
To summarize the main findings, our results suggest that domestic cats may get biological information about the sex and age of males via the specific volatile organic compounds of faeces left unburied.

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Disclosure statement

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