Vocal discrimination of potential mates by female giant pandas (*Ailuropoda melanoleuca*)

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In the current study, we used male giant panda (*Ailuropoda melanoleuca*) bleats in a habituation–discrimination paradigm to determine whether females discriminate between the vocalizations of different males. We found that females habituated to the bleats of a specific male showed a significant dishabituation when they were presented with bleats from a novel male. Further playbacks, in which we standardized the mean fundamental frequency (pitch) and amplitude modulation of male bleats, indicated that amplitude modulation is the key feature that females attend to when discriminating between male callers. Our results show that female giant pandas can discriminate between the vocalizations of potential mates and provide a platform for further studies investigating the functional role of caller identity in giant panda sexual communication.

Keywords: vocal communication; habituation; discrimination; vocal recognition

1. INTRODUCTION

The ability to discriminate between vocalizations from different individuals has been documented in several mammalian species (Hare 1998; Reby *et al.* 2001; Frommolt *et al.* 2003; Blumstein & Daniel 2004) and is postulated to confer several advantages. Gregarious mammals often need to recognize closely related kin (Rendall *et al.* 1996) and mother–offspring recognition can be crucial for young to survive (Charrier *et al.* 2003; Torriani *et al.* 2006). However, vocal recognition may also be important in sexual contexts and in species where individuals only associate with other conspecifics for mating purposes. For instance, over the course of a breeding season, females may become familiar with and prefer the vocalizations of certain males that invest greater vocal effort (McElligott *et al.* 1999). Moreover, this ‘familiarity’ may be a reliable indicator of male quality because it represents both the male's ability to produce high rates of vocalization and their ability to monopolize access to females around their fertile period (Zimmerman & Lerch 1993).

Giant pandas (*Ailuropoda melanoleuca*) are solitary, mono-oestrous mammals that rely heavily on communication to coordinate their annual mating activities (Peters 1982, 1985; Kleiman 1983; Schaller *et al.* 1985). The importance of olfaction in giant panda sexual communication is well documented (for review see Swaisgood 2004); however, a significant increase in vocal activity during the breeding season suggests that vocal signals are also likely to play a crucial role in reproductive contexts. The primary vocalization of the giant panda is a frequency- and amplitude-modulated ‘bleat’ that is thought to signal non-aggressive intent (Peters 1982; Kleiman 1983) and is produced at particularly high rates by males in mating contexts (Peters 1985). These vocalizations could be important for maintaining contact between courting individuals, but may also be used by males to familiarize females to their presence during the breeding season. However, for preferences based on caller familiarity to evolve and be maintained, two requirements must be met: first, male giant pandas must possess vocalizations that are individually distinctive; and second, females need to be able to distinguish between the vocalizations of different males.

Previous work showed that potential features for individual vocal recognition exist in the acoustic structure of giant panda bleats (Charlton *et al.* submitted), so the purpose of this study was to test the hypothesis that female giant pandas can discriminate between the bleats of different males. In addition, because the mean fundamental frequency (perceived as the pitch of the call) and aspects of amplitude modulation are the most highly individualized acoustic features of giant panda bleats (Charlton *et al.* submitted), we wanted to determine the relative importance of these acoustic features for individual discrimination. To this end, we used a habituation–discrimination paradigm (Swaisgood *et al.* 1999; Reby *et al.* 2001; Charlton *et al.* 2007) and hypothesized that after showing a diminishing response (habituating) to a series of bleat exemplars from one male, female giant pandas would show a renewal of response levels (dishabituate) to a bleat exemplar from a novel male. We also predicted that the ability of females to discriminate between male callers would be significantly impaired when resynthesis techniques are used to standardize mean fundamental frequency (F0) and remove amplitude modulation across the habituation–discrimination sequences.

2. MATERIAL AND METHODS

The study was conducted during March and April 2008/2009 at the China Research and Conservation Center for the Giant Panda, China. The subjects for the experiments were non-oestrous adult females (aged 6–19 years, mean = 9.81). To test the ability of female giant pandas to perceive the change in male caller, we used a habituation–discrimination paradigm and single male giant panda bleats of comparable duration from six adult males that were unfamiliar to the current females. To minimize the possibility of dishabituation occurring owing to size-related acoustic differences between exemplars, they were paired up based on their minimum body weight.

The playback sequences consisted of eight single bleats each separated by 20 s. In each sequence, the first six bleats comprising the habituation phase (H1–H6) and the eighth bleat, the rehabilitation stimulus (RH), originated from the same male exemplar. The seventh bleat of the playback sequence, the dishabituation stimulus (DH), originated from a different male from that of the habituation phase. In this way, we aimed to provoke habituation to bleats H1–H6 with discrimination inferred if the level of response significantly increased to DH and then fell back again after RH. The habituation phase comprised six playbacks and to ensure a symmetrical experimental design

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Figure 1. Log transformed means $\pm$ se of female first look duration (s) + 1 to the habituation–discrimination playback sequences for the three experiments: (a) unmodified bleats ($n = 16$); (b) mean FO standardized ($n = 13$); and (c) no amplitude modulation ($n = 13$).

each male was used alternately to habituate and dishabituate the females, giving us six unique sequences from the three male pairs.

We carried out a total of three playback experiments. For the first experiment, we used unmodified bleats to determine whether female giant pandas could discriminate between different male callers. We then conducted two further experiments in which we used resynthesis techniques and modified male bleats to test the importance of mean FO and amplitude modulation for individual discrimination (see electronic supplementary material for details on standardizing mean FO and amplitude modulation removal across bleat sequences). The playbacks were initiated when each female was settled and eating. To quantify the strength of the female's response to each stimuli, we measured the duration of the first look given to the playback source with the head maintained in a fixed position. Before statistical testing, the data were log transformed to normalize the distribution for parametric significance tests. To retain values of zero in the dataset, 1 was added to the data values before log transformation.

3. RESULTS

(a) Female discrimination of male caller

A significant increase in first look duration between DH and the last playback of the habituation phase (paired t -test: $t_{15} = -3.333$, $p = 0.002$) was detected. In addition, a significant reduction in first look duration between DH and RH was observed (paired t -test: $t_{15} = 4.026$, $p < 0.001$, see figure 1a).

(b) Female discrimination of male caller after standardization of mean F0

First look duration was significantly longer to DH than the last playback of the habituation phase (paired t -test: $t_{12} = -2.929$, $p = 0.007$) and a significant reduction in first look duration between DH and RH was observed (paired t -test: $t_{12} = 1.839$, $p = 0.046$, see figure 1b).

(c) Female discrimination of male caller after removal of amplitude modulation

No differences in first look duration between DH and the last playback of the habituation phase (paired t -test: $t_{12} = 0.622$, $p = 0.273$) or between DH and RH were detected (paired t -test: $t_{12} = 0.379$, $p = 0.356$, see figure 1c).

4. DISCUSSION

Our results show that female giant pandas can discriminate between the vocalizations of different males and indicate that the characteristic amplitude

modulation of giant panda bleats is crucial for this. Although the ability to discriminate vocalizations from different individuals is well documented (see §1) to our knowledge, few experimental studies have investigated which acoustic features are actually used for individual discrimination in mammals (but see Charrier *et al.* 2003; Searby & Jouventin 2003). Whereas female giant pandas still strongly dishabituated to the change in male caller when mean F0 was controlled for (indicating that this acoustic feature is not important for vocal discrimination of male callers), they could not do so when we removed the amplitude modulation of bleats. Amplitude modulation is a highly individualized component of giant panda bleats (Charlton *et al.* submitted) and contributes to vocal recognition in birds (Aubin & Jouventin 2002); however, to our knowledge, this is the first direct demonstration of its importance for mammal vocal recognition. Interestingly, mean F0 is in fact the most highly individually distinctive acoustic feature of giant panda bleats (Charlton *et al.* submitted) but does not appear to be essential for vocal recognition in this species, possibly because of its propensity to change according to the motivational state of the caller. Nevertheless, standardizing mean F0 across sequences did expedite female habituation and lessen their ability to remain habituated to the initial caller (as evidenced by the smaller rebound in response to the rehabituation control), suggesting that it may be incorporated into the vocal recognition process at some level.

Whether females are able to recognize different males vocally or if they just discriminate between the vocalizations of different callers is less clear. However, because they react to a change of caller it seems likely that the identity of male callers is biologically relevant to female giant pandas during the breeding season. Although giant panda bleats are low amplitude and features of amplitude modulation would be particularly susceptible to attenuation in the densely forested natural habitat that giant pandas inhabit (Wiley & Richards 1978), these vocalizations could be used for close-range signalling of identity in direct encounters. Future work could re-record bleats in bamboo forest environments to assess the degradation of the acoustic detail of giant panda bleats and determine the

distances over which information on caller identity could realistically be broadcast in this species natural environment.

Most other studies of this type concerning individual vocal recognition in mammals have focused on mother–offspring recognition (Charrier *et al.* 2003; Torriani *et al.* 2006), contact calls (Rendall *et al.* 1996) and alarm calls (Hare 1998; Blumstein & Daniel 2004). Very few have considered whether females can discriminate between the vocalizations of potential mates (but see Reby *et al.* 2001). Giant panda bleats are often delivered alternately as males and females approach each other, and male giant pandas bleat at particularly high rates when they encounter oestrous females (Kleiman 1983). These observations suggest that bleats have an active role in initiating and controlling the interactions of a male and female pair prior to mating. Furthermore, females may be more receptive to males with whom they are already vocally familiar during their fertile period.

Free-ranging male giant pandas compete for females during the breeding season and are known to associate with females for up to one month before copulation and subsequent separation (Schaller *et al.* 1985; Zhu *et al.* 2001). It is conceivable, therefore, that female giant pandas may become progressively familiarized to the vocalizations of high-quality males that can outcompete other rivals and maintain close contact. Our demonstration that female giant pandas react to changes in male callers suggests that vocal recognition of potential mates may occur in the natural environment and that female preferences based on male familiarity could exist. The next step requires the use of playback experiments, in which females are presented with males of differing familiarity, to determine whether females preferentially associate with ‘familiar’ males as they approach and enter oestrous.

The procedures used in the research did not affect the housing, diet or management of the animals and comply with the law of the People’s Republic of China. The director of CRCCGP and Zoo Atlanta’s Institute for Animal Care and Use Committee approved the research.

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