BEHAVIORAL RESPONSIVENESS OF CAPTIVE GIANT PANDAS (*AILUROPODA MELANOLEUCA*) TO SUBSTRATE ODORS FROM CONSPECIFICS OF THE OPPOSITE SEX

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1. INTRODUCTION

Chemical signals are supposed to play major roles for the mammalian social and reproductive behavior. Conover and Gittleman (1989) have classified scent-markings' potential functions into six categories: 1) identity--information regarding individual, group, or sexual identification (Rasa, 1973; Gorman, 1976); 2) status--information on dominance status (MacDonald, 1979; Erlinge et al., 1982); 3) reproductive status--related to courtship and breeding (Gorman, 1980; Gorman and Trowbridge, 1989); 4) spatial information--serves a territorial function (Peters and Mech, 1975; Kruuk, 1978); 5) temporal information--reveals when individuals are at particular locations (Rasa, 1973); and 6) foraging--serves a bookkeeping function by informing an individual of whether it has previously looked for food in a particular area (Henry, 1977; MacDonald, 1979). Indeed, there is a wealth of evidence that the anal gland secretion contains information concerning individual identity, such as gender, age and family (for beavers, Sun and Müller-Schwarze, 1998a,b; for giant pandas, Hagey and MacDonald, 2003; Yuan et al., 2003; for wolves, Raymer et al., 1984; for lions, Andersen and Vulpius, 1999). Secretions of the anogenital gland in the giant panda (*Ailuropoda melanoleuca*) contain information about male pandas' sexual ability and sexual performance (Liu et al., 2003).

The giant panda is a rare animal that inhabits and feeds on dense bamboo forests among isolated mountains in Sichuan, Shananxi and Gansu provinces in China (Hu et al., 1985). Captive giant pandas had a low rate of 30% breeding success before 1991

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(Wolong Nature Reserve and China Conservation and Research Center for the Giant Panda, 1993). One reason is the unsuccessful technique for hand raising the abandoned cub by unsuccessful mother or mother of the twin; another reason is the low rate of successful mating and pregnancy. More than two-thirds of males are poor in sexual performance and about one-third of females do not show typical estrous behavior in captivity (Zhang et al., 1994). Giant pandas are typically solitary and only get together during mating season. Olfaction is believed to play an important role in giant pandas' communication, especially during the mating season. Both male and female pandas recognize individual odors in anogenital gland secretions, and the gender and reproductive conditions have significant effects on discrimination tests (Swaisgood et al., 1999, 2000). Yet little is known about their behavioral responsiveness to substrate odors from conspecifics of the opposite sex, the effects of sexual experiences of females on their behavioral responsiveness, and mating success at the time of being first paired with males. To answer these questions, a behavioral experiment on 11 captive sub-adult and adult giant pandas was conducted during the mating and non-mating season.

2. METHODS

2.1 Subjects and Study Site

We conducted this study at the China Conservation and Research Center at Wolong, Sichuan province, China during the mating season (March – June) and non-mating season (August-November) of 1995, using two males and nine females at least 4.5 years of age. The subjects were adult and sub-adult giant pandas. Table 1 shows the numbers of subjects in our study. They were housed individually in cages. A cage contained a night pen (5.8 x 2.3 m) and an outdoor yard (5.8 x 13 m) with grass, climbing apparatuses, and a small pond as water source. Each outdoor enclosure adjoined two others via a cement wall on which there was a small wire mesh fence door (1 x 1m). Therefore, the subjects could see, smell, hear, and even have some limited physical contact through the mesh fence with neighboring animals of the opposite sex. Based on their reproductive records, all females were divided into sexually experienced and inexperienced groups. Those females who had been paired with males or gave birth in the past were grouped into the experienced group. Otherwise they would be grouped into the sexually inexperienced group. There were two sexual experienced females and seven sexual inexperienced females in the mating season of 1995. Besides, females and males housed as neighbors were thought to be familiar. Otherwise they were thought to be strange to each other.

2.2 Experimental Design, Behavioral Observation, Recording, and Analysis

The subjects were exposed to the opposite sex's substrate odor for 30 minutes. The exposure experiment was repeated twice a week. Methods of focal sampling and continuously recording were used throughout the study. Pandas' behaviors were observed and recorded by mini-tape cassette recorder. Pandas' behavior in their own cages was used as the control. The data were then transferred into computer by OBSERVER 3.0 (Noldus Company).

Table 1. Subjects used in the study.

Observation stages	Sub-adults		Adults	
	Female	Male	Female	Male
Mating season	3		6	2
Non-mating season	3			2

The durations of the following behavioral categories were recorded: scent-marking - rubbing the anogenital area around or up and down on the surface of an object or on the wall; ingesting, handling and eating steamed bread, apples and grass and drinking water and milk; investigating - investigating an object with a distance of 0.1m or longer between the end of nose and the object; sniffing - investigating an object with the distance of less than 0.1 m between the end of nose and the object and with a response of Flehmen; grooming - scratching and licking of the pelage; resting – inactive behaviors including sleeping and non-sleeping; urinating/defecating – urinating in a squat, leg-cock, handstand, or standing posture on the wall or ground and/or defecating at the meantime; locomotion - rapidly pacing and moving around the enclosure without placing feet in the same position each time and following the same path; and playing - rolling and somersaulting with manipulation of objects, such as food dishes, bamboo stalks, tree-branches or toys provided by the keeper.

Data distribution was examined by *One-Sample Kolmogorov-Smirnov test*, and transformed by square root to fit the normal distribution (Sokal and Rohlf, 1995). The behavioral comparisons between the control and experiment were analyzed by Wilcoxon Rank test, and comparisons between sexual experience and odor effects on pandas' behavioral responsiveness were analyzed by two-way ANOVA. Finally, the mating duration of familiar mates and strange mates were analyzed by Mann-Whitney U test. Significance level was 0.05.

3. RESULTS

During the non-mating season, we found both the male and female pandas exposed to the opposite sex substrate odor showed similar behavioral patterns in scent-marking, ingesting, investigating, sniffing behavior, but different patterns in locomotion, grooming, playing, resting and urinating/defecating. Females spend more time playing and resting instead of urinating or defecating in their own cages, while males spend more time in locomotion and grooming. We found no significant effects of substrate odor both on male and female pandas' behavioral responsiveness (Figure 1).

During the mating season, however, the females exposed to males substrate odor spent significantly more time locomotion, scent-marking and sniffing ($P<0.05$, $P<0.01$ and $P<0.05$, respectively), and remarkably less time resting than when they were in their own cages ($P<0.05$). Females also spent less time feeding and drinking when exposed to the male's substrate odor, yet the difference did not reach the statistical level ($P=0.069$). Besides, females also communicated with males more vocally. They showed significantly less chomping (0.07 ± 0.07 vs. 1.28 ± 1.14 times/30 minutes, $P<0.05$), and more bleating

(29.48±12.62 vs. 25.72±14.22 times/30 minutes) and chirping (23.06±7.69 vs. 9.06±3.89 times/30 minutes) which are indicative of courtship, being receptive and invitation to mating. However, the differences of the last two vocal behaviors did not reach statistical level (both $P>0.05$). Males' substrate odor has significant effects on females' behavior. Males, on the other hand, did not show any significant changes in behavior when they were exposed to females' substrate odor (Figure 2).

Females' responsiveness to males' substrate odor was greatly affected by the females' sexual experience (Table 2). Both the sexually experienced and inexperienced females exposed to the male substrate odor showed significantly more locomoting, whereas the sexually inexperienced females spent less time in locomoting and more time

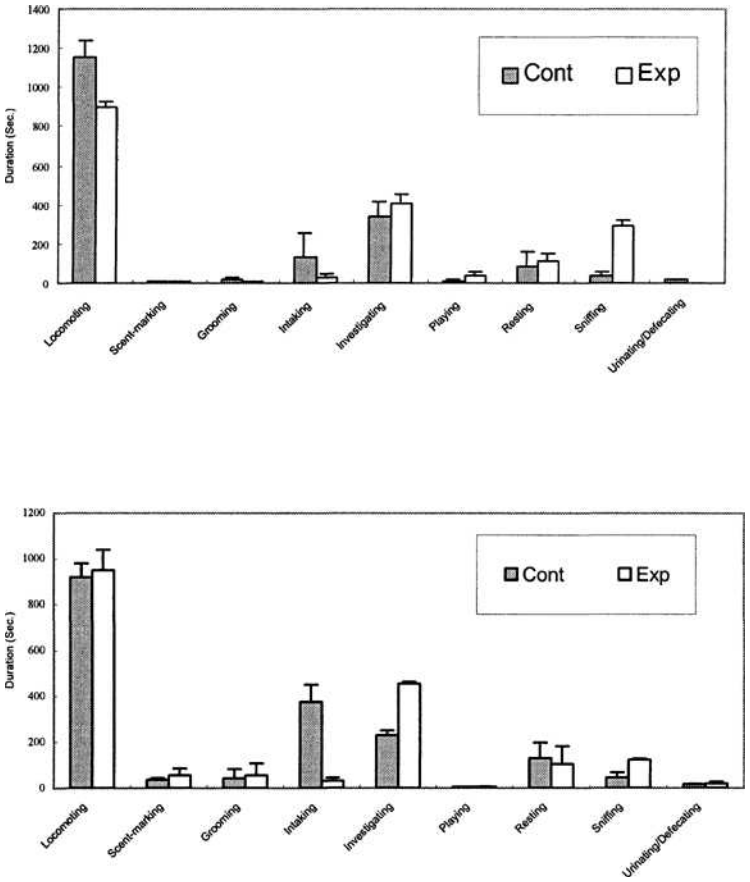

Figure 1. Behavioral responsiveness of female (upper panel) and male (lower panel) pandas to the substrate odor of the opposite sex (exp) and controls (cont) during the non-mating season.

investigating, resting and sniffing than sexually experienced females. Moreover, the sexually inexperienced females also uttered more chirping ($P=0.07$) while the experienced females displayed more chomping ($P<0.05$). We also found an interaction between sexual experience and odor in locomoting, chomping and investigating behavior. These results suggest that females' scent marking during mating season might be mainly to male pandas for advertising their state of estrus, and the happening of chomping behavior might indicate the females' sexual experiences and sexual ability.

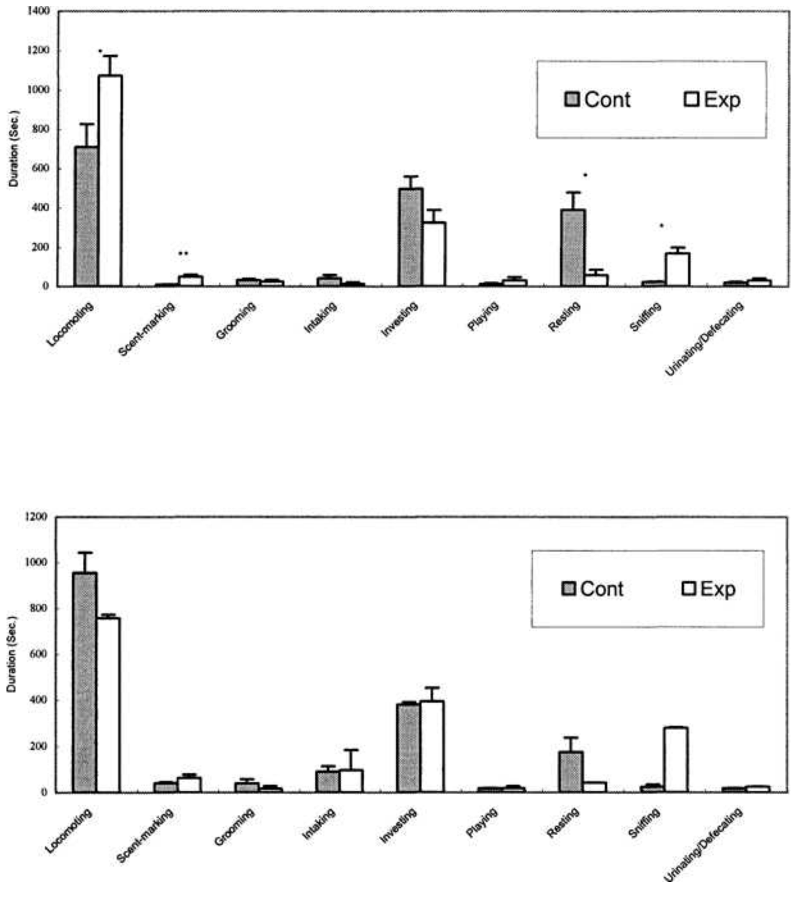

Figure 2. Behavioral responsiveness of female (upper panel) and male (lower panel) pandas to the substrate odor of the opposite sex during the mating season.

There were four females in the mating season that exhibited receptive state and paired with male pandas. We found that only one of the four females could naturally mate with a male at the time when she was paired with a male for the first time in 1995, and the mating lasted 998 seconds after intromission. She gave birth to twins finally in the fall. Surprisingly, we found that the average mating duration after intromission for familiar pairs is 517.00±312.00 seconds (n=2), while the average mating duration for strange pairs is 307.75±112.25 seconds (n=2) ($P>0.05$, Mann-Whitney U test). The percentage of natural mating success for familiar pairs is 60%, while it is 42.85% for strange pairs. The communication between the male and female pandas by neighboring or pre-experiencing before being paired might have important impact on the success of natural mating in captive giant pandas.

Table 2. The effects of sexual experience and odor on behavioral responsiveness of female pandas during the mating season.

Behavioral Measure	Sexually inexperienced (N=7)		Sexually experienced (N=2)		SE[a]	OE[b]	SE-OE
	Cont.	Exp.	Cont.	Exp.	*p*	*p*	*p*
Locomotion	765.45 ± 145.07[c]	953.88 ± 76.07	516.33 ± 12.63	1492.05 ± 110.81	*ns*	**	*
Scent-Mark	9.47 ± 2.56	53.95 ± 12.46	8.39±1.85	31.64 ± 19.69	*ns*	*	*ns*
Bleat[d]	29.24 ± 18.35	35.47 ± 15.58	13.39 ± 3.39	8.50 ± 8.50	*ns*	*ns*	*ns*
Chirp[d]	10.58 ± 4.86	29.57 ± 8.34	3.71 ± 3.71	0.25 ± 0.25	*ns*	*ns*	*ns*
Chomp[d]	0.04 ± 0.02	0	5.62 ± 4.75	0.30 ± 0.30	*	*	*
Groom	26.28 ± 4.69	29.13 ± 11.82	51.15 ± 22.54	4.30 ± 4.30	*ns*	*ns*	*ns*
Intake	41.39 ± 18.45	14.39 ± 12.78	41.87 ± 40.44	1.38 ± 1.38	*ns*	*ns*	*ns*
Investigate	451.69 ± 66.48	378.41 ± 67.97	654.31 ± 129.28	135.94 ± 112.89	*ns*	*	*
Play	15.13 ± 5.96	38.81 ± 19.55	0.02 ± 0.02	1.65 ± 0.41	*ns*	*ns*	*ns*
Rest	388.03 ± 110.35	74.79 ± 33.15	400.67 ± 146.40	0.00 ± 0.00	*ns*	**	*ns*
Sniff	24.82 ± 3.00	193.94 ± 34.55	14.06 ± 4.17	81.35 ± 16.90	*ns*	**	*ns*
Urinate/ Defecate	20.83 ± 6.09	24.08 ± 10.42	17.20 ± 1.80	45.59 ± 46.17	*ns*	*ns*	*ns*

Note: [a] Mean±SE
[b] OE: Odor Exposure Experiment
[c] SE: Sexual Experience
[d] Behavioral categories measured by frequencies (times per 30minutes)
(ns---- not significant, *--- $p<0.05$, **-- $p<0.01$)

4. DISCUSSION

Chemical communication plays major roles in mammalian social life and reproduction (Ralls, 1971). In many species, males can detect the reproductive status of females either by urine or scent-marks, and promote a synchrony of estrus and therefore facilitating a successful mating (American bison, *Bison bison,* see Berger et al., 1992; golden hamster, *Mesocricetus auratus* see Tang-Martinez et al., 1993; house mice, *Mus musticulous,* see Sipos et al., 1995). Giant pandas also use scent-markings to maintain social contacts and territorial demarcation in the wild (Schaller et al., 1985). The males have a complex of four distinct postures to deposit scent-marks all year round, while the females leave scent-marks frequently only during mating season (Kleiman, 1985; Schaller et al., 1985; Liu, 1996). The composition of those scent-marks deposited on the ground was identical to that of anogenital glands, and the scent-marks contain information about gender, age group and individual membership (Hagey and MacDonald, 2003; Liu et al., 2003). The females' scent-marks during the mating season also contain some vaginal secretions (Liu, unpublished data). The substrate odor from captive pandas contains a combination of anogenital gland secretions, urines/vaginal and body odors. Females displayed more locomotion and scent-marking during the mating season and thus, might convey estrous information to their potential mates. More sniffing in females might be related with the discrimination of the conspecific membership, gender and age. Female pandas can discriminate between males individually by males' scent-marks (Swaisgood et al., 1999). In our study, male pandas exposed to the females' substrate odor also performed more sniffing though the differences did not reach statistical level. Moreover, we found no significant differences in any of the behavioral categories of male and females before and after being exposed to the odor of the opposite sex during the non-mating season. These results indicate that both females' reproductive condition and substrate odor have significant effects on their chemosensory responsiveness. The increase in locomotion, scent-marking and sniffing was mainly related to sexual activities and breeding, and females' scent-marking at this time is for sexual advertisement (Hudson and Voldermager, 1992). A similar result was found in the subsequent study of Swaisgood et al. (2000) at the same place and on the same subjects.

Giant pandas also use the visual and auditory channels to communicate in the wild besides chemical communication, and their behavioral patterns differ with different seasons and reproductive status (Schaller et al., 1985). For captive pandas, females exposed to the male's substrate odor also exhibited less chomping, more chirping and bleating although the differences in the later two behavioral categories were not significant. These changes in vocal behaviors showed that females' amicable behavior to substrate odor donors may be an invitation of mating. This is supported by the observation that females show the most frequent chirping and bleating when they are in the receptive state (Liu, 1996).

Unlike other mammals such as tigers and wolves, male giant pandas seldom patrol their territories and demarcate territory by depositing scent-markings. Their social dominance is determined and maintained mainly by scent-markings and occasionally fighting (Hu et al., 1985). The height of odor deposition may be associated with body size, a major determinant of competitive ability (White et al., 2002). We found no significant changes in scent-marking in male subjects exposed to females' substrate odor both in the mating season and non-mating season. One reason for this result is that the males' scent-marking behavior might be directed to other males to show their competitive ability although the females may detect the male's social status and competitive ability by scent-marks. Another reason is the small sample size for males in our experiment. The exposure of sexually inactive male pandas to the females' substrate odor was avoided because of management limits.

The giant panda is not typically sexually dimorphic but we could find many sexual differences both in morphology, ecology and behavioral ecology (Hu et al., 1985). In captivity, male and female pandas exposed to the opposite substrate odor displayed a different behavioral responsiveness in locomotion and play behavior (Figures 1, 2). Those differences might represent sexually dimorphic behavior. Other behavioral differences in chemosenory responsiveness to the substrate odor of the opposite sex may be due to seasonal effects and sexual experience. The different behavioral responsiveness between sexually experienced and inexperienced females indicates that the sexual experience or chemical communication with males may have an effect on successful natural mating. The significant behavioral responsiveness of females with sexual experiences might also be related to the females' mate choice. Both captive males and females were found to prefer certain mates during the mating season (Zhang Hemin, personal communication). A management technique for keeping pandas individually and alternatively by sex was recommended and conducted for promoting the chemical communication at Wolong Breeding Center since 1992. Both our current results and the subsequent breeding success records at Wolong show that the more pandas are allowed to communicate chemically between males and females, the larger the possibility would be for a successful natural mating and breeding in captivity.

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