Department of Physics, Chemistry and Biology

Master Thesis

Behavioral responses of African wild dogs *(Lycaon pictus)* and Dholes *(Cuon alpinus)* to the mammalian blood odor component trans-4,5-epoxy-(E)-2-decenal

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Sammanfattning/Abstract:

So far it is largely unknown whether single volatile components are as efficient for eliciting behavioral responses in animals as the whole complex mixture of a behaviorally relevant odor. Recent studies analysing the composition of volatiles in mammalian blood found the odorant trans-4,5-epoxy-(E)-2-decenal to evoke a typical “metallic, blood-like” odor quality in humans. Wooden logs were impregnated with the blood odor component trans-4,5-epoxy-(E)-2-decenal, or horse blood, or a fruity odor or an odorless solvent and placed randomly into the enclosures of African wild dogs (*Lycaon pictus*) and Dholes (*Cuon alpinus*). The frequency of occurrence as well as the duration of all log-directed behaviors were recorded and compared between odors and species. Both species displayed the highest number of interactions with the odorized logs when presented with the blood component or with the horse blood compared to the other odors. The results from this study indicate that the blood component trans-4,5-epoxy-(E)-2-decenal was as interesting as the whole complex mixture of real blood odor and may also be associated with prey or food by the two canine species. Each of the three odorous stimuli used in the present study elicited a significantly higher number of log-directed behaviors compared to the odorless stimulus in both species. This indicates that the use of odors can be an efficient way of enriching the captive environment of these two species.

Nyckelord/Keyword:
Lycaon pictus, Cuon alpinus, blood odor, trans-4,5-epoxy-(E)-2-decenal, olfactory enrichment

Content

1 Abstract

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2 Introduction

A wide range of bodily fluids is used in chemical communication between mammals. The chemicals in secreted bodily fluids may convey information such as species identity, sexual identity, individual identity, reproductive state and social status about the odor donor (Ralls 1971, Eisenberg & Kleiman 1972, Wyatt 2003). Although their purpose might be to communicate with conspecifics, the same chemical cues can be used by hunting predators or by prey species trying to avoid predation (Conover 2007).

The odor of all bodily fluids is comprised of a complex mixture of volatile components (Wyatt 2003). However, the components of the odor that elicit a behavioral response in the recipient might constitute a minority of the total chemical mixture of the odor (Apps *et al*. 2013). Ferrero *et al*. (2011) reported that it was the presence of the component 2-phenylethylamine, found in the urine of bobcats (*Lynx rufus*) and lions (*Panthera leo*), which elicited avoidance behavior in rodents. So far only a few examples are known in which single components are sufficient for eliciting the same behavioral responses as the whole complex mixture of an odor does. Such knowledge however, would allow for the production and use of synthetic chemicals as effective predator chemoattractants and prey repellents, for example along plantations, protected areas and highways (Wyatt 2003, Apps *et al*. 2013).

The odor of blood has been found to act as a warning signal to conspecifics in prey species, eliciting antipredator behavior such as increased vigilance and avoidance of areas marked by that odor (Eisenberg & Kleiman 1972, Cocke & Thiessen 1986, Wyatt 2003, Sherbrooke & Middendorf 2004, Lonnstedt & McCormick 2012). The odor of blood released from an injured prey might also be used by predator species to detect and locate prey. There is not much known about the volatiles that comprise the odor of blood in mammals or about the components of blood odor that elicit behavioral responses in predators and/or prey species. Recent studies identified the odorant trans-4,5-epoxy-(E)-2-decenal as a volatile component in mammalian blood and in meat. Interestingly, humans have a low detection threshold of 0.078 ppt – 0.33 ppt (parts per trillion) for this particular odorant and it has been described to evoke a typical "metallic, blood-like" odor quality in humans (Konopka & Grosch 1991, Buettner & Schieberle 2001, Rachamadugu 2012).

It is known that the sense of smell is well developed in the Canidae family and that they rely on their sense of smell for intra- and interspecific communication as well as for detecting and locating prey (Anisko 1976, Estes & Wilson 1991). Trained domestic dogs (*Canis familiaris*) are able to discriminate between the odor of blood from healthy humans and the odor of blood from patients with ovarian carcinoma (Horvath *et al*. 2010, Horvath *et al*. 2013). This indicates that dogs can respond to the odor of certain components in blood and not only to the odor of whole blood.

The Asian wild dog (*Cuon alpinus*), often referred to as the dhole, relies heavily on its sense of smell when hunting in the dense forests of Southeast Asia, tracking its prey by scent until it can pursue it by sight (Cohen 1978, Fox 1984). Dholes, as most canids use their sense of smell also in the context of social communication (Anisko 1976) They have been found to deposit their feces and urine at particular sites throughout the group's range producing latrines that may serve both as intragroup and territorial communication sources (Fox 1984, Karanth & Sunquist 1995, Durbin *et al*. 2004). Research on the use the sense of smell in dholes has been less intense compared to research on other canids (Venkataraman & Johnsingh 2004).

The African wild dog (*Lycaon pictus*), one of Africa's most endangered carnivores (Woodroffe *et al*. 2012) communicates using a wide range of unique vocalizations (Robbins 2000) as well as olfactory cues. They secrete a strong body odor, emanating from its anal glands, that is thought to aid in olfactory tracing by members that have lost visual contact with the rest of the pack during a hunt (Estes & Goddard 1967). They are, like most canids, territorial and deposit scent marks throughout their home range (Parker 2010). Jackson *et al*. (2012) found that by using urine deposited by a non-neighboring pack of African wild dogs they could create artificial territory boundaries keeping another pack of African wild dogs inside protected areas.

Considering that both dholes and African wild dogs strongly rely on their sense of smell in a variety of contexts, it seems reasonable to assume that olfactory stimuli can be an efficient way of enriching the captive environment of the two canine species. The use of odors as enrichment for captive carnivores has shown to be successful in increasing the level of activity in captive felids (*lions* Baker *et al*. 1997, *black-footed cat* Wells & Egli 2004, *cheetahs* Quirke & O'Riordan 2011). The use of odors as enrichment for captive canids has been studied to a lesser extent, with the exception of laboratory and kennel-housed domestic dogs (Graham *et al*. 2008, Clark & King 2008).

It was therefore the aim of the study:

- to assess behavioral responses of two canine species, African wild dogs *(Lycaon pictus)* and dholes *(Cuon alpinus)* to mammalian blood and the mammalian blood odor component trans-4,5-epoxy-(E)-2-decenal
- to compare their behavioral responses to those towards a fruity control odorant and an odorless control
- to compare the behavioral responses between the two canine species and to other carnivore species tested with the same odorants in a previous study
- to assess the suitability of the odor stimuli as environmental enrichment for captive African wild dogs and dholes.

3 Material & methods

3.1 Animals and housing conditions

The study was conducted at Kolmården Wildlife Park, Sweden, where a group of twelve Dholes (*Cuon alpinus*) was tested. The group comprised seven females and five males ranging from 1-8 years of age. All the dholes were offspring to the alpha pair in the group. One month before the study started seven additional dholes were born. These were not included in the study as they were too young to participate. The dholes were housed in an outdoor enclosure (2500m^2) that could be divided into one large and one small compartment for temporary keeping of the dholes during cleaning of one of the compartments. The outdoor enclosure contained huts, trees and rocks but consisted mainly of open grassland with a 0.5 m deep water moat along the visitors' walkway.

A group of twelve African wild dogs (*Lycaon pictus*) was also tested. The group comprised one female and eleven males ranging from 2-9 years of age. Nine out of the eleven males were offspring to the female in the group. The African wild dogs were housed in an outdoor enclosure (5000 m^2) with access to an indoor quarter, which was shut closed during opening hours (dogs outdoor) and cleaning (dogs indoor). The outdoor enclosure consisted of a rocky area with surrounding open grassland areas.

Figure 1. Two of the adult dholes at Kolmården Wildlife Park.

Figure 2. One of the African wild dogs at Kolmården Wildlife Park.

3.2 Odor stimuli

The four odor stimuli used in the present study were:

- Blood from domestic horse (*Equus caballus*) provided by Kolmården Wildlife Park. The blood had been taken directly after the horse was euthanized and immediately deep-frozen. The blood samples remained deep-frozen until immediate use in the present study.
- Trans-4,5-epoxy-(E)-2-decenal (CAS# 134454-31-2). This colorless odorant has been identified as a volatile component in mammalian blood and evokes a typical "metallic, blood-like" odor quality in humans (Rachamadugu 2012). It has also been found to be a potent odorant of many foodstuffs. Konopka and Grosch (1991) as well as Kerler and Grosch (1997) identified it as one of the odorants contributing to the aroma of beef and chicken that has been cooked, refrigerated and then reheated ("warmed-over" flavor). The odor sample used in the present study was obtained from Aromalab (Freising, Germany) as a stock solution of 5mg/ml.
- Isopentyl acetate (CAS# 123-92-2). This colorless odorant has been identified as a volatile component in a variety of fruits such as banana and apple. It has been described to evoke the odor quality of an over-ripe banana or a sweet "apple-like" odor in humans (Jordan *et al*. 2001, Komthong *et al*. 2006).
- Diethyl phthalate (CAS# 84-66-2). This colorless organic solvent is often used in fragrances and cosmetic ingredients (Api 2001) and is near odorless.

3.3 Preparations

A dilution series was prepared with both the blood odor component and the fruity odor. The two odorants were diluted with the near-odorless diethyl phthalate to concentrations that were clearly detectable for humans but not overwhelmingly strong. The blood odor component (which came as a stock solution of 5 mg/ml) was diluted 1:100 and the fruity odor was diluted 1:1000. The horse blood was not diluted.

3.4 Experimental procedure

On the morning of a given experimental day, five wooden logs of 48 x 7 x 4,5 cm were impregnated with 500 microliters of a given odor. Only one out of the four odor stimuli was used during each experimental day. The odor was applied on the two largest surfaces of the logs by using a pipette and a brush to spread it over the entire surface. Plastic gloves and a separate brush were used for each odor and for each of the two species to ensure that the logs would not be contaminated. The wooden logs were marked with an X on their short sides in a color representative of the odor used. Logs impregnated with horse blood were marked with red. Those with trans-4,5-epoxy-(E)-2-decenal (the blood odor component) were marked with black. Those with isopentyl acetate (the fruity odor) were marked with green and those with diethyl phthalate (solvent) were marked with blue. The logs were re-used for the same species and odor if they were still in good condition after an experimental day. Otherwise they were replaced with a new log the next experimental day.

At the start of each experimental day, five freshly odorized wooden logs were thrown randomly into the enclosure of the dholes allowing the animals to play or otherwise interact with the logs and transport them across the enclosure. The entire enclosure of the African wild dogs could not be overviewed. Therefore, the wooden logs were thrown into their enclosure while attached to the fence with a chain of 20 meters length. Hence, the wooden logs could not be transported across the entire enclosure but still allowed the animals to interact with them and transport them as far as the chain reached (Figure 3).

When the animals were allowed access into the enclosure they were observed for three hours in the morning and three hours in the afternoon. The observations took place from 8.00 AM – 4.00 PM. Experiments were only performed on non-rainy days (to prevent the odor samples from being immediately washed away by rain) with at least two days interspersed between consecutive experiments with a given species. If it started to rain the observation was terminated and the results not included in the study. Each of the four odors was presented to each of the two species five times in a pseudo-random order, which gave a total of 20 experimental days per species. The experiments were carried out between late May and the beginning of October 2013.

Figure 3. One of the wooden logs used with the African wild dogs.

To enable comparison of the behavioral responses in the present study with those of a previous Master's thesis, which used the same odorants but two other carnivore species, the same ethogram was used (Table 1).

Continuous sampling was used to record the frequency of occurrence as well as the duration of all behaviors on a check sheet (Altmann 1974).

Table 1. Ethogram of all recorded behavioral responses towards the odor stimuli (Sjöberg 2013)

Functional term	Descriptive term
Sniffing	Investigating site where odor is applied with nose.
Licking	Using tongue to investigate the site where odor is applied.
Biting	Using teeth to investigate the wooden log.
Pawing	Scratching site where odor is applied with paw and/or claws.
Toying	Moving or otherwise manipulating the wooden log.
Flehmen	Curling of upper lip to facilitate transfer of odors to the vomeronasal organ.
Impregnating	Rubbing face or other body parts where odor is applied.
Scent marking	Urinating or defecating on the wooden log where odor is applied.
Orientating	Turning head (ears and eyes) following other interaction with the wooden log.
Vocalizing	Making sounds following other interaction with the wooden log.

3.5 Statistical analysis

A Pearson chi-square goodness of fit test was used to compare the frequency of occurrence of the behavioral responses between odors, morning and afternoon and between species. To compare the duration of the recorded behaviors between odors and species a Mann-Whitney test was used (as assumptions were not met for a normal distribution) when the number of recorded durations was not equal between odors. A Wilcoxon signed rank test was used when the number of recorded durations was equal between odors. All of the statistical analyses were made using the software IBM SPSS Statistics 20 and with a significance level of 0.05 and 95% confidence intervals.

4 Results

4.1 Dholes

4.1.1 Behavioral responses

Across the 20 days of observations, with all four odors considered, the total number of interactions with the wooden logs was 458. These were distributed over seven of the ten behaviors recorded. Sniffing was, by far, the most frequently displayed behavior with all four odor stimuli whilst vocalizing, flehmen and scent marking were not observed at all (Table 2).

Table 2. Summary of the number of interactions with the odorized logs observed with the dholes. The percentage of the total number of interactions the behavioral response accounted for, for each odor stimuli in brackets.

	Sniff.	Lick.	Bit.	Paw.	Toy.	Flehm.	Impreg.	Scent.	Orient.	Vocal.	**Total**
Blood component	110 *(65%)*	3 *(2%)*	28 *(16%)*	3 *(2%)*	23 *(14%)*	0	2 *(1%)*	0	1 *(0.6%)*	0	**170**
Horse blood	75 *(75%)*	17 *(11%)*	51 *(32%)*	2 *(1%)*	13 *(8%)*	0	2 *(1%)*	0	1 *(0.6%)*	0	**161**
Fruity	41 *(54%)*	0	24 *(32%)*	1 *(1%)*	10 *(13%)*	0	0	0	0	0	**76**
Solvent	24 *(47%)*	0	16 *(31%)*	0	11 *(22%)*	0	0	0	0	0	**51**
Total	**250**	**20**	**119**	**6**	**57**	**0**	**4**	**0**	**2**	**0**	**458**

4.1.2 Comparisons between odor stimuli

The dholes displayed a significantly higher number of interactions when presented with the blood component compared to the fruity odor ($p<0.0005$, $\chi^2=35.92$) and the solvent ($p<0.0005$, $\chi^2=64.08$) (Table 2). There was no significant difference in the number of interactions between the blood component and the horse blood ($p=0.621$, $\chi^2=0.245$). When presented with the horse blood, the dholes displayed a significantly higher number of interactions compared to the fruity odor ($p<0.0005$, $\chi^2=30.49$) and the solvent ($p<0.0005$, $\chi^2=57.08$). When comparing the number of interactions between the fruity odor and the solvent there was a slightly significant difference ($p=0.027$, $\chi^2=4.29$).

4.1.3 Morning vs. Afternoon hours

Across the 20 days of observations, with all four odors considered, the total number of interactions with the wooden logs was significantly higher during morning hours compared to afternoon hours ($p<0.0005$, $\chi^2=109.56$) (Table 3). When considering the four odors separately, the dholes also displayed a significantly higher number of interactions during morning hours compared to afternoon hours when presented with the blood component ($p<0.0005$, $\chi^2=37.65$), the horse blood ($p<0.0005$, $\chi^2=76.53$) and the solvent ($p<0.0005$, $\chi^2=43.31$). There was no significant difference in the number of interactions between morning hours and afternoon hours for the fruity odor ($p=0.108$, $\chi^2=2.58$).

Table 3. The number of interactions performed by the dholes for the four different odor stimuli, subdivided into morning and afternoon hours.

	Blood component	Horse blood	Fruity	Solvent	**Total**
Morning	125	136	31	49	**341**
Afternoon	45	25	45	2	**117**
Total	**170**	**161**	**76**	**51**	**458**

Across all sessions with the blood component, the horse blood and the solvent all observed behaviors were displayed more often during morning hours than during afternoon hours. Across all sessions with the blood component and the horse blood six out of the seven behaviors observed were displayed both during morning hours and afternoon hours. During the five sessions with the solvent two out of three observed behaviors were displayed only during morning hours. During the five sessions with the fruity odor three out of four observed behaviors were observed both during morning and afternoon hours (Figure 4a-d).

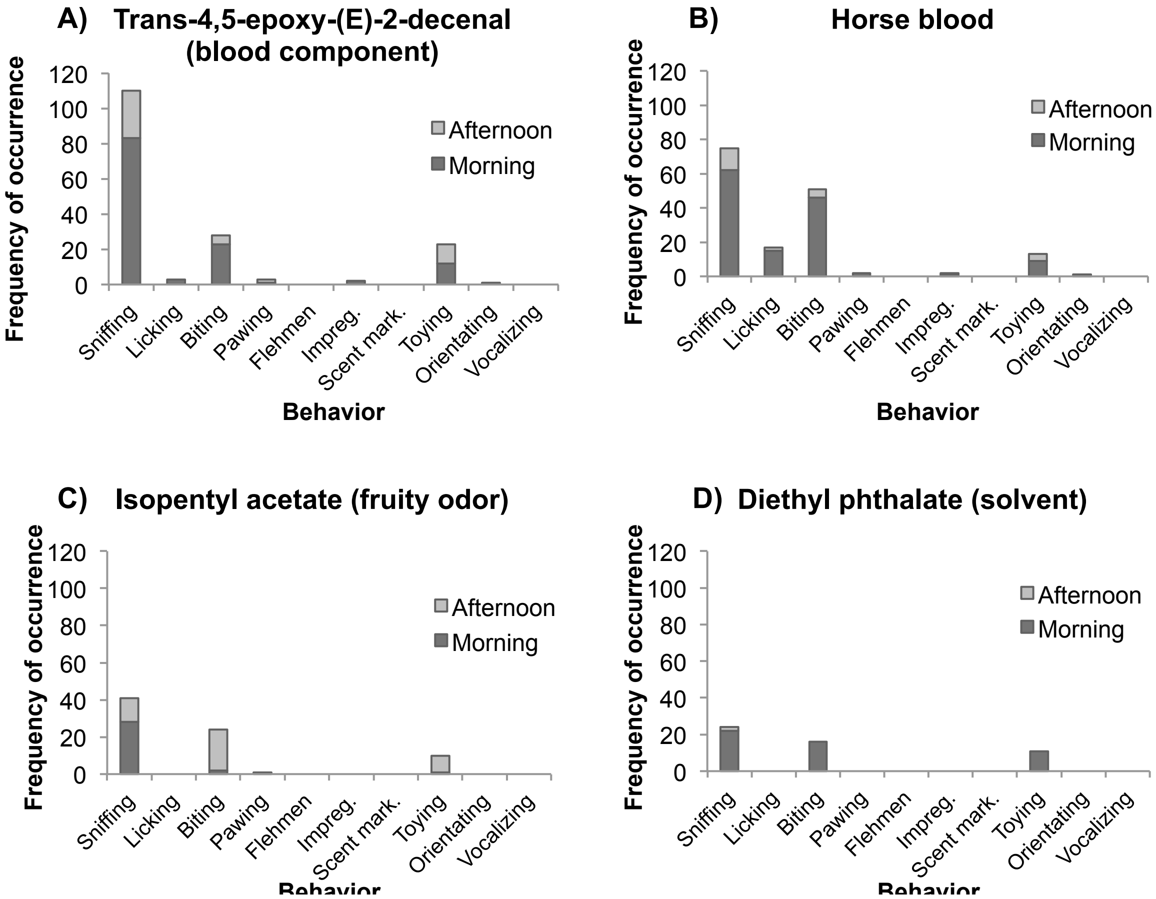

Figure 4. Frequency of occurrence of the behaviors displayed by the dholes across the five sessions with each of the four odor stimuli, subdivided into morning and afternoon hours for **a)** Blood component **b)** Horse blood **c)** Fruity odor and **d)** Solvent. Light grey bars indicate Afternoon hours and dark grey bars indicate Morning hours.

4.1.4 Duration of interactions

The duration of each interaction with the wooden logs was recorded when possible. As it was not possible to record the duration of all interactions, the results are based on the average time per interaction of those interactions for which it was possible to record their duration. The time per interaction is defined as the time (in seconds) that the animal interacted with the log by performing any of the behaviors listed in the ethogram (Table 1) and continued until the interaction ended, regardless if the animal changed behavior during the interaction. Across the five sessions with the blood component the dholes spent on average 3.6 seconds per interaction (mean value from n=22) (Table 7). This was not significantly different from the average duration spent per interaction for the horse blood (p=0.207, U=347), the fruity odor (p=0.983, U=98), or the solvent (p=0.848, U=104), respectively. The average time spent per interaction with the horse blood (mean value from n=26) was not significantly different from the average time spent with the fruity odor (p=0.446, U=96) or the solvent (p=0.540, U=100). Nor was there a significant difference in the average time spent per interaction with the fruity odor (mean value from n=9) and the solvent (mean value from n=9) (p=0.859, z=0.178) (Table 7).

4.1.5 Variability across sessions

The number of interactions and the duration of each interaction varied across the 20 days of observations for the dholes. Across the five sessions with all of the four odors considered the number of interactions decreased only slightly from 100 during the first session to 93 during the fifth session (Figure 5a). Sniffing and biting were the most frequently displayed behaviors across all five sessions for each of the four odor stimuli and accounted for 43%-100% of the total number of interactions during each session.

Trans-4,5-epoxy-(E)-2-decenal, (Blood component)
Across the five sessions with logs impregnated with the blood component the number of interactions varied, with the lowest number of interactions during the second session and the highest number during the fifth session (Figure 5b). The average time per interaction decreased from 8.2 seconds during the first session to 3.4 seconds during the fifth session.

Horse blood
Across the five sessions with logs impregnated with the horse blood the number of interactions varied, with the lowest number of interactions during the third session and the highest number during the second session (Figure 5c). The average time per interaction was not recorded during the first session. During the second session it was 5.3 seconds and then decreased to 2.1 seconds during the fifth session.

Isopentyl acetate, (Fruity odor)
Across the five sessions with logs impregnated with the fruity odor the number of interactions decreased from 24 during the first session to 7 interactions during the fifth session (Figure 5d). During the first session the average time per interaction was 2.2 seconds and increased to 6.9 seconds to the fifth session.

Diethyl phthalate, (Solvent)
Across the five sessions with logs impregnated with the solvent the number of interactions decreased from the first session to the fourth session. During the fifth session the number of interactions increased (Figure 5e). The average time per interaction decreased from 6.5 seconds during the first session to 1.6 seconds during the fifth session.

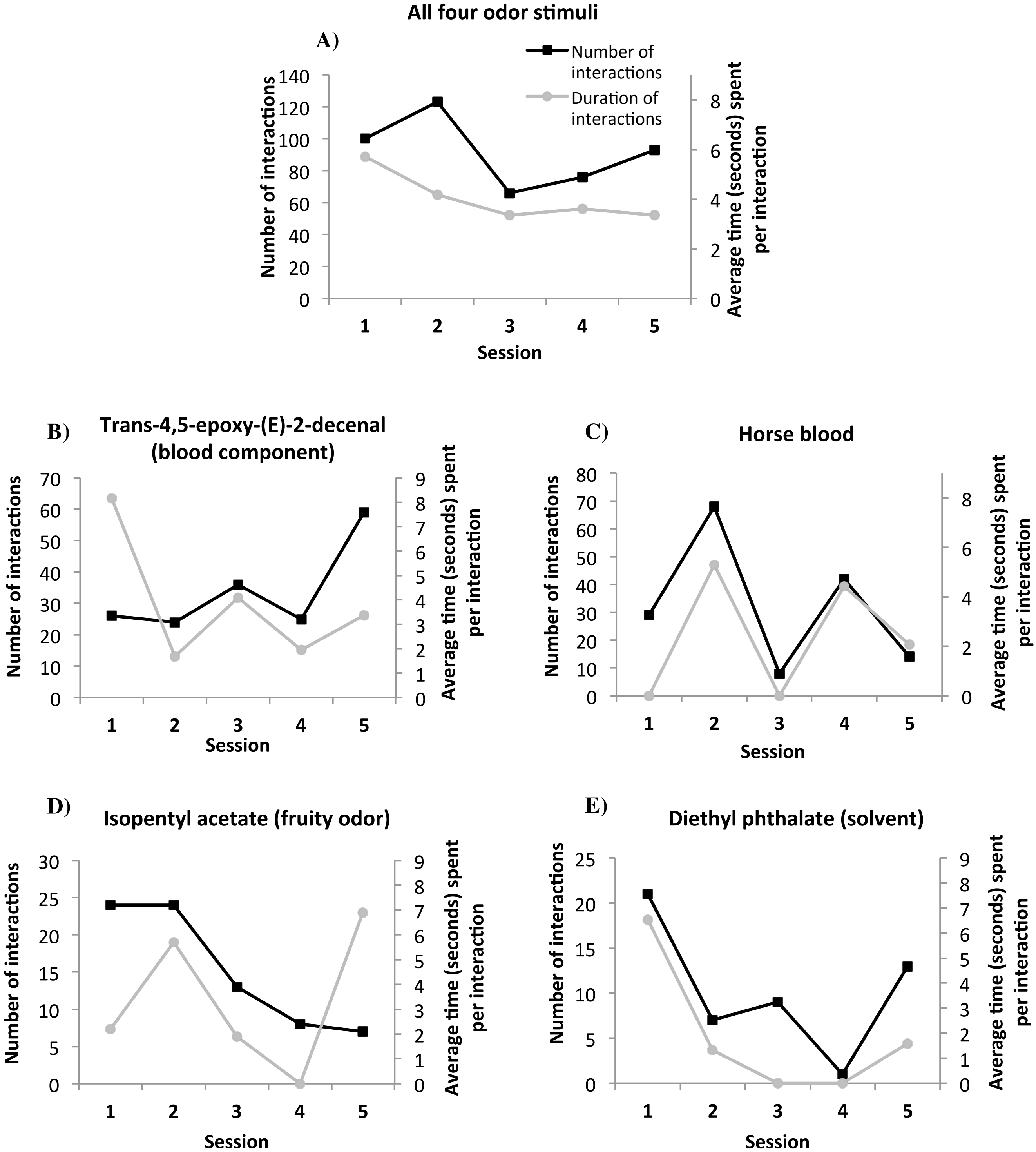

Figure 5. The total number of interactions and the average time spent per interaction displayed by the dholes across the five sessions of observations with **a)** all the four odor stimuli pooled, and logs impregnated with **b)** Blood component **c)** Horse blood **d)** Fruity odor and **e)** Solvent.

4.2 African wild dogs

4.2.1 Behavioral responses

Across the 20 days of observations, with all four odors considered, the total number of interactions with the wooden logs was 647. These were distributed over six of the ten behaviors recorded. Sniffing was, by far, the most frequently displayed behavior with all of the four odor stimuli whilst flehmen, scent marking, orientating and vocalizing were not observed at all (Table 4).

Table 4. Summary of the number of interactions with the odorized logs observed with the African wild dogs. The percentage of the total number of interactions the behavioral response accounted for, for each odor stimuli in brackets.

	Sniff.	Lick.	Bit.	Paw.	Toy.	Flehm.	Impreg.	Scent.	Orient.	Vocal.	**Total**
Blood component	173 *(61%)*	47 *(17%)*	44 *(16%)*	1 *(0.4%)*	8 *(3%)*	0	10 *(4%)*	0	0	0	**283**
Horse blood	104 *(73%)*	10 *(7%)*	14 *(10%)*	1 *(0.7%)*	13 *(9%)*	0	0	0	0	0	**142**
Fruity	85 *(67%)*	32 *(25%)*	7 *(6%)*	0	3 *(2%)*	0	0	0	0	0	**127**
Solvent	54 *(57%)*	18 *(19%)*	15 *(16%)*	0	4 *(4%)*	0	4 *(4%)*	0	0	0	**95**
Total	**416**	**107**	**80**	**2**	**28**	**0**	**14**	**0**	**0**	**0**	**647**

4.2.2 Comparisons between odor stimuli

The African wild dogs displayed a significantly higher number of interactions when presented with the blood component compared to the horse blood ($p<0.0005$, $\chi^2=46.78$), the fruity odor ($p<0.0005$, $\chi^2=59.36$) and the solvent ($p<0.0005$, $\chi^2=93.50$). When comparing the horse blood and the solvent, the number of interactions was significantly higher for the horse blood than the solvent ($p=0.002$, $\chi^2=9.32$). There was no significant difference ($p=0.360$, $\chi^2=0.84$) in the number of interactions between the horse blood and the fruity odor. When comparing the number of interactions between the fruity odor and the solvent there was a slightly significant difference ($p=0.032$, $\chi^2=4.61$).

4.2.3 Morning vs. Afternoon hours

When comparing the total number of interactions displayed by the African wild dogs during morning hours and afternoon hours, with all four odors considered, the number of interactions was significantly higher during morning hours ($p=0.013$, $\chi^2=6.13$). The African wild dogs displayed a significantly higher number of interactions during morning hours compared to afternoon hours when presented with the blood component ($p<0.0005$, $\chi^2=14.93$) and the horse blood ($p=0.003$, $\chi^2=9.13$). When presented with the fruity odor, the African wild dogs displayed a significantly higher number of interactions during afternoon hours compared to morning hours ($p=0.006$, $\chi^2=7.57$). There was no significant difference in the number of interactions between morning hours and afternoon hours for the solvent ($p=0.473$, $\chi^2=0.52$) (Table 5).

Table 5. The number of interactions displayed by the African wild dogs for the four different odor stimuli, subdivided into morning and afternoon hours.

	Blood component	Horse blood	Fruity	Solvent	**Total**
Morning	174	89	48	44	**355**
Afternoon	109	53	79	51	**292**
Total	**283**	**142**	**127**	**95**	**647**

Across the 20 days of observations, with all four odors considered, six out of six observed behaviors were displayed both during morning and afternoon hours. All six behaviors were also displayed more often during morning hours than afternoon hours (Figure 6a-d). During the five sessions with logs impregnated with the blood component four out of six behaviors were displayed more often during morning hours than afternoon hours. During the five sessions with horse blood five out of five observed behaviors were displayed more often during morning hours than afternoon hours. During the five sessions with the fruity odor four out of four observed behaviors were displayed more often during afternoon hours than morning hours. During the five sessions with the solvent three out of the five observed behaviors were displayed more often during afternoon hours than morning hours.

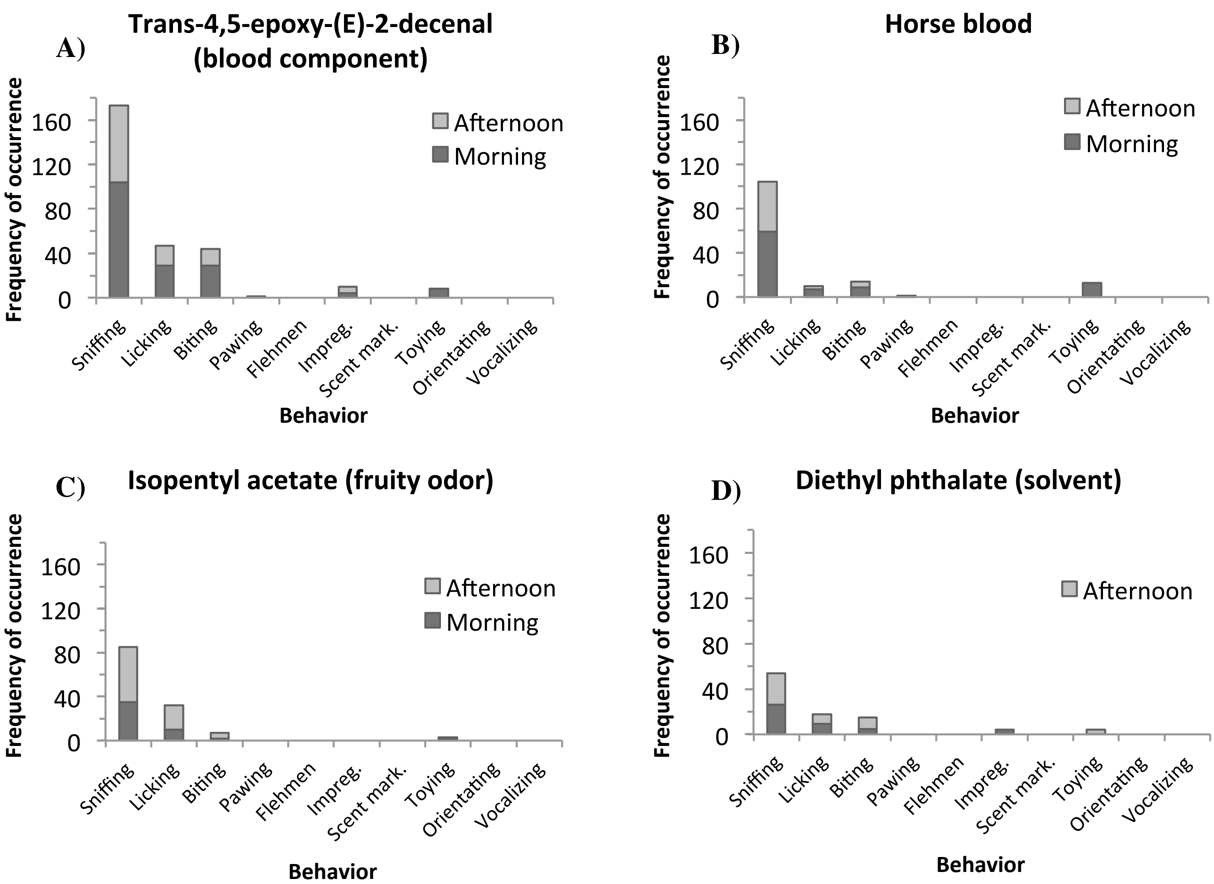

Figure 6. Frequency of occurrence of the behaviors displayed by the African wild dogs across the five sessions with each of the four odor stimuli, subdivided into morning and afternoon hours for **a)** Blood component **b)** Horse blood **c)** Fruity odor and **d)** Solvent. Light grey bars indicate Afternoon hours and dark grey bars indicate Morning hours.

4.2.4 Duration of interactions

The African wild dogs spent on average 3.8 seconds per interaction (Table 7). There was no significant difference in the average time spent per interaction between the blood component and the horse blood (p=0.922, U=251). The African wild dogs spent a significantly longer time per interaction when presented with the blood component (p=0.008, U=224) and the horse blood (p=0.036, U=116) compared to the fruity odor. There were no significant differences in average time spent per interaction between the solvent and the blood component (p=0.673, U=328) nor the horse blood (p=0.965, U=178). There was no significant difference in average time spent per interaction between the fruity odor and the solvent (p=0.104, U=338).

4.2.5 Variability across sessions

Across the five sessions with all of the four odors considered the number of interactions decreased from 263 during the first session to 78 during the fifth session (Figure 7a). Sniffing was the most frequently displayed behavior across all five sessions for each of the four odors and accounted for 40%-100% of the total number of interactions during each session. The second most frequent behavior varied between odors and sessions but was either licking or biting.

Trans-4,5-epoxy-(E)-2-decenal, (Blood component)
During the first session with logs impregnated with the blood component the total number of interactions was 146, it then decreased during the remaining sessions. The average time per interaction decreased from 9.0 seconds during the second session to 2.6 seconds during the fifth session with the blood component (Figure 7b).

Horse blood
Across the first four sessions with the horse blood the number of interactions increased from 16 during the first session to 53 during the fourth session. During the fifth session the number of interactions decreased to 26. The average time per interaction was not recorded during the first session (Figure 7c).

Isopentyl acetate, (Fruity odor)
Across the two first sessions with the fruity odor the number of interactions remained similar and then decreased to only nine during the fifth session. The average time per interaction decreased from 4.0 seconds during the second session to 1.6 seconds during the fifth session (Figure 7d).

Diethyl phthalate, (Solvent)
Across the five sessions with the solvent the number of interactions decreased from 52 interactions during the first session to 7 interactions the fifth session. The average time per interaction decreased from 8.2 during the first session to 3.4 seconds during the fifth session (Figure 7e).

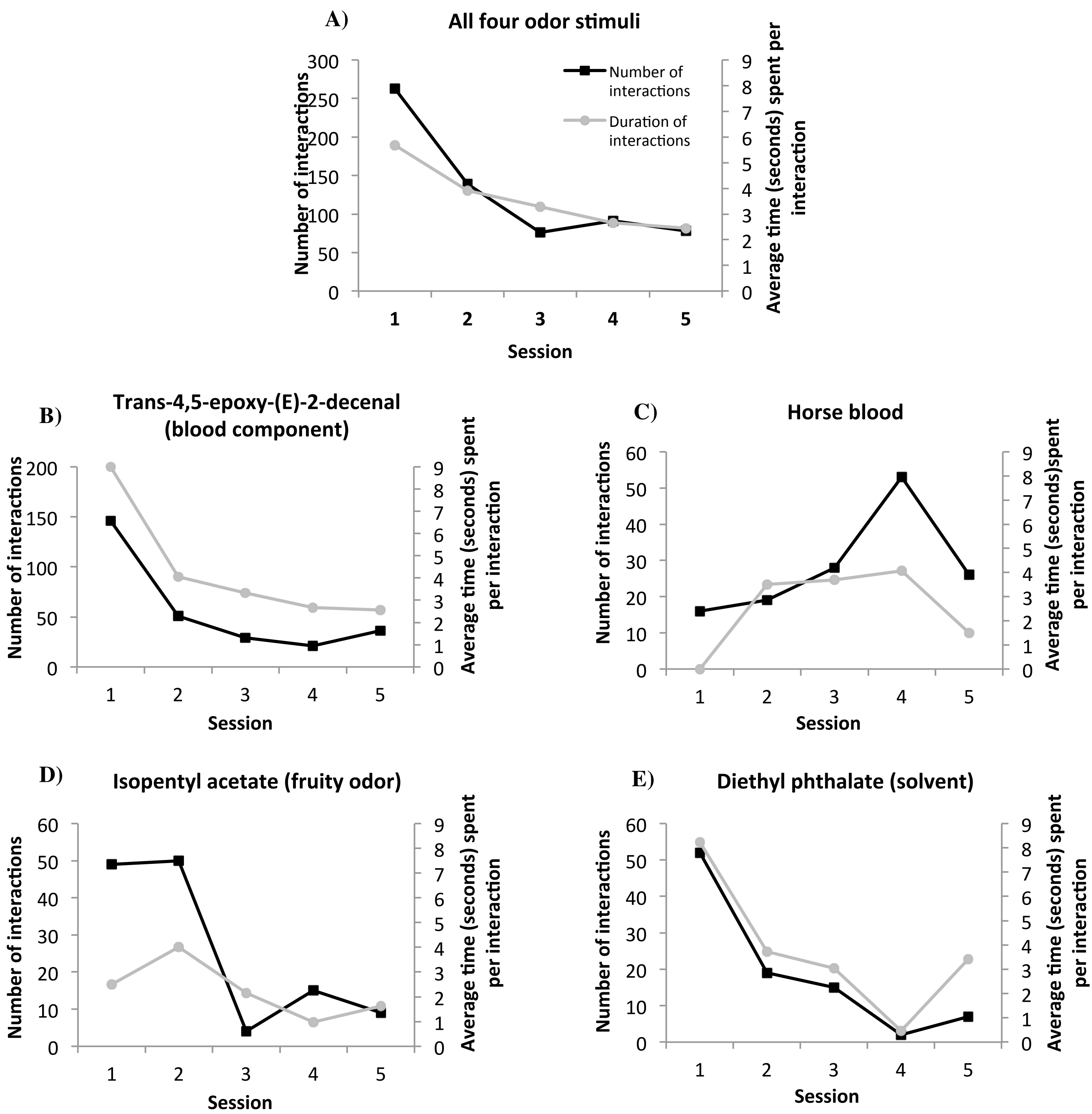

Figure 7 The total number of interactions and the average time spent per interaction displayed by the African wild dogs across the five sessions of observations with **a)** all the four odor stimuli pooled, and logs impregnated with **b)** Blood component **c)** Horse blood **d)** Fruity odor and **e)** Solvent.

4.3 Comparisons between the two canine species

The twelve African wild dogs displayed a total of 647 interactions across the 20 days of observations, distributed over six out of the ten behaviors recorded. This was a significantly higher number of interactions compared to the twelve dholes (458) ($p<0.0005$, $\chi^2=32.33$)(Table 6). The dholes' interactions were distributed over seven out of the ten behaviors recorded. For both species sniffing was the most frequently displayed behavior and accounted for 55% of the dholes and 64% of the African wild dogs total number of interactions, respectively. The African wild dogs displayed a significantly higher number of interactions when presented with the blood component compared to the dholes ($p<0.0005$, $\chi^2=28.19$). There was no significant difference between dholes and African wild dogs when comparing the number of interactions displayed with the horse blood ($p=0.275$, $\chi^2=1.19$). The African wild dogs displayed a significantly higher number of interactions compared to the dholes for both the fruity odor ($p<0.0005$, $\chi^2=12.81$) and the solvent ($p<0.0005$, $\chi^2=13.26$) (Table 6).

There were no significant differences between the two canine species in average time spent per interaction in total ($p=0.417$, $U=2868$), with the blood component ($p=0.320$ $U=295.5$), the horse blood ($p=0.492$ $U=234.5$), the fruity odor ($p=0.207$ $U=140$) or the solvent ($p=0.983$ $U=98.5$) (Table 7).

Table 6. Summary of the number of interactions displayed by the two species tested in the present study for the four odor stimuli.

	Blood component	Horse blood	Fruity	Solvent	**Total**
Dholes	170	161	76	51	**458**
African wild dogs	283	142	127	95	**647**

Table 7. Summary of the average time (seconds), with standard deviation, spent per interaction for the two species tested in the present study for the four odor stimuli.

	Blood component	Horse blood	Fruity	Solvent	**Total**
Dholes	3.6±2.6	4.7±3.0	3.9±3.1	4.3±3.6	**4.2±2.9**
African wild dogs	4.2±3.0	3.6±1.8	2.7±2.2	4.6±4.3	**3.8±3.0**

5 Discussion

5.1 Main findings

The African wild dogs displayed a significantly higher total number of interactions across the 20 days of observations compared to the dholes. Except for the horse blood, the African wild dogs displayed a significantly higher number of interactions with the logs for all odor stimuli, compared to the dholes. Both of the two species displayed the highest number of interactions when presented with the blood component. The blood component was significantly more interesting, in terms of number of interactions, than the fruity odor and the solvent for both species. The odorless solvent was the stimulus that both species showed the least interest in. There was no significant difference in the number of interactions between the blood component and the horse blood for the dholes. The African wild dogs, however, displayed a significantly higher number of interactions with the blood component compared to the horse blood.

The average time spent per interaction did not differ between the two species. The only difference in duration between the four odor stimuli was displayed by the African wild dogs that spent a significantly longer time interacting with logs odorized with the blood component and the horse blood compared to logs odorized with the fruity odor.

5.2 Dholes

Sniffing was, by far, the most frequently displayed behavior by the dholes across the five sessions with the four odor stimuli. This might indicate that the sense of smell is one of the most important senses for dholes when investigating their environment. The behavior biting was the second most frequently displayed behavior, indicating that the senses of taste and touch are also important parts of investigating novel objects and odors for dholes. The proportions and types of behavioral responses displayed by the dholes differed between the four different odor stimuli used. The dholes showed a higher variability in the types of behavioral responses displayed with the blood component and the horse blood compared to the fruity odor and the solvent. Only three behaviors, sniffing, biting and toying, were observed when presenting the odorless solvent (Figure 4d). The behaviors licking, impregnating and orientating were only observed when logs odorized with the blood component or horse blood was presented (Figure 4a-b). This might indicate that the dholes investigate novel objects both by their senses of smell and touch, as both biting and toying involved manipulation of the log with paws or mouth. When further investigating an interesting odor, they might also use their sense of taste, in the form of licking. The behavior impregnating, also called rolling, has been described by Drea *et al*. (2002) to be elicited by animal-based odors in the spotted hyena (*Crocuta crocuta*). As this behavior was only observed when presenting the horse blood and the blood component to the dholes, this might indicate that the dholes perceive both the horse blood and the blood component as prey or food-related and do not display this behavior to odors per se. It also indicates that the dholes do not only respond to the odor of whole blood but also to a single component of blood odor.

The relatively high interest, in terms of number of interactions, and the same behavioral responses displayed by the dholes for both the horse blood and the blood component indicates that the dholes may perceive trans-4,5-epoxy-(E)-2-decenal as blood-like, as in the case for humans (Konopka & Grosch 1991).

5.3 African wild dogs

Similar to the dholes, the most frequently displayed behavior by the African wild dogs was sniffing. The second most frequently displayed behavior was licking. The African wild dogs displayed the behavior licking with all of the four odor stimuli (Figure 6a-d). This indicates that the sense of taste, as well as the sense of smell, is one of the most important senses when investigating the environment for the African wild dogs. This is comparable to the results presented by Drea *et al*. (2002), where a group of spotted hyenas investigated both animal-based and plant-derived odors by frequently licking the odor source. The same authors suggested that licking (i.e. tasting) might be part of a generalized series of behaviors for investigating novel objects and odors in some species.

One, of few, studies investigating olfactory enrichment in African wild dogs (Price 2010) reported a significant increase in active behavior, such as sniffing and locomotion, when presenting a blood trail to the enclosure of a group of 14 captive African wild dogs. A relatively high number of interactions with logs odorized with horse blood were also expected in the present study. The African wild dogs were significantly more interested, in terms of number of interactions, in the horse blood compared to the solvent. However, the total number of interactions for the horse blood was surprisingly low, compared to the much higher number of interactions for the blood component. The caretakers at Kolmården do on occasion use blood trails as enrichment for the African wild dogs (Cecilia Håkansson, personal communication) and this might have removed some of the novelty of the horse blood odor used in the present study (Clark & King 2008).

In comparison to the dholes, the types of behavioral responses were similar for the African wild dogs with the four different odor stimuli used (Figure 6a-d). As such it cannot be stated that the African wild dogs for certain perceive the blood component as prey-related. They did however spend a significantly longer time interacting with logs odorized with horse blood and the blood component compared to logs odorized with the fruity odor. They also displayed a significantly higher total number of interactions for the blood component compared to all other odors. This indicates that African wild dog may find trans-4,5-epoxy-(E)-2-decenal more interesting than the whole complex mixture of real blood and that it should be further investigated whether it is perceived as prey or food-related or if the interest is due to the novelty of the odor.

5.4 Comparisons between the two canine species and previous findings

A previous Master's thesis (Sjöberg 2013) assessed the behavioral responses of Siberian tigers (*Panthera tigris altaica*) and of South American bush dogs (*Speothos venaticus*) towards the same odorants used in the present study. The groups of African wild dogs and dholes used in the present study were made up of twelve individuals, respectively. The group of bush dogs was made up of ten individuals and the group of Siberian tigers was made up of six individuals. As such, the average number of interactions per individual animal was calculated to enable comparison between the four species (Table 8).

Table 8. The number of interactions displayed per individual animal of the two species tested in the present study and the two species from a previous study for the four different odor stimuli. (* Data from Sjöberg 2013)

	Blood component	Horse blood	Fruity	Solvent	**Total**
Dholes	14.17	13.42	6.33	4.25	**38.17**
African wild dogs	23.58	11.83	10.58	7.92	**53.92**
Tigers*	27.16	27.66	5.66	4.83	**65.30**
Bush dogs*	52.10	54.0	29.70	22.4	**158.20**

The bush dogs used in the study by Sjöberg (2013) displayed a significantly higher number of interactions per animal compared to both the dholes and the African wild dogs used in the present study with all four odors combined as well as when each odor was compared separately (Table 9). This indicates that the bush dogs might be more interested in novel objects and odors in general, than the dholes and the African wild dogs. The three species displayed the highest number of interactions when presented with the blood component (dholes and African wild dogs) and with the horse blood (bush dogs) compared to the other odors. There were no significant differences in number of interactions displayed between the blood component and the horse blood for the dholes or the bush dogs (Sjöberg 2013). This indicates that the bush dogs, similar to the dholes, might perceive trans-4,5-epoxy-(E)-2-decenal as blood-like.

Table 9. Results of the Chi-square analysis of the number of interactions per animal between the two species tested in the present study and two species from a previous study (Sjöberg 2013). Values highlighted in grey show a significant difference ($p<0.05$).

	Dholes					African wild dogs				
p-value (χ^2)	Blood comp.	Horse blood	Fruity	Solvent	**Total**	Blood comp.	Horse blood	Fruity	Solvent	**Total**
Bush dogs	**<0.05** (21.88)	**<0.05** (25.09)	**<0.05** (16.0)	**<0.05** (12.46)	**<0.05** (73.47)	**<0.05** (10.32)	**<0.05** (26.73)	**<0.05** (8.81)	**<0.05** (6.53)	**<0.05** (51.02)
Tigers	**<0.05** (4.12)	**<0.05** (5.49)	1.000 (0.00)	0.739 (0.111)	**<0.05** (7.08)	0.674 (0.18)	**<0.05** (6.40)	0.225 (1.471)	0.405 (0.692)	0.313 (1.017)

The Siberian tiger used in the study by Sjöberg (2013) displayed a significantly higher number of interactions per animal with all four odor stimuli combined as well as when presented with only the blood component compared to the dholes (Table 9). The tigers also displayed a significantly higher number of interactions per animal than both the dholes and the African wild dogs when presented with the horse blood. The tigers and the African wild dogs were not significantly different in the number of interactions per animal displayed for the blood component, the fruity odor or the solvent. This indicates a similar relatively high interest in investigating novel objects and odors by the tigers and the African wild dogs.

All of the three species displayed the highest number of interactions per animal when presented with the blood component (dholes and African wild dogs) or with the horse blood (tigers) compared to the other odors.

There were no significant differences in the number of interactions per animal displayed between the blood component and the horse blood for the dholes or the tigers (Sjöberg 2013). This indicates that the tigers, similar to the dholes, might perceive trans-4,5-epoxy-(E)-2-decenal as blood-like.

5.5 Suitability of odors as enrichment for captive canines

Environmental enrichment is defined as an animal husbandry principle that aims to increase species-specific behaviors, increase explorative and interactive behaviors with the environment and reduce abnormal behaviors of a captive species (Sheperdson 1998). Olfactory enrichment is defined as the addition of scents or scented material to the enclosure of a species (Swasigood & Sheperdson 2005). There are relatively few published papers investigating the use of odors as enrichment for captive canids compared to studies made on captive felids (Clark & King 2008). However, it seems reasonable to assume that introducing odors can be an efficient way of enriching the captive environment of canine species as the sense of smell is well developed in the Canidae (Anisko 1976, Estes & Wilson 1991).

The most frequently observed log-directed behavior in the present study was, by far, sniffing for both the dholes and the African wild dogs. This indicates that both species strongly rely on their sense of smell when investigating novel objects and odors. The presence of an odorous stimulus (blood component, horse blood or fruity odor) elicited a significantly higher number of log-directed behaviors compared to the presentation of an odorless stimulus (solvent) in both species. Presenting odors and novel objects might therefore be an efficient way of increasing the level of explorative and interactive behaviors of these two species, and therefore enriching their environment.

Across the five sessions, with all of the four odor stimuli considered, the dholes did not seem to loose their interest, as the total number of interactions only decreased slightly from 100 during the first session to 93 during the last session (Figure 5a). The interest for the blood component, in terms of number of interactions, increased across the five sessions (Figure 5b). This indicates that this particular odor stimulus can be an efficient way of enriching the environment of this species. The dholes spent on average 3.6 seconds with logs odorized with the blood component (Table 7). By varying the way that the blood component is presented to the dholes it might be possible to increase the amount of time that the dholes spend scent exploring the odor as well as their surroundings.

With all of the four odor stimuli considered the African wild dogs did, in comparison to the dholes, seem to loose their interest in the odor stimuli across the five sessions (Figure 7a). They seemed to loose their interest in both the blood component and the fruity odor quickly after the first two sessions (Figure 7b and d). The effects of olfactory enrichment can decrease quickly over time (Wells & Egli 2004). To prevent habituation to an odor, the odor stimuli used in the present study were presented in a semi-randomized order and with at least two days interspersed between consecutive experiments with a given species. However, in a review by King and Clark (2008) it was suggested that a sufficient interval length between odor stimuli used should be approximately three weeks to prevent habituation. Such a long interval was however not possible in the present study due to time restriction.

A previous study by Price (2010) found that when presenting the novel object ”an empty boomer ball” to a group of captive African wild dogs it did not attract any interest in the dogs, whilst the presentation of a blood trail and hidden food stimulated both foraging and feeding behaviors. Rafacz and Santymire (2013) found that the presentation of fecal material from potential prey species increased the activity levels as well as the level of social interactions of captive African wild dogs, whilst fecal material from lions did not. The findings from previous enrichments studies (Price 2010, Rafacz & Santymire 2013) and the results from the present study indicate that the use of odors can be an efficient form of enrichment for captive African wild dogs. The type of odor used as well as the time interval between presented enrichments should however be reviewed before providing captive African wild dogs with such enrichments.

5.6 Limitations and further research

As the entire enclosure of the African wild dogs could not be overviewed, the odorized logs were chained to the fence. This limited the possible distribution of the logs across the enclosure. When the caretakers performed their daily tasks in the enclosure next to the African wild dogs they quickly generated the interest of the African wild dogs. At several occasions this resulted in the African wild dogs spending large parts of the sessions in sections of the enclosure with the logs out of sight. During late August, fights started breaking out between African wild dogs resulting in the need to immobilize two dogs to treat severe bite injuries. The group was divided into two smaller groups for a period of time but later on reunited into one group. However, after the reunion the group still seemed nervous and were, during some nights, kept in separate indoor quarters. The observations were paused during the separation of the group, but when resumed, the nervousness of the group could have had an effect on the results of the study.

It is known that different odors elicit different behavioral responses in captive felids but not much is known about the effects of different odors in captive canids. For future research it would be of interest to assess the behavioral responses to a wide range of odors, both prey-related and non prey-related, and their efficiency as enrichment.

The volatiles that comprise the odor of urine and the behavioral responses of several prey species to components of predator urine have been previously studied. It would also be of interest to study the behavioral responses of predator species to volatile components of prey urine as both the odor of urine, as well as the odor of blood, can be used for detecting and locating prey.

5.7 Conclusions

In conclusion, the behavioral responses and the high interest in the horse blood and the blood component displayed by the dholes indicates that they may perceive trans-4,5-epoxy-(E)-2-decenal as blood-like, as is the case for humans (Konopka & Grosch 1991) and may also associate it with prey or food. The African wild dogs displayed a significantly higher total number of interactions with the blood component compared to all other odors. This indicates that African wild dog may find trans-4,5-epoxy-(E)-2-decenal more interesting than the whole complex mixture of real blood.

The results from the present study and a previous master's thesis using the same odor stimuli for two additional carnivores species (Sjöberg 2013) indicate that carnivores may not only respond to the odor of whole blood but also to a single component of blood odor.

Presenting an odorous stimulus (blood component, horse blood and fruity odor) elicited a significantly higher number of log-directed behaviors compared to presenting an odorless stimulus (solvent) in both the dholes and the African wild dogs. This indicates that the use of odors can be an efficient way of enriching the captive environment of these two species.

6 Societal and ethical aspects

The experiments reported here comply with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication no. 86-23, revised 1996) and also with current Swedish laws.

Chemical characterization of the active components of odors may provide researchers with a better understanding of the chemosensory dimension of predator/prey relationship and of intraspecific chemical communication. It may also allow for the production and use of synthetic chemicals as effective predator chemoattractants and prey repellents, for example along plantations, protected areas and highways.

The research field of olfactory enrichment for captive canids is lacking published studies. The present study indicates that it would be worthwhile to further examine the use of olfactory enrichment for canine species.

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