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Effects of odors on behaviors of captive Amur leopards *Panthera pardus orientalis*

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Abstract Captive environments often fail to resemble the wild environment in respects of limited space, unchanging habitat, lack of stimulus and contingency. Common animal welfare problems which occur in captive animals include low behavioral diversity, abnormal behavior and excessive inactivity. Environmental enrichment, as an effective strategy to tackle these problems and promote mental health of captive animals, has been recognized as an important principal for captive animal management. Among all the enrichment techniques, olfactory enrichment is a simple and effective method for improving the well-being of the olfactory sensitive felids. Behavioral problems were observed in six Amur leopards *Panthera pardus orientalis* at Beijing Zoological Garden. These were held in the older type exhibits which have now been rebuilt. These behaviors include stereotypic behavior and excessive inactivity caused by the spatially limited enclosures with low levels of stimuli. To determine the effects of predator, prey, and herb odors as potential enrichment materials for captive leopards, we conducted olfactory enrichment experiments for the leopards and tested the effects of nutmeg *Myristica fragrans*, feces of roe deer *Capreolus capreolus* and urine of Amur tiger *Panthera tigris altaica* to test for an increase in behavioral repertoire and activity. Odors provided in this study were also believed to improve the psychological and physiological health of individuals. To standardize the method of presentation the odors were introduced to the enclosures by rubbing or spraying onto a clean towel. Our results show that the selected three odors effectively increased the behavioral diversity. Ten new behavior types were observed in the nutmeg experiment, eight in the feces of roe deer experiment and six in the tiger urine experiment. Among the three odors, cats responded to nutmeg for the longest duration, followed by tiger urine and feces of roe deer. Leopards showed more play behavior in presence of nutmeg while more investigatory behavior in presences of feces of roe deer and tiger urine. Providing novel odors increased the spatial use of the exhibit and the animal's increased use of the logs, sleeping platforms and bars in the cages. Novel odors also significantly increased the overall activity of the leopards, but the effects were diminished in about three hours [*Current Zoology* 55 (1): 20－27, 2009].

Key words *Panthera pardus orientalis*, Environmental enrichment, Olfactory enrichment, Behavioral diversity, Stereotypic behavior, Animal welfare

Environmental element is one of the three elements of animal behavior, which facilitates normal behavioral development (Jiang, 2004). However, appropriate environmental elements are often neglected in *ex situ* conservation facilities. Animals are not able to display species-specific behaviors without necessary environmental elements (Li et al., 2007). Lack of behavioral diversity may lead to unsuccessful *ex situ* conservation, because behavioral diversity is an integral part of the conservation of endangered species (Lyles and May, 1987). May and Lyles (1987) pointed out the behavioral challenges facing the program of reintroducing tamarin *Leontopithecus rosalia*, such as disorientation, incapability of selecting appropriate food, and avoiding predators. Hellstedt and Kallio (2005) compared behavioral differences between captive-born and wild-caught weasels *Mustela nivalis nivalis*, reporting that captive-born weasels were more visible and less timid in the field than wild-caught individuals, which indicated that individuals living in captivity lacked experience conducive to survival. Salvanes and Braithwaite (2005) reported the behavioral deficits in coastal cod *Gadus morhua* reared in standard, impoverished hatchery environment, such as weak anti-predatory responses. Shepherdson (1994) proposed two factors that are critical for reintroduction of captive animals, the proficiency obtained from earlier experience and the ability to learn new skills and adjust them to variable environment, both of which can be achieved by

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an enriched prerelease environment. It has been suggested that black-footed ferrets *Mustela nigripes* exposed to more complex environments (enriched cages, including encouragement of food-searching behaviors) were more likely to kill their prey than the ferrets kept in an under-stimulated environment (Vargas and Anderson, 1999).

Environmental enrichment is defined as "an animal husbandry principle that seeks to enhance the quality of captive animal care by identifying and providing the environmental stimuli necessary for optimal psychological and physiological well-being" (Shepherdson, 1998). Enrichment can also be defined in terms of "behavioral enrichment". Captive-bred animals can be provided with opportunities to perform species-appropriate behaviors that otherwise "disappear" in a deprived captive environment. Behavioral enrichment through provision of devices which enable an animal to display a natural behavior also attempts to provide opportunities to express species-specific behaviors. By addressing the optimal planning in social behavior, fully enriched captive environments can reinforce an animal's behavior, including: learning, social communication, physical activity, and investigation (Dinse, 2004), while reducing abnormal behavior of mammals caused by lack of stimuli in barren environement (Shyne, 2006). Plastic objects, a burlap sack full of straw, spruce branches, a fruitcicle and a puzzle feeder provided to fourteen giant pandas *Atluropoda melanoleuca*, significantly reduced stereotypic behavior and feeding anticipation, while increasing activity and behavioral diversity (Swaisgood et al., 2001). Jenny and Schmid (2002) reported that stereotypic pacing of two Amur tigers *Panthera tigris altaica* was significantly reduced in the presence of controlled feeding boxes compared with conventional feeding, which supported the hypothesis that frustration of appetitive foraging behavior caused stereotyped pacing of adult tigers. A feeding device with honey in a bottle markedly increases foraging opportunities while reducing inactivity of captive chimpanzees (*Pan troglodytes*; Celli, 2003). Similar positive change in behavior patterns has also been reported in spectacled bear (*Tremarctos ornatus*; Renner and Lussier, 2002), and blue foxes (*Alopex lagopus*; Korhonen and Niemelä, 2000).

Environmental enrichment has been suggested to reduce the chronic stress caused by limitations of captive environment, failure to express appetitive behaviors, and disturbance from other animals and humans. Mice reared in enriched condition were more active in elevated plus maze and open field compared to those reared in a standard environment, and had lower corticosterone levels during the cat odor test (Roy et al., 2001). Boinski et al. (1999) used plasma and fecal cortisol measures, and behavioral measures to evaluate the effectiveness of four levels of toy and foraging enrichment on the brown capuchin *Cebus apaella*. They reported that cortisol concentration of the brown capuchins significantly decreased in enrichment treatments, indicating a positive impact of enrichment on physiological responses to stress and improved psychological well-being. It has been suggested that environmental enrichment may improve an animal's responsiveness to social, physical and psychological stimuli through (1) developmental process, (2) modulation of stress and arousal, and (3) modification of social interactions (Carlstead and Shepherdson, 1994).

Olfactory enrichment has been widely used, and can be achieved by introducing odors and objects with odors scented to animal enclosures. Food, essential oil, herb, spice, animal feces, urine as well as some other animal products and allure scent are all examples of the odors that can be provided to captive animals (Clark and King, 2008). Recent studies have suggested its value in stimulating inherent behaviors and decreasing the excess inactivity. Both activity and exploratory behaviors of snow leopards *Uncia uncia* increased after rotation of enclosure, in which the cats could detect the trace of other individuals (Stelvig, 2002). Introducing the odors of male fruit bats *Pteropus rodricensis* led to the change in female's estrus cycle, while causing an increase of territory marking behavior of males (Stevens et al., 1996). Predator odors may be beneficial to animals as an olfactory enrichment by increasing chemical interaction. Zhang et al. (2008) found that cat *Felis catus* urine increased the mark urine production and male pheromones of male house mice *Mus musculus*. Male mouse urine was also more attractive to females compared to the other two groups of mice that were exposed to rabbit urine and water. Wells and Egli (2004) investigated the value of scents to enrich the life of small captive felids and their study indicated the success of various scents introduced to the cats' environment, all of which increased the cats' activity, reduced sedentary behavior and encouraged exploration.

We observed behavioral problems with the six Amur leopards *Panthera pardus orientalis* at Beijing Zoological Garden. Problems included stereotypic behavior and excessive inactivity caused by spatially limited enclosures with low stimuli. To determine the effects of herb, prey, and predator odors as potential enrichment materials for captive leopards, we conducted olfactory enrichment experiments for the leopards and tested the effects of nutmeg *Myristica fragrans*, feces of roe deer *Capreolus capreolus* and urine of Amur tiger on increasing behavioral repertoire and activity.

1 Materials and methods

1.1 Subjects

This study was conducted at Beijing Zoo during March to May and September to October, 2006. The subjects were six Amur leopards in Beijing Zoo, 1 male and 5 female leopards. #1 (male) and #2 (female) were introduced from North Korea, and the others were the offspring of #1 and #2 born in Beijing Zoo. The leopards' enclosures consisted of two parts, the inner resting enclosure and the outer cage for exhibition. We closed the door that connected the two parts of the enclosure during observation because the leopards could not be observed when they were inside. But the doors for #5 and #6 were broken and the two leopards were invisible sometimes during the study. The enclosures of #1 and #2 were both rectangular with an area of 9.5 m^2. The enclosure of #3 was cylindrical with an area of 14.6 m^2. The area of the enclosure of #4 was 6.5 m^2, square, while the enclosure areas of #5 and #6 were both 15 m^2. All the enclosures were enriched with sleeping boards, rocks, and logs, and sometimes branches. The leopards were fed daily, five times each week. Food included beef and lamp, with vitamins and calcium.

1.2 Treatment

Three odors used in the study were nutmeg, feces of roe deer as a source of prey odor, and the urine of Amur tiger as the predator odor. Nutmeg trees produce two spices, one of them is nutmeg which is the seed kernel inside the fruit. The essential oils extracted from the trees are mainly applied in food and drinks, but also in cologne, soap, and cosmetics (Yang, 2001). Nutmeg has been widely used in zoos as a scent to enrich the captive environment of captive animals. Roe deer are the main prey of Amur leopards, while Amur tigers are the top predator throughout the Amur leopard distribution (Http: //www.amurleopard.org).

1.3 Hypotheses

Hypothesis 1: Nutmeg, roe deer odor, and tiger odor would increase behavioral diversity, but tiger odor would stimulate fewer behaviors.

Hypothesis 2: Captive Amur leopards would show interest in nutmeg and roe deer odor, while avoiding tiger odor.

All three odors were impregnated on a piece of a clean white towel (60 cm × 27 cm, length × width) before introduced to the enclosure. Nutmeg was purchased from a local drug store. Five kernels of nutmeg were smashed and rubbed onto the towels ten times. Fresh roe deer feces and tiger urine were collected 24 hours before they were used and kept at 4℃. Feces were collected randomly at the roe deer enclosure, in which the sex and age of the individuals were unknown. Fifty grams of feces were put in 200 mL water for 24 hours, and 15 mL was collected and sprayed on the towels. Tiger urine was collected from two adult male Amur tigers, 15 mL of which was also sprayed on the towels. Towels were discarded after use. New gloves and plastic bags were used every day by the experimenter to avoid possible contamination of the cloth.

We provided scented material for four consecutive days each week and had three days without any odors to eradicate the impact of last odor. Nutmeg, prey odor and predatory odor were introduced to the leopards in the following three weeks after a week of control condition. #2 female mated with #1 in the winter of 2005, and was kept in the indoor enclosure, which made observation impossible. Therefore, the nutmeg experiment was carried on #2 after all the other trials were finished.

1.4 Behavior observation and data analysis

We spent one week observing in order to make the cats completely accustomed to the observer, while using *ad libtum* sampling to develop a behavior ethogram (Teng et al., 2003; Table 1). The leopards were accustomed to humans because of long-term exhibition. We chose the observation periods based on: (1) the peak of stereotypic behavior, and (2) the peak of activity, so that individuals could notice the scented towels (Swaisgood et al., 2001). According to the results of pilot study, we determined that 08: 00 – 11: 00 was the observation period. In the first 30 minutes we used videos to record the behaviors, and analyzed the behaviors in laboratory. We described and coded the behaviors with PAE code (Jiang, 2000; Table 2 – 4), and compared the behavioral diversity for different odor treatments. We used two types of software to record behaviors (developed by Hui Zhu and Bengui Xie), including continuous recording of the duration time and frequency of different types of behavior. We recorded the duration time of behaviors that are state, such as stereotypic behavior, resting, moving, and grooming. We only recorded the frequency of event behavior, including territory marking, exploration behavior, and rubbing. Behavior was recorded every 2 minutes in the rest of the 150 minutes to estimate the percent of time spent in each behavior. Before observation, 5 – 10 minutes was spent to acclimate the animals to observers and cameras. We define "responsiveness" as (1) individuals contacted the towels directly; (2) sniffed or extended paws to within 5 cm of the towels. On the second day of nutmeg trial, #5 and #6 were only recorded by video cameras for the first 30 min while the rest of the 150 min was missed because of bad weather.

A repeated measures design ANOVA was conducted on the data using SPSS 13 for windows with "odor" being with-subjects and "subject" being between-subjects. If the assumptions of population normality and homogeneity of variance were not met, Friedman non-parametric tests were conducted.

2 Results

2.1 Behavioral diversity

The Amur leopards explored the scented towels first after they were placed in the enclosures, mainly sniffing and occasionally touching with forelimbs. Biting,

Table 1 Ethogram for Amur leopard in Beijing Zoological Garden

Behavior	Description
Stereotypic behavior	Individual walks or runs at the same location, three or more times Individual stands on hind legs, places paws on fence, and turns head at the same location repeatedly for three or more times; observed in some individuals
Sleep	Eyes closed, no movement
Rest	Individual lays on stomach or side
Sit	Individual sits on the posterior, hind legs tucked under the body
Stand	Individual stands on all four limbs
Walk	Individual moves limbs to make forward motion
Run	Individual moves limbs to make quick forward motion
Leap	Individual leaps from ground to the sleeping board or vice versa
Climb	Individual climbs the enclosure with limbs
Stand Erect	Forelimbs on objects while supporting the whole body with hind legs
Roll	Individual lays on the ground, moves from the dorsal side to the ventral side repeatedly
Exploration behavior	Individual sniffs objects with nose less than 5 cm from the objects
Groom	Lick, bite, or rub certain part of body
Eat	Acquire, cut, chew, moisture, and swallow
Scent-mark	Tail raised, urine or gland excretion sprayed to objects; cheek rubbing; claw
Urinate or defecate	Urinate or defecate
Invisible	Subjects were indoor and therefore invisible

Table 2 Posture code of Amur leopard

Postures	Descriptions	Codes
Standing	Individual stands on all four limbs	1
Sitting	Individual sits on the posterior, hind legs tucked under the body	2
Lying	Individual lays on stomach	3
Lying upward	Individual lays on back	4

Table 3 Act code of Amur leopard

Acts	Descriptions	Codes
Head and neck		
Head up	Head raised up	1
Head down	Head down	2
Shake	Individual shakes head with towel in month	3
Rub	Individual rubs head, neck or cheek on the towels	4
Mouth		
Bite	Individual bites towels	5
Chew	Individual chews towels in month	6
Lick	Individuals licks the towels	7
Flehmen	Upper lip raised, month slightly open	8
Tear	Individual tears the towels with month and paw	9
Hold in mouth	Individual holds the towels in month	10
Nose		
Touch	Individual approaches towels with nose	11
Sniff	Sniff	12
Limbs		
Touch	Individuals touches the towels with forelimbs	13
Tear	Individual tears the towels with month and paw	14
Hold	Individual holds the towels with forelimbs for more than two seconds	15
Paw	Individuals grabs the towels with paw	16
Jump	Individual jumps after sniffing the towels	17
Walk forward	Individual moves limbs to make forward motion	18

Table 4 Environment code of Amur leopard

Odor condition	Codes
Nutmeg	1
Feces of roe deer	2
Tiger urine	3

playing, cheek rubbing, and other behaviors sometimes occurred afterwards. All three odors increased the behavioral diversity of Amur leopards. The results supported hypothesis 1. We observed 10 new behaviors when the cats were exposed to nutmeg, which is the highest among all three odors; 8 when exposed to roe deer feces; and 6 when exposed to tiger urine (Table 5). The cats showed more exploration and playing behavior with nutmeg and roe deer feces. The results supported hypothesis 2.

The frequency of exploration behavior was not significantly different when the cats were exposed to different odors (Friedman, $P = 0.067$, $n = 24$), whereas the frequency of marking was highly variable among odors (Friedman, $P = 0.004$, $n = 24$). Marking behavior increased when nutmeg and roe deer feces were provided, but reduced when the cats were exposed to tiger urine. Our results supported hypothesis 2. Novel scents did not have an effect on the frequency of rolling (Friedman, $P = 0.539$, $n = 24$), nor resting or grooming (Repeated Measures ANOVA, $F_{3,69} = 1.566$, $P = 0.206$; $F_{3,69} = 1.374$, $P = 0.258$, Table 6).

Table 5 PAE codes of Amur leopard in three odor experiments

Behavior	Codes		
	P	A	E
1) Exploration behavior			
Sniff	1, 2, 3	2, 11, 12	1, 2, 3
Flehmen	1, 2	8	1, 2, 3
Touch	1	2, 13	1, 2, 3
2) Locomotive behavior			
Carry	1	1, 10, 18	1
Approach	1	2, 12, 18	1, 2, 3
Jump	1	17	1
3) Other behaviors			
Lick	1, 2, 3	7	1, 2, 3
Bite	1, 2, 3	3, 5, 6, 9, 14	1, 2, 3
Play	1, 2, 3, 4	9, 13, 14, 15, 16	1, 2
Cheek rub	1, 2, 3	4	1, 2

Table 6 Duration and frequency of different behaviors of Amur leopard in four odor experiments

Frequency of behavior (times/30 min)	Control ($n = 24$)	Nutmeg ($n = 24$)	Roe deer feces ($n = 24$)	Tiger urine ($n = 24$)
Exploration behavior	1.04 ± 0.25	3.92 ± 1.0	2.38 ± 0.73	2.92 ± 0.61
** Scent-mark	1.31 ± 0.37	5.50 ± 0.01	2.79 ± 0.73	1.21 ± 0.34
Roll	0.33 ± 0.21	0.79 ± 0.47	0.33 ± 0.25	0.08 ± 0.06
Duration of behavior (s)				
Resting	895.50 ± 120.86	647.88 ± 128.09	723.42 ± 132.95	619.83 ± 125.38
Grooming	88.375 ± 28.74	93.25 ± 34.46	70.417 ± 24.22	87.625 ± 27.13

** Friedman, $P < 0.01$.

2.2 Preference over different odors

The response time of Amur leopards to four odor conditions is significantly different (Friedman, $P < 0.001$, $n = 24$). The leopards showed no response to the blank towels without novel scents, while they showed interest in the three novel scents. To compare the preference of the leopards over different odors, we compared the response time of the cats to the three odors we provided. We found that the leopards responded to the three scents differently (Friedman, $P = 0.019$, $n = 24$). The average response time for nutmeg is 72.13s ± 22.91s, followed by tiger urine, 39.88s ± 15.85s. The shortest response time of the cats was to roe deer feces, which was 15.21s ± 5.32s. The response time decreased with time. On the fourth day of each odor trial, the leopards barely responded to scented towels except those scented with tiger urine (Fig.1).

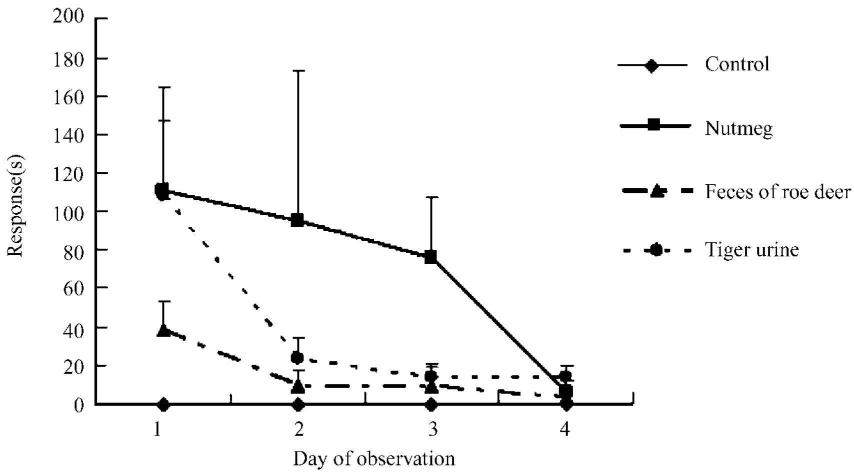

Fig. 1 Response time of Amur leopards to four odors during the experiments (Mean ± *SE*)

3 Discussion

3.1 The effects of olfactory enrichment on behaviors

Exploratory behavior is very important to animals (Mench, 1998). Animals explore the environment to gather information about potential food and resource distribution. Our results indicated that the leopards showed more exploration when novel scents were provided, and the frequency of exploration was the highest with nutmeg, followed by tiger urine and roe deer feces. Individuals tended to display more play behavior with nutmeg, and more exploration or marking behavior with tiger urine and roe deer feces. We suggest that the odors of prey and predator stimulated the behaviors of investigation and marking. Other observations by caretakers in zoos also found that various scents encouraged exploration, social interaction and some aspects of predatory behavior of lions (Powell, 1995; Baker et al., 1997; Schuett and Frase, 2001; Pearson, 2002). Our results indicated that the three novel scents used in this study could be used for enriching the captive environment of Amur leopards, and could be extended to other captive felids.

The leopards showed the most behavioral diversity with nutmeg. The main active ingredients of nutmeg are volatile oils with myristicin and elemicin as the most important chemicals, which can be metabolized to compounds similar to amphetamine with hallucinogenic effects (Pytte and Rygnestad, 1998). Cases have been reported that people use nutmeg as a recreational substance to achieve a euphoric and hallucinogenic state (Demetriades et al., 2005). Nutmeg may exert a similar hyper-stimulation effect on the leopards, which led to change in their behavior pattern. In this study nutmeg also promoted exploratory behavior in the leopards. But it could also be the psychotropic effects of nutmeg on animals, or individuals were simply looking for more nutmeg in the environment. Wells and Egli (2004) also reported the positive effect of nutmeg on the behavior of black-footed cats *Felis nigripes*. The behaviors of exploring the scented cloth and of the enclosure both increased after the cats were exposed to nutmeg compared to controls. However, this does not necessarily indicate that nutmeg is superior to tiger urine and roe deer feces in terms of stimulation, because the alteration of behaviors could be due to chemicals *per se* that affect the central nerves system. Second, an animal's responsiveness to different odors is based on the species' physiology and ecology. For example, tiger urine did not induce as many behaviors as the other two scents, in terms of playing and cheek rubbing, this is perhaps because animals try to avoid predatory odor, which have also been reported in mice (Roy et al., 2001), and rats (Dielenberg and McGregor, 1999).

The frequency of exploration behavior with prey odor was lower than that with tiger urine, which may suggest that predatory odor is more effective in inducing exploration of environment information by the animals in a captive environment in which plenty of food is provided. Although predator odor can cause stress, stressful stimuli provided short term could be beneficial to animals (Moodie and Chamove, 1990; Zhang et al., 2008). Zoo training programs and captive environments should make efforts to provide animals a habitat that is close to wild, including potential stresses (Castro et al., 1998).

Nutmeg increased the marking behavior of the leopards, the frequency of which was higher than other odors. The leopards also showed an increased frequency of marking with prey odor. This may be related to their behavior of marking territory and food in the wild. This study showed that captive felines may still keep this behavior. We expected a decrease of marking with tiger urine, which was supported by our results. #1 and #2 were introduced from North Korea, so we do not know if they had any contact with tigers. But the other four leopards that were born in Beijing Zoo had never been exposed to tigers. We suggest that the decline in marking indicated an avoidance of predators by the leopards, and this is a natural instinct built in evolution because they had never learned about the predators in captive-bred environment. Both environment and genetics affect behavior, and the leopards' response to predator scent were based on both instinct and learning. The avoidance of animals to predators is based on genetics, but learning can reinforce this behavior (Müller-Schwarze, 1995). Therefore, captive-bred animals may still have the potential to exhibit the natural behavior repertoire despite of the deficiencies in their current behavior pattern. We suggest that enriched environment or training that aim to rebuild behavior repertoire could increase the survival capability and benefit the reintroduction programs.

Plants can increase rolling, which could be caused by the psychotropic effects of nutmeg. Rolling can increase activity, which is one goal of environmental enrichment. The frequency of rolling was the highest with nutmeg, markedly higher than the other odors. The frequency of rolling reduced with tiger urine compared to control and roe deer feces. This may be because certain types of behaviors are concealed in the presence of predators. There was no significant difference among the four odors for resting and grooming, but the time of grooming was the longest with nutmeg. We observed that the leopards rubbed their head and neck on the nutmeg scented towel, which increased grooming afterwards.

3.2 Preference over different odors

Captive animals may prefer some odors to the others. For example, lions showed the most attention to the feces of Dorcas gazelle *Gazelle dorcas* and nyala antelope *Tragelaphus angasii*, while ignoring samples of other herbivore species (Baker et al., 1997). Therefore, odors that animals would interact with the most should be chosen for enrichment programs. In this study, the leopards didn't interact with blank towels, but showed interest in the other scents. Nutmeg, feces of roe deer, and tiger urine appreared to attract the cats and increase their behavior diversity. Among the three scents, nutmeg received the most interaction, followed by tiger urine according to the average interaction time.

Olfactory enrichment has been widely used in zoo management. Plants have been proved effective scent source for enrichment purposes, such as spearmint, peppermint, oregano, creeping marjoram, English lavender, catnip, basil, thyme, lemon balm, and rosemary (LeBlanc, 1998). Blount and Taylor (2000) found that cloths sprayed with rose scent combined with manipulable feeders promoted the positive behaviors and space utilization of captive Kinkajous *Potos flavus* while reducing their stereotypic and inactive behaviors. For our study when comparing the effects of the three odors, all individuals showed the most interest in nutmeg, showing the longest length of time spent in direct interaction and exhibiting more playing behavior around this scent cue. Rubbing on the towels and other objects also increased. The behaviors expressed with tiger urine were different, with more exploration, such as sniffing and Flehmen, instead of direct contact with scented towels. The cats usually smelled the towel but rarely touched them. It seemed they tried to avoid the tiger urine scented objects. Swihart (1991) also found bobcat urine reduced the stem gnawing of fruit trees by woodchucks *Marmota monax* by 98.3% compared to untreated trees. Shed snake skins have been used as a novel odor source for a variety of species in zoos, such as meerkats *Suricata suricata* which attached the snake skins, and zebras *Equus grevyi* which avoided them (Tresz et al., 1997). The results showed that captive Amur leopards responded strongly to herb odor while avoiding the predator scent.

The leopards showed the least interaction with roe deer feces in this study according to response time, and an abundance of food in captive environments could be one reason the leopards showed low interest levels in this scent cue. The behavioral responses of the leopards to the odors decreased with time, which suggests that individuals habituate to the scents provided. This is common in environmental and behavioral enrichment practice, which is why enrichment items must be changed periodically. It should be noted that evaluating the effect of odors on animal behavior by direct contact with scented objects could be biased because the effects could only be expressed as behaviors aimed at other objects, such as rubbing logs and marking at bars. This calls for a more comprehensive framework to assess the effects of olfactory enrichment on the psychological health of animals.

3.3 The effects of odors on the use of other enrichment items by Amur leopard

The introduction of odors also increased the use of other enrichment facility in the enclosure by Amur leopards. It was observed that the enrichment objects in the enclosures, logs, branches and rocks were barely used by the leopards before scents were installed. These objects were noted to often limit the movement of the leopards. For example, #2 was observed pacing in a corner of the enclosure while trying to avoid the logs, branches, and rocks. After scents were provided, the leopards used these objects frequently for marking, such as cheek rubbing, scratching, and body rubbing. Therefore, we suggest that zoo managers combine the existing enrichment objects with other enrichment methods, such as olfactory enrichment, to increase the use of enrichment items. Since certain habitat elements are required for animals to express typical behaviors, physical enrichment of the enclosures should be addressed. The goals of environmental enrichment would not be achieved unless we understand the science of animal behavior and the necessary habitat elements.

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