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# Giant pandas use odor cues to discriminate kin from nonkin

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## Abstract

Sociality is an important factor in both the mechanism and function of kin recognition, yet it is little explored in solitary species. While there may be future opportunities for nepotistic functions of kin discrimination among solitary species, the ability to discriminate kin from nonkin may still have important roles in social regulation. The solitary giant panda *Ailuropoda melanoleuca* offers a good model system to explore kin discrimination in a solitary mammal. As kin discrimination in many other mammals is olfactorily mediated, we investigated whether giant pandas are able to discriminate odor cues from daughters even after months and years of separation. Our results indicate that giant pandas are capable of discriminating between kin and nonkin using odor cues available in urine and body odor. Daughters preferentially investigated the odors of unrelated adult female pandas over the odors of their mothers, and mothers spent more time investigating the odors of unrelated age-matched female pandas over those from their daughters. Because these studies were conducted months or years after the mother–daughter period of dependency ended, it is still unclear what mechanism is used for recognition. Long-term olfactory memories and phenotype matching should both be considered, and further studies are required for such determination.

**Key words:** chemical communication, giant panda, kin discrimination.

Chemical signals are broadly used in regulating social and reproductive behavior across many taxa (Wyatt 2005). One important function of these signals is to mediate kin recognition (Holmes and Sherman 1982), the ability to identify or classify conspecifics based on genetic relatedness (Penn and Frommen 2010). If kin recognition results in kin-biased actions, it can serve several potential functions, including favoritism toward kin (nepotism) as predicted by inclusive fitness models (Hamilton 1964) and inbreeding avoidance. Nepotism takes many forms, including parental care (and avoiding providing parental care to unrelated young), tolerance of conspecific proximity, and avoidance of competition and aggression with kin (Penn and Frommen 2010). Two principal mechanisms that allow animals to recognize (defined as an internal process that facilitates assessment of genetic relatedness) and discriminate (defined as an observable behavior of differential treatment) kin from nonkin are

familiarity and phenotype matching (Hauber and Sherman 2001; Mateo 2003; Penn and Frommen 2010). The familiarity mechanism involves the ability to recognize kin through prior direct association with conspecifics and associative learning of individually distinct cues. Phenotype matching occurs when animals learn their own phenotypes (self-referent phenotype matching or “armpit effect”) or the phenotypes of social companions and match this template to unknown individuals encountered in the future. In addition, nonconspecific contextual cues can also be used if kin are reliably found in a particular location (e.g., a den or nest).

Degree of sociality is hypothesized to govern functions and mechanisms of kin recognition (Sherman et al. 1997; Mateo 2002). Nepotism is often more fully developed in more social species, and thus, kin discrimination mechanisms are predicted to be better expressed. For more solitary species, in contrast, nepotism may not

extend beyond maternal care for dependent young, although kin discrimination may still be necessary to optimize outbreeding during mate choice. However, even among solitary species, closer examination may reveal kin-biased spatial structure raising the possibility of more subtle forms of nepotism, such as tolerance of kin's presence in areas that provide access to resources (Stoen et al. 2005; Manel et al. 2004). The mechanisms contributing to these kin-related patterns of social distribution are largely unstudied, but chemical signals are a primary mode of communication in many solitary mammals and are a key signaling modality in conveying relatedness in other mammalian species (Mateo 2003).

Here we examine, for the first time, the role of scent signals in kin recognition in the giant panda *Ailuropoda melanoleuca*, a solitary species that has no known nepotistic behavior other than care of dependent offspring (Schaller et al. 1985). Chemical signaling is well studied and figures prominently in the regulation of social and reproductive behaviors in this species (review in Swaisgood et al. 2004), but the role scent may play in governing kin-biased behavior has not been evaluated. While solitary, giant pandas' home ranges overlap extensively and pandas use communal areas for scent communication (Schaller et al. 1985; Nie et al. 2012; Zhang et al. 2014). Within these overlapping ranges, however, pandas appear to avoid encounters with conspecifics. It is unknown whether pandas may respond to contact with conspecific differentially based on genetic relatedness. Pandas have female-biased dispersal, occurring several years after independence (Zhang et al. 2006; Zhang et al. 2014), so mother–daughter recognition may be important for mediating social relationships prior to the daughter's dispersal, and may also facilitate the daughter's dispersal outside the mother's range. Thus, kin recognition mechanisms may be favored to support nepotism in the form of maternal tolerance of the daughter's presence in the mother's range and/or to avoid reproductive competition in adulthood. Scent signals are used by a variety of mammalian species for mother–offspring recognition (Leon 1983) although few studies have examined parent–offspring recognition beyond the period of maternal dependency.

As a relatively asocial mammal, theoretical considerations predict less well-developed kin recognition systems as low sociality is associated with fewer opportunities for nepotism (Sherman et al. 1997). However, some empirical evidence diverges from this prediction (Mateo 2002), and more empirical evidence for kin discrimination by olfactory and other cues is needed to understand the evolution of these mechanisms. In light of these considerations, we tested the hypothesis that pandas will discriminate between odor cues of female kin and nonkin.

## Materials and Methodology

### Subjects and study site

The study was conducted during the nonbreeding season in June and July 2004 at the China Conservation and Research Center for the Giant Panda (CCRCGP) at the Wolong Nature Reserve, Sichuan Province, China. Subjects consisted of 4 female cubs under 2 years of age (ranged 10–11 months) and 7 adult female giant pandas 4–8 years old. Cubs were separated from their mothers for >5–6 months prior to the commencement of the study with no further contact from the day of separation. All subjects were housed in indoor–outdoor enclosures of varying sizes. For general husbandry, see Swaisgood et al. (1999). As the management of pandas in the center is dynamic, we took measures to ensure that donor and subject pandas had no close olfactory contact for >30 days: subjects were not

housed in adjacent enclosures and were not housed in an enclosure that had been recently occupied by the scent donor.

### Experimental Protocol

Experimental scent stimuli consisted of body odor and urine collected on fir boards (13 × 10 × 2 cm). Body odor was obtained by rubbing the board on the body of the donor immediately prior (< 1 h) to experimental presentation. Fresh urine was collected from the ground using sterile syringes and applied directly to the board. Unused urine was stored in plastic vials and kept refrigerated at 4 °C until needed (< 24 h). Scent stimuli were collected from 7 adult females and 4 subadult females (cubs). To control for inherent differences in individual attractiveness of particular subjects' odors, the same donor's odors were used as both kin and nonkin stimuli with different subjects. Adult subjects were presented with odors from kin (daughters) and nonkin (unrelated) adult and subadult donors. Including adult daughters as donors addresses kin discrimination abilities among adult age categories. Subadult subjects were tested with odor stimuli from adults only, using kin (mother) and nonkin (unrelated adult females) as donors.

We presented all scent stimuli in simultaneous preference tests using the same odor type (urine or body odor) in each discrimination trial. To address random variation in responsiveness to odors, subjects were included in multiple trials (up to 3) for each discrimination task and results were averaged to yield a single pair of matched values for kin and nonkin for each odor type. Each trial consisted of placing the paired scent stimuli on the ground of the subject's enclosure approximately 30 cm apart. The trial commenced when the animal approached to within 5 cm, and its behavior indicated that the stimulus was detected. The behavioral response was recorded for a minimum of 10 min or until the panda ceased to direct behavior toward the stimulus for >10 consecutive minutes. Behavioral responses included sniff (nose <5 cm from board), flehmen (deep inhalation with upper lip curled), lick (tongue made contact with the board), scent anoint (board rubbed on face, neck, shoulders, other body part), and chew (using teeth to bite board or break board). For each behavior, the stimulus type that the behavior was directed toward was recorded. Observations were video recorded from <2 m away providing for detailed observation of behavior.

### Statistical analysis

Data for all chemoresponse behaviors were summed into a single dependent measure representing the amount of time the subject spent with its behavior directed toward the scent stimulus, which included responses to both urine and body odors combined into a single measure; we labeled this composite measure “time spent investigating.” Due to the small sample size and large effects of statistical outliers, we conducted a visual assessment of the data and removed a single outlier. By eliminating this variance-increasing outlier and log-transforming the data, we were able to ensure that the assumptions of normality (Shapiro–Wilk's test; Sokal and Rohlf 2012) and homoskedasticity of variance (Hartley's  $F_{\max}$  test; Sokal and Rohlf 2012) were met. We fitted a linear mixed-effect model using treatment (kin vs. nonkin) and scent type (urine vs. body odor) as fixed effects, and subject identity and subject age class (adult or subadult) as random effects. We used the Satterthwaite's correction to adjust denominator degrees of freedom alpha was set to 0.05 for all tests, and analyses were conducted using JMP v. 11.0 (SAS Institute Inc., Cary, NC, USA).

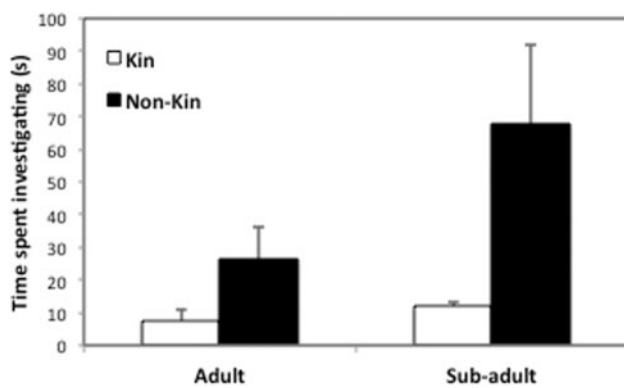
## Results

Adults and subadults (cubs) responded to chemosignals with sniffing, licking, chewing, and rubbing the scent stimulus on the body, especially the head area. Our analysis showed evidence of behavioral discrimination of odors: pandas spent significantly more time engaged in olfactory investigation of odors collected from nonkin than from kin ( $F_{1,14.02} = 14.47$ ,  $P = 0.002$ , Figure 1). Further, pandas did not discriminate odor types on the basis of their origin (scent type:  $F_{1,14.02} = 0.94$ ,  $P = 0.34$ ), indicating that combining data from body and urine for analysis was merited. The interaction between odor type and kin treatment was not significant ( $F_{1,14.02} = 3.07$ ,  $P = 0.10$ ), and the pattern of discrimination between scents from kin versus nonkin was the same for adult and subadult subjects. However, subadults investigated nonkin scent more than twice as long as adults (subadults:  $67.63 \pm 24.54$  sec vs. adult:  $26.44 \pm 9.99$  sec), whereas time spent investigating scent from kin was similar for the 2 age-classes (cubs:  $11.88 \pm 1.33$  sec vs. adult:  $7.56 \pm 3.18$  sec).

## Discussion

Our results indicate that giant pandas are capable of discriminating between kin and nonkin using odor cues available in urine and body odor. Daughters preferentially investigated the odors of unrelated adult female pandas over the odors of their mothers, and mothers spent more time investigating the odors of unrelated age-matched female pandas over those from their daughters. Our study was conducted months or years after the mother–daughter period of dependency ended, and therefore does not represent mother–daughter recognition for the purpose of maternal care. Instead, our findings should be interpreted in the light of relationships among mothers, daughters, and nonkin females outside the period of maternal dependency.

One implication of these results may be that pandas retain long-term olfactory memories of the period of mother–daughter association as our results show a preference for investigating presumably less familiar odors. At Wolong, where the study subjects were kept prior to and during our study, mother–daughter pairs were held together for approximately 6 months following birth (wild pandas remain in a mother–offspring pair for up to 2 years), and thus had ample opportunity to become familiar with one another's odors. However, the paired odor stimulus in most trials (odor from nonkin) could have been somewhat familiar to the subjects given the relatively small area in which pandas are kept at the breeding center and



**Figure 1.** Mean ( $\pm$ standard error) time (in seconds) adult and cub assessors spent engaged in olfactory investigation.

the frequent movement of pandas among pens. Therefore, it might be most accurate to state that pandas preferred to investigate the less familiar odor in discrimination trials. This finding is also consistent with a previous study using a habituation–discrimination paradigm, demonstrating that pandas preferentially investigated the more novel of 2 paired conspecific odor stimuli (Swaisgood et al. 1999). However, in contrast to the present study, familiarity was manipulated over a period of a few days rather than months or years. No study to date with pandas has shown that memory of odor familiarity can be retained for years.

If odor familiarity through direct mother–daughter contact during rearing is the mechanism in operation for discriminating kin from nonkin odors, then memory of these familiar odors is long-lasting. It is also possible that pandas are using the phenotype matching mechanism for recognizing shared odor characteristics governed by genetic relatedness, but further study is required (e.g., father–offspring discrimination) in order to establish such a supposition. Prior association is most often the mechanism governing lasting parent–offspring recognition in species with small litter sizes and where chances for recognition mistakes are limited, as when few nonkin are encountered during the learning phase (Mateo 2003). In the wild, panda mothers rear young in isolated dens far from other females (Schaller et al. 1985; Zhu et al. 2001); thus, there are ample opportunities for prior association to govern parent–offspring recognition which may be the mechanism in operation here although selection for phenotype-matching mechanisms should also be considered.

Whether the ability of pandas to recognize kin is due to phenotype matching or olfactory memory, it suggests that this ability has functional value. Even relatively solitary species often have established relationships with neighboring individuals (Yoerg 1999), and some communities are structured by genetic relatedness, making possible more subtle forms of nepotism among related individuals (Manel et al. 2004; Støen et al. 2005; Moyer et al. 2006). However, in pandas, females are the dispersing sex (Zhan et al. 2007; Zhang et al. 2014) and so the species' spatio-social structure does not support this interpretation: females, on average, will not have the opportunity to display nepotistic behavior toward female kin, except during the period between independence and dispersal. It is possible that mother–offspring recognition promotes greater social tolerance and sharing of resources within the home range, alternatively, as a species with female-biased dispersal, it is plausible that female pandas discriminate kin odors in order to avoid settling in areas with close female relatives. This interpretation is consistent with the hypothesis that female pandas avoid female kin as part of an optimal outbreeding strategy. Because female pandas share overlapping ranges with males with whom they mate (Schaller et al. 1985), the presence of the mother is predictive of the presence of the father, which in pandas provides no paternal care and are therefore unfamiliar to offspring. Lastly, it is possible that kin discrimination by odor cues has no adaptive value in pandas, and is the by-product of selection for other functions. Indeed, kin recognition has been found in other species lacking nepotism and it has been suggested that selection for mate choice cues in the major histocompatibility complex may lead to the evolution of these abilities as a byproduct (Mateo 2002).

Sociality is an important factor in both the mechanism and function of kin recognition, yet kin recognition is little explored in solitary species (Mateo 2003), no doubt in part due to the logistical challenges of obtaining adequate sample sizes for solitary living species. The present study, although limited in scope, contributes to a

better understanding of kin recognition in solitary species, and adds to the growing body of biological knowledge necessary to manage the endangered giant panda in the wild and captivity (Swaigood et al. 2010, 2011).

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