Detective mice assess relatedness in baboons using olfactory cues

Aurélie Célérier[1,2,*], Elise Huchard[1,3,4], Alexandra Alvergne[1,3], Delphine Féjan[1,3], Floriane Plard[2],
Guy Cowlishaw[5], Michel Raymond[1,3], Leslie A. Knapp[6] and Francesco Bonadonna[2]

[1]University Montpellier II, Place Eugène Bataillon, 34095 Montpellier Cedex 5, France, [2]CNRS, Centre d'Ecologie Fonctionnelle et Evolutive UMR 5175, 1919 Route de Mende, 34293 Montpellier Cedex 5, France, [3]CNRS, Institute of Evolutionary Sciences, CC065, Place Eugène Bataillon, 34095 Montpellier Cedex 5, France, [4]Department of Behavioral Ecology and Sociobiology, German Primate Center, Kellnerweg 4, 37077 Göttingen, Germany, [5]Institute of Zoology, Zoological Society of London, Regent's Park, London NW1 4RY, UK and [6]Department of Biological Anthropology, University of Cambridge, Cambridge, UK

*Author for correspondence (aurelie.celerier@cefe.cnrs.fr)

SUMMARY

The assessment of relatedness may be crucial in the evolution of socio-sexual behaviour, because it can be associated with fitness benefits mediated by both nepotism and inbreeding avoidance. In this context, one proposed mechanism for kin recognition is 'phenotype matching'; animals might compare phenotypic similarities between themselves and others in order to assess the probability that they are related. Among cues potentially used for kin discrimination, body odours constitute interesting candidates that have been poorly investigated in anthropoid primates so far, because of a mixture of theoretical considerations and methodological/experimental constraints. In this study, we used an indirect approach to examine the similarity in odour signals emitted by related individuals from a natural population of chacma baboons (*Papio ursinus*). For that purpose, we designed an innovative behavioural tool using mice olfactory abilities in a habituation–discrimination paradigm. We show that: (i) mice can detect odour differences between individuals of same sex and age class in another mammal species, and (ii) mice perceive a higher odour similarity between related baboons than between unrelated baboons. These results suggest that odours may play a role in both the signalling of individual characteristics and of relatedness among individuals in an anthropoid primate. The 'biological olfactometer' developed in this study offers new perspectives to the exploration of olfactory signals from a range of species.

Key words: kin recognition, olfaction, odour cues, baboons, mice, habituation–discrimination task.

INTRODUCTION

Since Hamilton's work on inclusive fitness theory (Hamilton, 1964), significant advances have been made in understanding the role of genetic relatedness and kin recognition in both nepotism (the preferential treatment of kin) and inbreeding avoidance in animals (Bateson, 1983; Hepper, 1991; Sherman et al., 1997). In many group-living mammals, including some primates, individuals adjust their behaviour according to the degree of relatedness they share with those individuals with whom they interact (Dugatkin, 1997; Smith et al., 2003; Chapais, 2006). The mechanisms by which animals discriminate conspecifics may be multiple, including indirect mechanisms through contextual cues (e.g. spatial or temporal proximity) and direct mechanisms through phenotype recognition. Among direct mechanisms, two main hypotheses for kin recognition have been formulated: (i) familiarity (or association) among individuals that have been closely associated during early development (Waldman, 1988), and (ii) 'phenotype matching', i.e. the assessment of phenotypic similarities (including allele recognition, a particular case of phenotype matching) (Hepper, 1991; Mateo and Johnston, 2003). This latter capacity is expected to evolve in situations where familiarity does not necessarily correlate with genetic relatedness (Widdig, 2007). This mechanism may, for instance, have been favoured in some primates living in multimale multifemale groups where females are highly promiscuous and consequently paternity uncertainty is high. In particular, male baboons apparently recognise their genetically related offspring among familiar

juveniles, suggesting the use of phenotype matching (Buchan, 2003).

Kin-recognition systems based on phenotypic similarities involve three components: the production of kin labels (also called expression), the perception of these labels by another individual, and the action (behavioural responses) of this individual according to the perceived similarities (e.g. nepotism or inbreeding avoidance) (Holmes and Sherman, 1982; Beecher, 1988; Reeve, 1989; Gamboa et al., 1991; Sherman et al., 1997; Hauber et al., 2001). The nature of kin labels supporting these mechanisms is probably variable between and within species but potentially includes visual appearance (Dasser, 1988; Vokey et al., 2004; Alvergne et al., 2009), acoustic cues through vocalisations (Rendall et al., 1996), personality traits (Gosling, 2001; Weiss et al., 2006) and body odours (Hübener and Laska, 1998; Widdig, 2007; Alvergne et al., 2009).

Olfaction is a sensory modality that is important in social communication across a wide array of species from insects to humans. Social odours, which are here defined as chemical signals involved in regulating social interactions (Snowdon, 2006), have a correspondingly broad range of functions. Odours can provide directional cues for orientation, serve as signals of alarm, mark territory boundaries, unify groups, direct foraging behaviour, attract mates, indicate reproductive and social status, and provide information about species, subspecies, group, kin and individual identity (Wyatt, 2003). Kin recognition and discrimination through olfactory cues occurs in several species across various taxonomic groups, ranging from invertebrates (Greenberg, 1979; Gamboa et

al., 1996) to vertebrates, including fish (Reynolds and Sheldon, 2003; Neff, 2003) and birds (Bonadonna and Nevitt, 2004; Mardon and Bonadonna, 2009). More specifically, in mammals, the importance of olfactory signals in the context of kin recognition is gaining appreciation, as olfactory signals have been shown to advertise individual identity in rodents (Schwagmeyer, 1988; Todrank et al., 1998; Mateo, 2002; Busquet and Baudouin, 2005; Hurst et al., 2001) and carnivores (Zhang, 2005) and Old World monkeys (Setchel et al., 2010), as well as relatedness in prosimians [ring-tailed lemurs (Charpentier, 2008; Boulet et al., 2009)] and New World monkeys [common marmosets (Smith, 1997)].

Nevertheless, progress in understanding the importance of olfactory signals is limited by methodological difficulties, because it is difficult to study a sensory modality that we, as observers, cannot perceive directly. In contrast with the optical and acoustical properties of an object, there is as yet no easy way to 'record' and quantify the olfactory properties of objects and chemical signals, especially under field conditions (Epple, 1986). Although significant advances in semiochemistry (Zhang et al., 2005; Scordato et al., 2007) have taken us a step forward in revealing the information potentially available within a chemosignal (Belcher et al., 1986; Belcher et al., 1990; Smith et al., 2001), the analysis of the composition of chemical signals through gas chromatography–mass spectrometry still remains relatively expensive and often technically challenging, especially for non-volatile components such as proteins. However, these compounds have been shown to play a crucial role in communication in many species (Belcher et al., 1990; Hurst et al., 1998; Nevison et al., 2003). Another possible approach to investigating olfactory cues is to perform behavioural assays, which can reveal the information perceived by the signal receiver through behavioural, physiological or neural responses under controlled conditions (Drea et al., 2002; Mateo, 2003; Mateo, 2006a; Mateo, 2006b; Nevitt and Bonadonna, 2005). However, although behavioural assays are informative about both the production and the perception of a chemical signal, the experimental settings they require are difficult to implement, particularly in natural populations, because they require extensive manipulations as well as a long phase of conditioned learning for the animals involved.

In addition to these methodological constraints, the production and perception of olfactory signals have been poorly investigated in some taxa, particularly anthropoid primates (Heymann, 2006). This is largely due to the view that olfactory cues may not represent promising candidates for kin discrimination in a taxon believed to have shown a decreasing reliance on smell during its evolution due to the increasing importance of trichromatic vision (Dominy and Lucas, 2001; Gilad et al., 2004). However, a growing body of evidence indicates that the sense of olfaction in humans is more important than previously thought (Weisfeld et al., 2003; Shepherd, 2004), and it seems possible that a similar pattern may be found in our close relatives. Moreover, it has been proposed that the evolution of kin discrimination might have preceded the reduction in olfactory sensitivity of anthropoid primates (Widdig, 2007), suggesting that the use of olfactory cues by anthropoid primates in the context of kin discrimination is plausible.

The goals of this study are twofold. Our first aim is to develop and validate a cost-effective tool to assess similarity between animal odours, through an indirect behavioural method. Our approach uses mice as 'noses' in an improved habituation–discrimination paradigm conducted in a hole-board apparatus. We call this tool a 'biological olfactometer' because it allows qualitative measurement ('meter') of odours ('olfacto') using the olfactory abilities of mice as a

'biological' analyser. Mice were chosen to act as 'noses' because a number of experiments have shown that rodents have a highly efficient sense of smell (Breer, 2001; Novotny, 2003; Hurst and Beynon, 2004). Additionally, mice can both discriminate between odours of congenic mice differing only at one major histocompatibility complex (MHC) locus, and smell slight environmental differences among individuals (Penn and Potts, 1998). Furthermore, a recent study using a basic classical habituation–discrimination paradigm demonstrated that rats were able to discriminate human odours varying in their degree of relatedness (Ables et al., 2007). Overall, these data indicate a prodigious olfactory acuity in rodents in general and mice in particular, coupled with a good capacity to work in the context of the habituation–discrimination paradigm. The behavioural apparatus and procedures described in our study constitute significant methodological innovations to the classical habituation–discrimination paradigm. The second aim of the study is to use our validated 'biological olfactometer' to investigate the similarity in odour production in related individuals from a natural population of chacma baboons (*Papio ursinus*), a species where chemical communication has rarely been investigated but might be important (Clarke et al., 2009). If individual odours can serve as objective kin labels, we predict that individual odour patterns, as perceived by mice, will show higher similarity between kin than between non-kin.

MATERIALS AND METHODS
Baboon data collection
Baboon odours were collected from a total of 77 wild chacma baboons (*Papio ursinus* Kerr 1792), including adults and juveniles, males and females, living in Tsaobis Leopard Park, on the edge of the Namib Desert in Namibia, Southern Africa [for details of the site and population, see Cowlishaw (Cowlishaw, 1999)]. The baboons, belonging to two troops (containing 32 and 57 individuals, respectively), were captured in October 2006 in order to gather biological samples (including tissue samples for microsatellite genotyping) using individual cages baited with corn cobs, and which were set-up at dusk. The baboons were captured at dawn, anaesthetised using tiletamine–zolazepam, and they were all processed within a day in order to be released together the following morning when fully awake. For each baboon, a swab made of viscose microfibres (obtained from the French forensic police and used for criminal investigation) was applied against the axillaries (armpits) for two minutes (one minute on each side), and then against the inguinal region (groin) for two minutes (one minute on each side), using new vinyl gloves for each individual. Once impregnated with baboon odours, the swabs were individually stored in opaque glass bottles, and refrigerated at 4°C. For this study, only those odours from adult females were used, to avoid sex and age effects. Tests were then performed on the odours collected from a subsample of 14 adult females (out of the 77 odour samples available).

Baboon microsatellite typing
Sixteen tetra- and di-nucleotide human microsatellite markers were polymorphic in *P. ursinus* with reproducible results, and thus retained for relatedness analysis. Briefly, DNA was extracted using a DNeasy Tissue Kit (Qiagen, Crawley, UK), following the manufacturer's instructions. PCR amplification was performed using a Qiagen Multiplex PCR kit, following the manufacturer's instructions. PCR conditions were as follows: initial denaturation (15 min, 95°C) and then either (i) 10 cycles of denaturation at 94°C

for 30 s, annealing at 60°C for 2.5 min and elongation at 72°C for 45 s, followed by 26 cycles of 94°C for 30 s, 58°C for 3 min, 72°C for 1 min, or (ii) 36 cycles of denaturation at 94°C for 30 s, annealing at 47°C for 2.5 min and elongation at 72°C for 45 s and then a final elongation at 60°C for 7 min. Multiple PCR products with different fluorescent labels were run together on either an ABI373 or ABI377 sequencer (Applied Biosystems, Foster City, CA, USA). The software Genotyper (Applied Biosystems) was used for automatic analysis of allele size, combined with visual analysis. Apparent homozygotes were genotyped at least three times from independent amplifications to minimise the risk of genotyping error. The number of alleles per microsatellite locus ranged from 3 to 11 (mean ± s.d.=5.25±1.82), and observed heterozygosity ranged from 0.55 to 0.77 (mean ± s.d.=0.68±0.08) for the microsatellite loci. Further details on microsatellite genotyping in this population can be found elsewhere (Huchard et al., 2010).

Estimation of pairwise relatedness between baboons

Pairwise coefficients of relatedness based on microsatellite typing similarity were calculated between pairs of individuals, using a triadic likelihood estimator of relatedness (TL) based on a likelihood method that uses the genotypes of a triad of individuals to estimate pairwise relatedness (Wang, 2007). Using TL relatedness coefficients, the mean (±s.d.) value of pairwise relatedness (r) between 34 mother–offspring pairs known from behavioural observations was found to be 0.48±0.08 (the TL coefficients range in value from zero to one). Pairwise relatedness coefficients ranged from 0 to 0.80 (median=0.02; mean ± s.d.=0.07±0.12, N=21.945 dyads across six baboon groups for 210 individuals).

Pairs of females for which the pairwise coefficient of relatedness was higher than the average pairwise relatedness of known mother–offspring pairs minus one standard deviation were matched as relatives in this sample (mean pairwise relatedness ± s.d.=0.48±0.08), whereas pairs for which the pairwise coefficient of relatedness was lower than the average pairwise relatedness in the population were matched as non-relatives (mean pairwise relatedness of this sample ± s.d.=0.03±0.03).

Apparatus

Odour tests were performed in a 4 hole-board apparatus (45.0 cm length × 45.0 cm width × 30.0 cm height) enclosed by grey Plexiglas. We used 0, 1 or 2 holes among the 4, according to the phase of the odour test. The hole-board apparatus was placed on the floor of the room (3.0 m length × 3.0 m width × 2.4 m height). Holes [3.0 cm diameter (Ø) × 2.5 cm depth] were located in each corner of the board and were 6.0 cm away from the sidewalls, each hole delimiting an open space in which the odour sample was inserted (see Table 1 for apparatus configuration). When not used, the holes were sealed. The start box consisted of a grey PVC tube (20.0 cm Ø × 20.0 cm height) placed in the centre of the board where the mice were enclosed for 15 s at the beginning of each phase of the test. The apparatus was placed in a room exposed to 2 lx lights. Before each mouse was tested, and between each phase of a test, the apparatus was cleaned with 50% ethanol and then with water. Photocells placed in each hole were used to automatically evaluate the number of sniff bouts (head dips) in each hole (Célérier et al., 2004). When only one hole was active (i.e. contained one odour sample), the parameter used to evaluate odour investigation was the number of sniff bouts. When two holes were active, the parameter used to evaluate the investigation of one odour relative to the other was the percentage of sniff bouts, considering that the percentage expected by chance is 50%. This parameter allowed us to correct for differences between individual mice in their general exploratory behaviour, which is probably more dependent on variations of locomotor activity and personality across individuals than on the sensorial perception of odour differences.

Preparation of odour samples

An odour stimulus consisted of a 48–52 g swab sample impregnated with baboon odours. Each odour sample was placed in a clean phial, inserted between two squares of gauze (3 cm length × 3 cm width) and held in position by a perforated cap (2 cm Ø), so that mice could come into direct contact and perceive both the volatile and non-volatile components of the odour (Cheetham, 2007). The phial was

Table 1. Behavioural procedure of habituation–discrimination
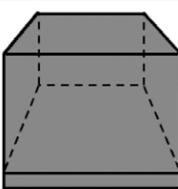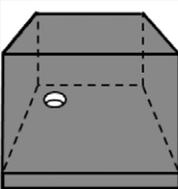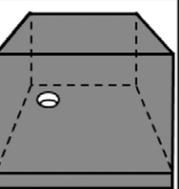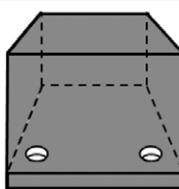
		Apparatus configuration				
Phase of behavioural task		FREE EXPLORATION	HABITUATION		TEST	
			Trial 1	Trial 2		
Duration (minutes)		2 min	10 min	10 min	5 min	
Odour Sample	Exp.1	None	Referent odour	Referent odour	First hole : odour A Referent odour (*r=1*)	Second hole : odour B Unrelated baboon (*r<0.06*)
	Exp.2	None	Referent odour	Referent odour	First hole : odour A Related baboon (*0.56>r>0.40 ; r = 0.48 ± 0.08*)	Second hole : odour B Unrelated baboon (*r<0.06 ; r = 0.03 ± 0.03*)

inserted at the bottom of the active hole in the apparatus and fixed so that it could not be moved by the animals. The odour samples were stored at 4°C in hermetically sealed bottles and placed at room temperature 10 min before testing. Each odour sample was prepared and manipulated using new vinyl gloves.

Mice and housing

The subjects used as 'noses' in our 'biological olfactometer' were 24 naïve adult male Swiss mice (two months old; 28–32 g) obtained from DEPRE (France). Animals were housed in groups of 12 with access to food and water *ad libitum*. They were kept in a temperature (21°C) and humidity (50%) controlled facility on a 12 h:12 h light:dark cycle. All test procedures were conducted during the light phase of the cycle, between 09:00 h and 16:00 h in a sound-attenuated and air-regulated experimental room, to which the mice were habituated at least 12 h before behavioural testing.

Habituation–discrimination tests

Habituation–discrimination is a procedure used to assess the perception of differences in odour signals (Halpin, 1986). Using this procedure, we assessed (i) whether mice investigate unfamiliar baboon odours more than familiar ones (Experiment 1), and (ii) whether mice are able to discriminate baboon odours based on their relatedness (Experiment 2). Before each experiment, mice were allowed to freely explore the apparatus in the absence of odour stimuli for two minutes, in order to get used to the apparatus. Then the animals were submitted to a habituation phase, followed by a discrimination phase.

Habituation phase

For both Experiment 1 and Experiment 2, this phase consisted of the display of one referent baboon odour to a mouse for two trials of 10 minutes each, separated by a two-minute interval (the referent odour was replaced by a fresh odour, i.e. a new piece of swab from the same individual, between trials 1 and 2 to ensure that habituation was not due to odour degradation). A subject was considered habituated when it displayed a reduced interest in the odour between trials 1 and 2. Only habituated mice were tested in the second discrimination phase.

Discrimination phase

In Experiment 1, the mouse was simultaneously presented with two odours for five minutes: the referent odour (a fresh sample, i.e. odour A) and the test odour (the odour of a baboon unrelated to the referent baboon, to which the animal was not familiar, i.e. odour B). In Experiment 2, the mouse was simultaneously presented with two odours for five minutes: odour A belongs to a baboon closely related to the referent baboon ($0.56>r>0.40$) whereas odour B belongs to a baboon unrelated to the referent baboon and unrelated to odour A ($r<0.03$). Note that the referent odour used in the habituation phase was not used in Experiment 2. For both Experiment 1 and Experiment 2, if the subject perceived odour B as more different in quality from the referent odour than odour A, it should investigate odour B more often than expected by chance (>50%).

Each odour triad formed by the association between a referent odour (habituation phase) with an odour A and an odour B (discrimination phase) was tested by three mice, and each mouse was used only once. The habituation and test odours were always placed in different holes, their locations were counterbalanced across tests, and the experimenter was blind to the identity of odours.

Statistical analysis

Data were analysed using an exact permutation test for paired samples. For habituation data analysis, the number of sniff bouts in trials 1 and 2 were compared: the null hypothesis H0 is that the number of sniff bouts is identical in trial 1 and trial 2; the alternative hypothesis H1 is that the number of sniff bouts in trial 2 is lower than the number in trial 1.

According to standard habituation–discrimination protocols, in the discrimination phase, if the subject perceives a greater difference between odour B (unrelated) and the referent odour than between odour A (related) and the referent odour, it is expected to investigate odour B more often than expected by chance (50%). Thus, for the discrimination data analysis, the percentage of sniff bouts of the unrelated odour (odour B) was compared with the percentage expected by chance (50%): the null hypothesis H0 is that the percentage of sniff bouts of the unrelated odour is at chance level (50%); the alternative hypothesis H1 is that the percentage of sniff bouts of the unrelated odour is above chance level.

RESULTS

The mice actively investigated all of the odours presented and showed no sign of avoidance to any stimuli in the two experiments.
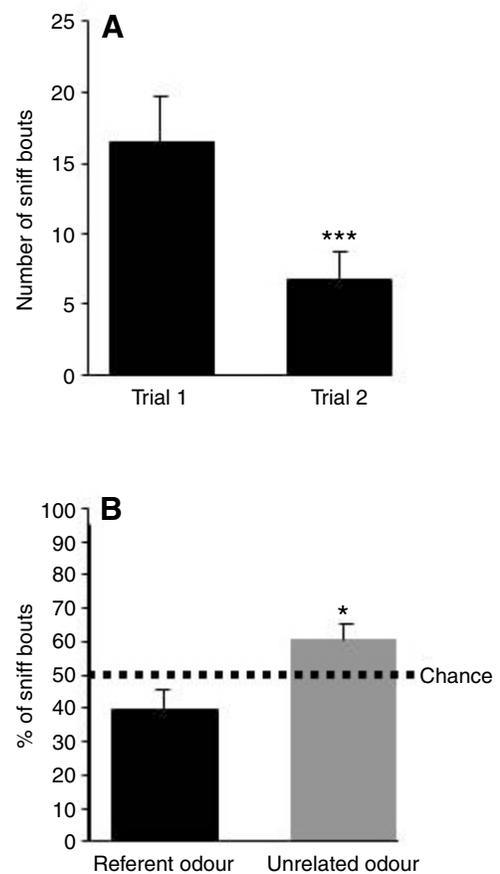
Fig. 1. Results of Experiment 1: did the mice investigate unfamiliar baboon odours more than familiar ones? Habituation phase (Fig. 1A): results are expressed as mean (±s.e.m.) number of sniff bouts during trial 1 and trial 2; (***) *P*<0.001. Discrimination phase (Fig. 1B): results are expressed as mean (±s.e.m.) percentage of sniff bouts of referent familiar odour (black) and unrelated unfamiliar odour (grey); (*) *P*<0.05 as compared with chance level (indicated by the broken line).

Three mice out of 24 were excluded from the data analysis: one because it did not fulfil our habituation criterion, and two because they did not investigate the two holes at least once during the test phase.

Experiment 1: did the mice investigate unfamiliar baboon odours more than familiar ones?

The subjects showed a significant decline of interest for the referent odour between trial 1 (16.4±3.3 sniff bouts) and trial 2 (6.8±1.8 sniff bouts) during the habituation phase ($P=0.0002$; Fig. 1A). Subsequently, the mice investigated the unfamiliar odours more than expected by chance during the discrimination phase (60.2%; $P=0.045$; Fig. 1B). The mice are thus able to detect odour similarities in baboons.

Experiment 2: were the mice able to discriminate baboon odours on the basis of relatedness?

The subjects showed a significant decline of interest for the referent odour between trial 1 (25.3±5.5 sniff bouts) and trial 2 (13.5±3.2 sniff bouts) during the habituation phase ($P=0.002$; Fig. 2A). Subsequently, the mice investigated the unrelated odours more than expected by chance during the discrimination phase (64.4%; $P=0.03$; Fig. 2B). Thus, according to the behaviour of the mice, the similarity
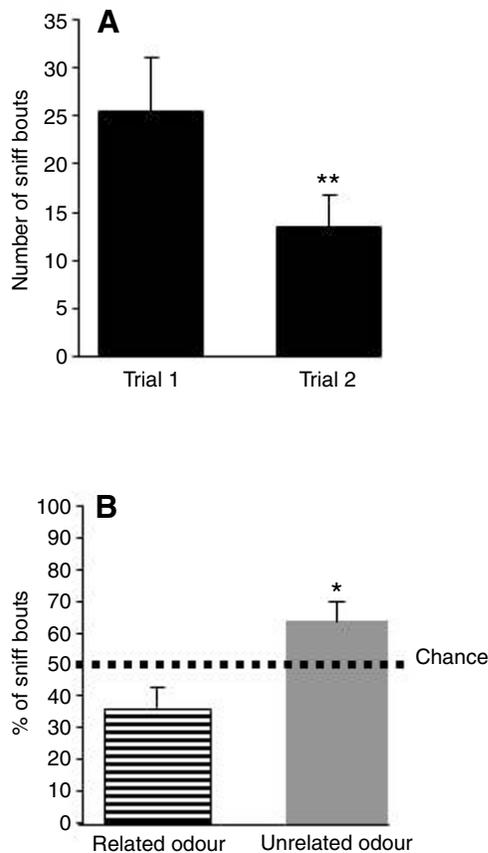
Fig. 2. Results of Experiment 2: were the mice able to discriminate baboon odours on the basis of relatedness? Habituation phase (Fig. 2A): results are expressed as mean (±s.e.m.) number of sniff bouts during trial 1 and trial 2; (**) $P<0.01$. Discrimination phase (Fig. 2B): results are expressed as mean (±s.e.m.) percentage of sniff bouts of related odour (striped) and unrelated odour (grey); (*) $P<0.05$ as compared with chance level (indicated by the broken line).

of body odours between two baboons is higher for related pairs than for unrelated pairs.

DISCUSSION

In this study, we designed and validated a cost-effective tool to assess the degree of similarity between animal odours. Using mice olfactory abilities in a new protocol of habituation–discrimination, and a highly sensitive apparatus automatically recording exploration behaviours, our results indicate that: (i) baboon odours are not considered repulsive for mice because avoidance behaviours were not observed, (ii) mice can detect odour differences between individuals of the same sex and age class in another mammal species (Experiment 1), and (iii) mice perceive a higher odour similarity between related baboons than between unrelated baboons (Experiment 2).

The method of habituation–discrimination developed in this study is an improvement of the classical procedure (Halpin, 1986) used in a previous study assessing objective odour qualities (Ables et al., 2007). Our method, using a hole board, offers several advantages. First, data collection is entirely automatic, which generates strict and unbiased measurements of behaviour. Second, it allows the tests to be conducted in a soft light and silent atmosphere without human presence; thus, minimising stress for the mice (Célérier et al., 2004). Third, this method is particularly well suited for mice because it is based on the exploration of a novel environment, on locomotor activity and on head dipping in a hole-board, all of which are behavioural aptitudes spontaneously expressed by mice (Kliethermes and Crabbe, 2006). Finally, it does not require any reinforcement or training. The ability of mice to detect individual odour differences in baboons confirms their prodigious acuity in olfaction coupled with a good capacity to work in the context of the habituation–discrimination paradigm. Further investigations are now needed to determine if this method is similarly efficient when applied to other sex and age classes of individuals, for instance using odours from males and juveniles. Taken together, these results suggest that the behavioural procedure we developed, using mice as 'noses', may be used in a quantitative way, to assess the degree of similarity between animal odours. Indeed, our method, which was tested here on baboon odours, is potentially suitable for other species.

Our findings also show that related adult females from a wild population of chacma baboons (*P. ursinus*) produce more similar odour cues than unrelated individuals of the same sex and age class, at least from a mouse's point of view. The next step is to identify the composition and the chemical properties of odour labels produced by baboons and used by mice, thanks to complementary experiments with mice, possibly coupled with chemical analyses. Previous studies, essentially in rodents, indicate that the scents associated with the MHC can be used to recognise kin (Yamazaki et al., 1983; Roser and Singh, 1991; Potts et al., 1991; Manning et al., 1992; Brennan and Peele, 2003), although similar effects are not documented in baboons. The MHC is a highly polymorphic gene complex that has been shown to influence the volatile scent profiles of mice (Yamazaki et al., 1979; Carroll et al., 2002), rats (Singh et al., 1987) and humans (Wedekind and Furi, 1997; Jacob et al., 2002). It is thus possible that individual odour differences perceived by mice result from MHC variation among individuals. Although attention has classically and largely focused on volatile compounds (such as odour variation influenced by MHC loci or by genes linked to these loci), it has lately become increasingly apparent that non-volatile proteins and peptides are also important in scent signals (Hurst et al., 2001). On the one hand, non-volatile components are

more stable and persistent than volatile components, which have a reduced longevity and can rapidly deteriorate. On the other hand, the detection of non-volatile components occurs through the vomeronasal system, which requires a longer scent contact than the detection of volatile components through the main olfactory system (Halpern and Martinez-Marcos, 2003; Hurst and Beynon, 2004). Both types of components (volatile and non-volatile) may differentially contribute to the communication of information on individual identity. In our experiments, mice were allowed to investigate odours at close contact; thus, both volatile and non-volatile scent components of the olfactory signal were likely to have been detected. One possibility to test whether the olfactory signal of baboons detected by our mice contains some non-volatile components would be to compare our results with a similar experiment where the mice are not allowed to make physical contact with the odour source.

Across taxonomic groups, the ability to assess the degree of relatedness among conspecifics appears essential in the evolution of behaviour such as nepotism and mate choice [in insects (Greenberg, 1979; Gamboa et al., 1996); in fishes (Neff and Sherman, 2003; Neff and Sherman, 2008); in mammals (O'Riain and Jarvis, 1998; Heth et al., 1998; Mateo, 2003)]. Odour is one of the possible cues used to detect kin. Such an ability relies on phenotypic comparisons and occurs in three steps: (i) similarity in odour production by related individuals, (ii) perception of such similarity, and (iii) behavioural reaction to this perception. Our data indicate that the first step of this process is effective in chacma baboons because variability in baboon odour production is influenced by their relatedness. Thus, a baboon could potentially use such cues to identify his own kin by comparing a known phenotype (either his own odour phenotype or one learned under unambiguous circumstances) with the odour phenotype of another individual, or to assess kinship between two individuals that are not directly related to him (but might be related to each other) by matching their respective body odours. In any case, the effective use of olfactory cues by baboons to identify their kin remains to be investigated. It remains possible that the olfactory abilities of baboons may limit their capacity to detect cues that are perceived by mice (Dominy and Lucas, 2001; Gilad et al., 2004) or that mice may not focus on the same olfactory components that baboons do.

Our study opens perspectives for both biochemistry and behavioural ecology. First, we hope that this research, by contributing a simple and cost-effective tool to the study of chemical communication in animals, will encourage its exploration in a variety of taxa and contexts. Second, our finding that odours may play a role in both the signalling of individual characteristics and relatedness among female baboons suggest that this cue is possibly used for kin recognition in this species and possibly other anthropoid primates, and we hope it will encourage future research to investigate both intra-specific perception of odour similarity and its associated behavioural response in non-human primates.

ACKNOWLEDGEMENTS

REFERENCES

Ables, E. M., Kay, L. M. and Mateo, J. M. (2007). Rats asses degree of relatedness from human odors. *Physiol. Behav.* **90**, 726-732.

Alvergne, A., Faurie, C. and Raymond, M. (2009). Father–offspring resemblance predicts paternal investment in humans. *Anim. Behav.* **78**, 61-69.

Bateson, P. (1983). *Mate Choice*. Cambridge: Cambridge University Press.

Beecher, M. D. (1988). Kin recognition in birds. *Behav. Genet.* **18**, 465-482.

Belcher, A. M., Smith, A. B., III, Jurs, P. C., Lavine, B. and Epple, G. (1986). Analysis of chemical signals in a primate species (*Saguinus fuscicollis*): Use of behavioral, chemical, and pattern recognition methods. *J. Chem. Ecol.* **12**, 513-531.

Belcher, A. M., Epple, G., Greenfield, K. L., Richards, L. E., Küderling, I. and Smith, A. B. (1990). Proteins: biologically relevant components of the scent marks of a primate (*Saguinus fuscicollis*). *Chem. Senses* **15**, 431-446.

Bonadonna, F. and Nevitt, G. A. (2004). Partner-specific odor recognition in an Antarctic seabird. *Science* **306**, 835-835.

Boulet, M., Charpentier, M. J. E. and Drea, C. M. (2009). Decoding an olfactory mechanism of kin recognition and inbreeding avoidance in a primate. *BMC Evol. Biol.* **9**, 281.

Breer, H. (2001). Olfaction. In *Encyclopedia of Life Sciences* (ed. L. C. J. W. Sons), pp. 1-71. London: Nature Publishing Group.

Brennan, P. A. and Peele, P. (2003). Towards an understanding of the pregnancy-blocking urinary chemosignals of mice. *Biochem. Soc. Trans.* **31**, 152-155.

Buchan, J. C., Alberts, S. C., Silk, J. B. and Altmann, J. (2003). True paternal care in a multi-male primate society. *Nature* **425**, 179-181.

Busquet, N. and Baudouin, C. (2005). Odour similarities as a basis for discriminating degrees of kinship in rodents: evidence from *Mus spicilegus*. *Anim. Behav.* **70**, 997-1002.

Carroll, L. S., Penn, D. and Potts, W. K. (2002). Discrimination of MHC-derived odors by untrained mice is consistent with divergence in peptide-binding region residues. *Proc. Natl. Acad. Sci. USA* **99**, 2187-2192.

Célérier, A., Piérard, C., Rachbauer, D., Sarrieau, A. and Béracochéa, D. (2004). Contextual and serial discriminations: A new learning paradigm to asses simultaneously the effects of acute stress on retrieval of flexible or stable information in mice. *Learn. Mem.* **11**, 196-204.

Chapais, B. (2006). Kinship, competence and cooperation in primates. In *Cooperation in Primates and Humans: Mechanisms and Evolution* (ed. P. M. Kappeler and C. P. van Shaik), pp. 47-64. Berlin, Heidelberg: Springer-Verlag.

Charpentier, M. J. E., Boulet, M. and Drea, C. M. (2008). Smelling right: the scent of male lemurs advertises genetic quality and relatedness. *Mol. Ecol.* **17**, 3225-3233.

Cheetham, S. A., Thom, M. D., Jury, F., Ollier, W. E. R., Beynon, R. J. and Hurst, J. L. (2007). The genetic basis of individual-recognition signals in the mouse. *Curr. Biol.* **17**, 1771-1777.

Clarke, P. M. R., Barrett, L. and Henzi, S. P. (2009). What role do olfactory cues play in chacma baboon mating? *Am. J. Primatol.* **71**, 493-502.

Cowlishaw, G. (1999). Ecological and social determinants of spacing behaviour in desert baboon groups. *Behav. Ecol. Sociobiol.* **45**, 67-77.

Dasser, V. (1988). A social concept in Java monkeys. *Anim. Behav.* **36**, 225-230.

Dominy, N. J. and Lucas, P. W. (2001). Ecological importance of trichromatic vision to primates. *Nature* **410**, 363-366.

Drea, C. M., Vignieri, S. N., Kim, H. S., Weldele, M. L. and Glickman, S. E. (2002). Responses to olfactory stimuli in spottedhyenas (*Crocuta crocuta*): II. discrimination of conspecific scent. *J. Comp. Psychol.* **116**, 342-349.

Dugatkin, L. A. (1997). *Cooperation Among Animals: An Evolutionary Perspective*. Oxford: Oxford University Press.

Epple, G. (1986). Communication by chemical signals. In *Comparative Primate Biology*, vol. 2A (eds G. Mitchel and J. Erwin), pp. 531-580. New York: Alan R. Liss.

Gamboa, G. J., Berven, K. A., Schemidt, R. A., Fishwild, T. G. and Jankens, K. M. (1991). Kin recognition by larval wood frogs (*Rana sylvatica*): effects of diet and prior exposure to conspecifics. *Oecologia* **86**, 319-324.

Gamboa, G. J., Grudzien, T. A., Espelie, K. E. and Bura, E. A. (1996). Kin recognition pheromones in social wasps: combining chemical and behavioural evidence. *Anim. Behav.* **51**, 625-629.

Gilad, Y., Wiebe, V., Przeworski, M., Lancet, D. and Pääbo, S. (2004). Loss of olfactory receptor genes coincides with the acquisition of full trichromatic vision in primates. *PLoS Biol.* **2**, 120-125.

Gosling, S. D. (2001). From mice to men: what can we learn about personality from animal research? *Psychol. Bull.* **127**, 45-86.

Greenberg, L. (1979). Genetic component of bee odor in kin recognition. *Science* **206**, 1095-1097.

Halpern, M. and Martínez-Marcos, A. (2003). Structure and function of the vomeronasal system: an update. *Prog. Neurobiol.* **70**, 245-318.

Halpin, Z. T. (1986). Individual odors among mammals: origins and functions. *Adv. Study Behav.* **16**, 39-70.

Hamilton, W. D. (1964). The genetical evolution of social behaviour I & II. *J. Theor. Biol.* **7**, 1-52.

Hauber, M. E. and Sherman, P. W. (2001). Self-referent phenotype matching: theoretical considerations and empirical evidence. *Trends Neurosci.* **24**, 609-616.

Hepper, P. G. (1991). *Kin Recognition*. Cambridge: Cambridge University Press.

Heth, G., Todrank, J. and Johnston, R. E. (1998). Kin recognition in golden hamsters: evidence for phenotype matching. *Anim. Behav.* **56**, 409-417.

Heymann, E. W. (2006). The neglected sense of smell in primate behavior, ecology and evolution. *J. Primatol.* **68**, 519-524.

Holmes, W. G. and Sherman, P. W. (1982). The ontogeny of kin recognition in two species of ground squirrels. *Am. Zool.* **22**, 491-517.

Hübener, F. and Laska, M. (1998). Assessing olfactory performance in an Old World primatete, *Macaca nemestrina. Physiol. Behav.* **64**, 521-527.

Huchard, E., Alvergne, A., Fejan, D., Knapp, L. A., Cowlishaw, G. and Raymond, M. (2010). More than friends? Behavioural and genetic aspects of heterosexual associations in wild chacma baboons, *Behav. Ecol. Sociobiol.* doi:10.1007/s00265-009-0894-3.

Hurst, J. L. and Beynon, R. J. (2004). Scent wars: the chemobiology of competitive signalling in mice. *BioEssays* **26**, 1288-1298.

Hurst, J. L., Robertson, D. H. L., Tolladay, U. and Beynon, R. J. (1998). Proteins in urine scent marks of male house mice extend the longevity of olfactory signals. *Anim. Behav.* **55**, 1289-1297.

Hurst, J. L., Payne, C. E., Nevison, C. M., Marie, A. D., Humphries, R. E., Robertson, D. H. L., Cavaggioni, A. and Beynon, R. J. (2001). Individual recognition in mice mediated by major urinary proteins. *Nature* **414**, 631-634.

Jacob, S., McClintock, M. K. and Zelano, B. (2002). Paternally inherited HLA alleles are associated with women's choice of male odor. *Nat. Genet.* **30**, 175-179.

Kliethermes, C. L. and Crabbe, J. C. (2006). Genetic independence of mouse measures of some aspects of novelty seeking. *Proc. Natl. Acad. Sci. USA* **103**, 5018-5023.

Manning, C. J., Wakeland, E. K. and Potts, W. K. (1992). Communal nesting patterns in mice implicate MHC genes in kin recognition. *Nature* **360**, 581-583.

Mardon, J. and Bonadonna, F. (2009). Atypical homing or self-odour avoidance? Blue petrels (*Halobaena caerulea*) are attracted to their mate's odour but avoid their own *Behav. Ecol. Sociobiol.* **63**, 537-542.

Mateo, J. M. (2002). Kin-recognition abilities and nepotism as a function of sociality. *Proc. R. Soc. Lond., B, Biol. Sci.* **269**, 721-727.

Mateo, J. M. (2003). Kin recognition in ground squirrels and other rodents. *J. Mammal.* **84**, 1163-1181.

Mateo, J. M. (2006a). Development of individually distinct recognition cues. *Dev. Psychobiol.* **48**, 508-519.

Mateo, J. M. (2006b). The nature and representation of individual recognition cues in Belding's ground squirrels. *Anim. Behav.* **71**, 141-154.

Mateo, J. M. and Johnston, R. E. (2003). Kin recognition by self-referent phenotype matching: weighing the evidence. *Anim. Cogn.* **6**, 73-76.

Neff, B. D. (2003). Decisions about parental care in response to perceived paternity. *Nature* **422**, 716-719.

Neff, B. D. and Sherman, P. W. (2003). Nestling recognition via direct cues by parental male bluegill sunfish (*Lepomis macrochirus*). *Anim. Cogn.* **6**, 87-92.

Neff, B. D. and Sherman, P. W. (2005). *In vitro* fertilization reveals offspring recognition *via* self-referencing in a fish with paternal care and cuckoldry. *Ethology* **111**, 425-438.

Nevison, C. M., Armstrong, S., Beynon, R. J., Humphries, R. E. and Hurst, J. L. (2003). The ownership signature in mouse scent marks is involatile. *Proc. R. Soc. Lond., B, Biol. Sci.* **270**, 1957-1963.

Nevitt, G. A. and Bonadonna, F. (2005). Sensitivity to dimethyl sulphide suggests a mechanism for olfactory navigation by seabirds. *Biol. Lett.* **1**, 303-305.

Novotny, M. V. (2003). Pheromones, binding proteins and receptor responses in rodents. *Biochem. Soc. Trans.* **31**, 117-122.

O'Riain, M. J. and Jarvis, J. U. M. (1998). The dynamics of growth in naked mole-rats: the effects of litter order and changes in social structure. *J. Zool.* **246**, 49-60.

Penn, D. and Potts, W. K. (1998). Untrained mice discriminate MHC-determined odors. *Physiol. Behav.* **63**, 235-243.

Potts, W. K., Manning, C. J. and Wakeland, E. K. (1991). Mating patterns in seminatural populations of mice influenced by MHC genotype. *Nature* **352**, 619-621.

Reeve, H. K. (1989). The evolution of conspecific acceptance thresholds. *Am. Nat.* **133**, 407.

Rendall, D., Rodman, P. S. and Emond, R. E. (1996). Vocal recognition of individuals and kin in free-ranging rhesus monkeys. *Anim. Behav.* **51**, 1007-1015.

Reynolds, J. D. and Sheldon, B. C. (2003). 'Wise fathers'. *Nature* **422**, 669-670.

Roser, B., Brown, R. E. and Singh, P. B. (1991). Excretion of transplatation antigens as signals of genetic individuality. New York: Marcel Dekker.

Schwagmeyer, P. L. (1988). Scramble-competition polygyny in an asocial mammal: male mobility and mating success. *Am. Nat.* **131**, 885.

Scordato, E. S., Dubay, G. and Drea, C. M. (2007). Chemical composition of scent marks in the ringtailed lemur (*Lemur catta*): glandular differences, seasonal variation, and individual signatures. *Chem. Senses* **32**, 493-504.

Setchell, J. M., Vaglio, S., Moggi-Cecchi, J., Boscaro, F., Calamai, L. and Knapp, L. A. (2010). Chemical composition of scent-gland secretions in an Old World monkey (*Mandrillus sphinx*): influence of sex, male status, and individual identity. *Chem. Senses* **35**, 205-220.

Shepherd, G. M. (2004). The human sense of smell: are we better than we think? *PLoS Biol.* **2**, 572-575.

Sherman, P. W., Reeve, H. K. and Pfennig, D. W. (1997). *Behavioural Ecology: an Evolutionary Approach.* Oxford: Oxford University Press.

Singh, P. B., Brown, R. E. and Roser, B. (1987). MHC antigens in urine as olfactory recognition cues. *Nature* **327**, 161.

Smith, T. E., Abbott, D. H., Tomlinson, A. J. and Mlotkewicz, J. A. (1997). Differential display of investigative behavior permits discrimination of scent signatures from familiar and unfamiliar socially dominant female marmoset monkeys (*Callithrix jacchus*). *J. Chem. Ecol.* **23**, 2523-2546.

Smith, T. E., Tomlinson, A. J., Mlotkewicz, J. A. and Abbott, D. H. (2001). Female Marmoset monkeys (*Callithrix jacchus*) can be identified from the chemical composition of their scent marks. *Chem. Senses* **26**, 449-458.

Smith, K., Alberts, S. C. and Altmann, J. (2003). Wild female baboons bias their social behaviour towards paternal half-sisters. *Proc. R. Soc. Lond., B, Biol. Sci.* **270**, 503-510.

Snowdon, C. T., Ziegler, T. E., Schultz-Darken, N. J. and Ferris, C. F. (2006). Social odours, sexual arousal and pairbonding in primates. *Philos. Trans. R Soc. Lond., B, Biol. Sci.* **361**, 2079-2089.

Todrank, J., Heth, G. and Johnston, R. E. (1998). Kin recognition in golden hamsters: evidence for kinship odours. *Anim. Behav.* **55**, 377-386.

Vokey, J. R. and Rendall, D. (2004). Visual kin recognition and family resemblance in Chimpanzees (*Pan troglodytes*). *J. Comp. Psychol.* **118**, 194-199.

Waldman, B. (1988). The ecology of kin recognition. *Annu. Rev. Ecol. Syst.* **19**, 543-571.

Wang, J. (2007). Triadic IBD coefficients and applications to estimating pairwise relatedness. *Genet. Res.* **89**, 135-153.

Wedekind, C. and Furi, S. (1997). Body odour preferences in men and women: do they aim for specific MHC combinations or simply heterozygosity? *Proc. R. Soc. Lond., B, Biol. Sci.* **264**, 1471-1479.

Weisfeld, G. E., Czilli, T., Phillips, K. A., Gall, J. A. and Lichtman, C. M. (2003). Possible olfaction-based mechanisms in human in recognition and inbreeding avoidance. *J. Exp. Child Psychol.* **85**, 279-295.

Weiss, A., King, J. E. and Perkins, L. (2006). Personality and subjective well-being in orangutans (*Pongo pygmaeus* and *Pongo abelii*). *J. Pers. Soc. Psychol.* **90**, 501-511.

Widdig, A. (2007). Paternal kin discrimination: the evidence and likely mechanisms. *Biol. Rev.* **82**, 319-334.

Wyatt, T. D. (2003). *Pheromones and Animal Behaviour, Communication by Smell and Taste.* Cambridge: Cambridge University Press.

Yamazaki, K., Yamaguchi, M., Baranoski, L., Bard, J., Boyse, E. A. and Thomas, L. (1979). Recognition among mice. Evidence from the use of a Y-maze differentially scented by congenic mice of different major histocompatibility types. *J. Exp. Med.* **150**, 755-760.

Yamazaki, K., Beauchamp, G. K., Egorov, I. K., Bard, J., Thomas, L. and Boyse, E. A. (1983). Sensory distinction between H-2b and H-2bml mutant mice. *Proc. Natl. Acad. Sci. USA* **80**, 5685-5688.

Zhang, J. X., Soini, H. A., Bruce, K. E., Wiesler, D., Woodley, S. K., Baum, M. J. and Novotny, M. V. (2005). Putative chemosignals of the ferret (*Mustela furo*) associated with individual and gender recognition. *Chem. Senses* **30**, 727-737.