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Chemosensory discrimination of identity and familiarity in koalas

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ABSTRACT

Despite numerous descriptive accounts of scent marking in marsupials, rigorous experimentation is rare, and relatively little evidence exists to show that conspecifics use chemical signals to distinguish between individuals or different social groups. In this study a series of olfactory discrimination tests sought to determine whether: (1) male koala sternal scent gland secretions are individually distinctive; and (2) male koalas can differentiate between the scent of familiar and unfamiliar individuals. In the first experiment a habituation–discrimination trial demonstrated that male koalas discriminate between the scent gland secretions of different unfamiliar individuals. In a second experiment male koalas spent significantly more time investigating scent from unfamiliar males than familiar males, supporting the hypothesis that they differentiate between conspecifics based on their familiarity. Taken together these results suggest that male koalas are able to discriminate the identity and familiarity of conspecifics using chemical cues, and provide a platform for further studies investigating the functional role of olfactory communication in this species.

1. Introduction

Olfactory communication plays a central role in mammal social behaviour and reproduction (Beauchamp and Yamazaki, 2003; Petrulis, 2013; Wyatt, 2003). Chemical signals are particularly useful for animals with dispersed social systems because they can persist long after the signal is deposited, allowing individuals that would otherwise not encounter one another very often to exchange information long after the signaller has left the area (Alberts, 1992). This is especially important when competing conspecifics possess overlapping ranges but individually use specific locations within shared areas (primates, Johns, 1986; rhinoceros, *Rhinoceros unicornis*, Laurie, 1982; giant pandas, *Ailuropoda melanoleuca*, Schaller et al., 1985). In relatively solitary and aggressive species, chemical signals can also be important for recognising known rivals in order to avoid unnecessary territorial encounters (Gosling and Roberts, 2001). For instance, male snow voles (*Chionomys nivalis*) and house mice (*Mus* musculus) show less aggression towards males that have been recognised by "scent matching" than those that have not been recognised in this way (Gosling and McKay, 1990; Luque-Larena et al., 2001). Furthermore, because highly competitive individuals will typically produce more scent marks and mark out larger territories using olfactory signals (Gosling, 1982; Hurst and Rich, 1999), this type of scent matching behaviour can also be used to assess the likely outcome of a future encounter based on the spatial and temporal pattern of scent marks (Gosling and Roberts, 2001). A prerequisite for scent matching to occur though is the ability to discriminate between the odour cues of different individuals.

The koala (*Phascolartos cinereus*) is a solitary marsupial mammal that dwells in the *Eucalyptus* forests of Australia (Lee and Carrick, 1989). Because koalas typically occur in low abundance (Martin and Handasyde, 1999; Mitchell, 1990), visual communication is likely to be restricted to infrequent associations, and effective communication using vocal and olfactory signals could be crucial for coordinating social behaviour. Although koalas occupy overlapping ranges (Ellis et al., 2009) agonistic encounters appear to be quite rare (Mitchell, 1990). The relatively low rate of male agonistic interactions that are observed during the breeding season is most likely to be a direct consequence of this species' predominantly arboreal and nocturnal lifestyle which makes behavioural observations difficult (Martin and Handasyde, 1999; Melzer et al., 2010; Mitchell, 1990) but could also imply that competitive interactions are often resolved through signalling (as in other mammals: Bradbury and Vehrencamp, 2011). Consistent with this notion, recent work has revealed the importance of male bellow vocalisations in this species' vocal communication system for signalling identity and deterring potential rivals (Charlton et al., 2011; Charlton et al., 2013).

Chemical communication is also likely to play an important role in the koala's social organisation. Koalas possess a vomeronasal organ (Broom, 1896), an auxiliary olfactory organ that is used for sensitive chemical detection in mammals, and can discriminate sex and female reproductive state using chemical cues in urine

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(Charlton, 2014). Male koalas also have prominent scent glands located in the middle of the chest that they use to mark trees and substrates in their environment, particularly in unfamiliar surroundings (Mitchell, 1990; Smith, 1980). This observation suggests that males may be using scent marks to establish temporal and spatial relationships without direct physical or visual contact (Laurie, 1982; Natoli, 1985; Schaller et al., 1985). Furthermore, because male koalas occupy overlapping ranges (Ellis et al., 2009) they will interact with other individuals in neighbouring territories, and hence, could familiarise themselves with the odour of known rivals. Nevertheless, male koalas would need to produce individually distinctive scent marks and have the ability to distinguish between the scent marks of different males for this to occur.

Proving that scent marks are individually distinctive requires examining their chemical profile to determine whether semiochemical patterns are individually distinctive (Hagey and Macdonald, 2003; Lawson et al., 2001; Scordato et al., 2007). However, if animals can be shown to distinguish between the scent marks of different individuals using controlled experiments, this also provides good evidence that chemical cues to identity exist within olfactory signals. Although several descriptive accounts of scent marking behaviour in marsupials indicate that olfactory communication is very important in this group of mammals (Biggins, 1984; Croft, 1981; Eisenberg et al., 1975; Ewer, 1968; Fadem and Cole, 1985; Kaufmann, 1974; Oakwood, 2002; Schultze-Westrum, 1969) relatively few experimental studies have demonstrated that marsupials can discriminate individuality on the basis of odour cues (Blumstein et al., 2002; Walker and Croft, 1990). Indeed, whereas male scent marking is postulated to play a role in advertising territory and mediating the koalas dispersed social system (Zabaras et al., 2005), whether male koalas can actually discriminate between the scent gland secretions of different males remains an open question.

In this study, I conducted two separate experiments to examine the ability of male koalas to discriminate between individuals using chemical cues in sternal scent gland secretions. In the first experiment, I used a habituation–discrimination paradigm to investigate whether male koalas have the ability to discriminate between different males using chemical cues in sternal scent gland secretions. I then conducted a second olfactory discrimination experiment to examine whether the familiarity of scent donors affected the behavioural response of male koalas. In the habituation–discrimination paradigm a subject is initially habituated by repeated exposure to one stimulus before a dishabituation stimulus is provided (Charlton et al., 2009; Eimas et al., 1971; Swaisgood et al., 1999; Swartz, 1983). A renewal in the level of response to the dishabituation stimulus implies an ability to discriminate the factor that changed between the stimuli used to habituate subjects and the dishabituation stimulus. I therefore predicted that after habituating to a series of scent stimuli from a given male, male koalas would show a renewal of response levels to the scent of a novel male. In line with other studies (Cinkova and Policht, 2015; Drea et al., 2002; Krueger and Flauger, 2011; Natoli, 1985; Rubenstein and Hack, 1992) I also predicted that male koalas would show more attention to scent stimuli from unfamiliar males than familiar individuals with which they have a past history of competitive interactions.

2. Methods

2.1. Study site and subjects

The study was conducted at Lone Pine Koala Sanctuary (LPKS), Brisbane, Australia, during the 2013 breeding season (September–December). The subjects for the scent presentation tests were 22 adult male koalas aged 4–14 years. Seven males participated in both experiments. In the first experiment, five male koalas were housed individually in enclosures measuring approximately $2 \times 2 \times 2.5$ m and 11 were housed with 2–5 other individuals in larger enclosures measuring approximately $5 \times 4 \times 3$ m. The subjects for the second experiment were all housed with 2–5 other male koalas in enclosures measuring approximately $5 \times 4 \times 3$ m. All experimental testing was conducted during the day while subjects resided in these enclosures.

This study follows the Association for the study of Animal Behaviour/Animal Behaviour Society guidelines for the use of animals in research, and was approved by the Ethical Review Committee of the University of Sussex (ERC/34/E-CIRC/CHA). The koalas in the study were all fed a diet of *Eucalyptus* leaves once daily and provided with water *ad libitum*. The life history of all the animals was known from birth. All the koalas were individually recognizable, and although several koalas were present in the enclosures during the experiments, the scent presentation tests were only conducted when all the animals were resting in separate tree forks. In addition, test subjects always had an unoccupied tree fork well within jumping range (<2 m) so that any aversion responses were not restricted during the experiments. Care was also taken to avoid conducting experiments during periods of increased activity when other individuals would be more likely to move around the enclosure and interfere with the test subject (for example, just after the daily feed had been introduced).

2.2. Scent stimuli

To construct the scent presentation sequences for the olfactory discrimination tests a total of four scent samples were collected from each of 12 males (aged 6–10 years) on four separate days i.e. only one sample was collected from an individual during a 24-h period. Collecting four scent samples from each male scent donor on separate days minimized any effect that short-term fluctuations in physiological state could have on the chemical composition of the scent samples from a given individual, which may then affect the propensity of subjects to habituate and/or dishabituate to them during the habituation–discrimination tests. The scent samples to be used as experimental stimuli in the olfactory discrimination tests were collected by rubbing sterile 5×5 cm cotton swabs on male sternal scent glands. The cotton swabs were then sealed in plastic bags, frozen the same day as collection, and stored frozen at $-4\,^{\circ}$C for a maximum of 5 days until presentation. The male koalas in the study were all fed the same diet of *Eucalyptus* leaves on a given day; thus, obviating the potential confounding influence of leaf chemistry on individual scent profiles. In addition, to avoid potential contamination the experimenter wore disposable gloves during sample collection.

2.3. Scent presentation

Before scent presentation the samples were thawed and allowed to reach ambient temperature (*circa* 30 °C on the testing days). The cotton swabs were then attached to the end of a microphone stand (Proel, Italy) and placed approximately 20 cm from the subject's head to start each scent presentation. This allowed me to present the scent stimuli in a controlled manner to koalas that were resting on tree-forks in their enclosures (Charlton, 2014). To limit the possibility of cross-contamination the experimenter wore disposable gloves that were replaced after each stimulus presentation, and the end of the microphone stand was covered with thin polyethylene sheeting (GLAD Wrap, Australia) before each swab was attached.

Although some of the scent donors were also subjects in the presentation tests, at no time during either experiment were males presented with their own scent. In addition, because there is some