Banded mongooses discriminate relatedness and MHC diversity in unfamiliar conspecifics

Short title: Banded mongoose smell relatedness and MHC diversity

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ABSTRACT

Olfactory cues play a vital role in mammalian social communication, conveying fitness-relevant information such as genetic quality and relatedness. Kin recognition through scent can help avoid inbreeding and guide nepotistic behaviors, enhancing fitness. In banded mongooses, synchronized breeding disrupts familiarity-based kin recognition, potentially increasing reliance on phenotype matching, where individuals compare genetically determined odors to assess similarity. We tested whether banded mongooses use odors to assess genetic diversity and relatedness based on (1) major histocompatibility complex (MHC) genotypes and (2) neutral microsatellite loci. Results showed individuals responded differently to odors from unfamiliar conspecifics based on MHC diversity and relatedness. Specifically, less MHC-diverse and less related individuals attracted more interest, suggesting odor cues are used to evaluate intruder or competitor threat levels. Neutral genetic diversity did not affect odor responses and was not correlated with MHC diversity, indicating responses to MHC diversity are independent of overall genetic diversity. No effect of MHC similarity was observed, possibly due to sample size limitations. Our findings suggest MHC diversity may signal genetic quality, while other genomic regions might contribute to assessing relatedness. These results provide a foundation for further research into the role of MHC and other genes in social communication in species where phenotype matching offers adaptive benefits.

Keywords: MHC, relatedness, familiarity, *Mungos mungo*, chemical communication, social signaling, anal gland, secretion, inbreeding

INTRODUCTION

Scent marks in mammals play a key role in social communication, conveying fitness-relevant information such as relatedness, genetic quality, and compatibility (Charpentier et al., 2008; Stoffel et al., 2015). Recognizing related individuals through olfactory cues helps animals avoid inbreeding depression (Pusey and Wolf, 1996), especially in populations where delayed dispersal increases the risk of mating with close relatives (Koenig and Dickinson, 2004; Nichols, 2017; Russell, 2009). Additionally, these cues can guide nepotistic behaviors, supporting kin and enhancing indirect fitness (Hamilton, 1964). Scent marks may also signal genetic compatibility, influencing mate selection and improving offspring viability (Penn, 2002).

Odors reflecting genetic information are particularly intriguing because their nature suggests that they must have a genetic basis. One prominent system for such signaling is the major histocompatibility complex (MHC), which is both highly polymorphic and critical to immune function (Bjorkman et al., 1987; Klein, 1986). MHC molecules bind peptides for self/non-self recognition and initiate immune responses against pathogens. MHC class I (MHC-I) molecules, present on nearly all nucleated cells, detect intracellular peptides, while MHC class II (MHC-II) molecules, found on professional antigen-presenting cells (APC), bind extracellular peptides that have been ingested by the APC (Klein, 1986; Neefjes et al., 2011). Pathogen-driven evolution of the MHC underpins its extraordinary allelic diversity.

Three main mechanisms—heterozygote advantage, rare allele advantage, and fluctuating selection—are thought to maintain MHC diversity (Radwan et al., 2020; Spurgin and Richardson, 2010). Heterozygote advantage allows individuals with more diverse MHC alleles to bind a wider variety of peptides, enhancing pathogen defense (Pierini and Lenz, 2018). Rare allele advantage arises because pathogens adapt to common alleles, making rare alleles more effective (Lenz, 2018). The MHC's role in immunity also extends to mate choice, where studies across vertebrates indicate a preference for MHC-diverse partners, potentially enhancing offspring fitness (Kamiya et al., 2014; Winternitz et al., 2017; Winternitz and Abbate, 2022). Despite these findings, the mechanisms linking MHC diversity to mate choice and fitness remain poorly understood.

MHC-related information might be transmitted through odor in several ways: (1) MHC molecules shed from cells (Boehm and Zufall, 2006), (2) peptides bound by MHC molecules (Hinz et al., 2013; Milinski et al., 2005; Spehr et al., 2006), or (3) MHC-regulated changes in the microbial community (Singh et al., 1990) or metabolic pathway (Aksenov et al., 2012) that produce odorants (Milinski, 2022; Schubert et al., 2021). Animals may use these cues to assess kinship through two main strategies: familiarity or phenotype matching (Lacy and

Sherman, 1983; Moore and Ali, 1984; Todrank and Heth, 2003). Familiarity relies on early-life associations to avoid mating with close kin, such as parents or siblings (Berger and Cunningham, 1987). Phenotype matching, on the other hand, allows individuals to compare their own scent with that of others to estimate genetic similarity, enabling kin recognition even among unfamiliar individuals (Lacy and Sherman, 1983; Todrank and Heth, 2003). This flexibility highlights the potential for MHC-mediated cues to influence social and reproductive behavior in diverse contexts.

Banded mongooses are cooperative breeders that show limited dispersal and usually reproduce within their natal pack (Cant et al., 2016). Inbreeding thus occurs frequently and in the population observed for more than 25 years in the Queen Elizabeth National Park in Uganda, two thirds of the population are to some extent inbred, including 7.1% with inbreeding coefficients above 0.25, which results from full-sibling or parent-offspring matings (Wells et al., 2018). Despite inbreeding being widespread, it has been observed to incur a cost on individual fitness in the form of yearling body mass and male reproductive success (Wells et al., 2018). Female banded mongooses can synchronize their estrus resulting in all breeding females giving birth on the same day and combining their pups into a single communal litter (Cant et al., 2016). This breeding behavior likely disrupts familiarity cues (Marshall et al., 2021). However, banded mongooses choose mates that are less closely related than what would be expected by chance (Sanderson et al., 2015) and this pattern cannot be explained by the use of familiarity cues (Khera et al., 2021). Furthermore, banded mongooses appear to discriminate relatedness when evicting members from the group. Females are more likely to evict females that are younger, and they also appear to apply negative kin discrimination, as more closely related females are more likely to be targeted (Thompson et al., 2017). Another context in which discrimination of relatedness may be used is escorting. The synchronized reproduction leads to a communal litter and once the pups leave the den and join the group for foraging trips at approximately 3-4 weeks of age, adults provide pups with food (Cant et al., 2016). This pup-escort relationship is beneficial for the pup, as it increases survival, body weight and faster reproductive onset (Hodge, 2005). Although there is no evidence that pup-escort pairs are formed based on relatedness, male escorts have been observed to increase their care by spending more time escorting pups that are more closely related to them (Vitikainen et al., 2017).

Mitchell et al. (2018) showed that banded mongooses can assess relatedness among familiar group members even when familiarity cues are disrupted. Odor interest declined with increasing relatedness, suggesting phenotype matching may guide mate choice when familiarity is unreliable. However, this mechanism appears limited to familiar conspecifics, as responses to unfamiliar odors did not vary with relatedness. Beyond kin discrimination for

inbreeding avoidance, phenotype matching may be used to assess relatedness levels in an intra-sexual selection context as well. One of these contexts is the eviction described in the previous paragraph, during which females negatively discriminate relatedness when the evictees are older (Thompson et al., 2017). For males, assessing genetic quality of potential intruders might be particularly important. Compared to females, males have a greater role in territory defense (Cant et al., 2016) and subordinate males respond first to an intruder, are more aggressive towards them than dominant males and spend more time inspecting them (Cant et al., 2002). Gaining information about the genetic makeup of a potential intruder might aid in assessing the potential threat of the intruder or its potential for competing for matings with the defender and help allocate resources effectively.

Our study tested whether banded mongooses use odor cues, potentially stemming from the MHC, for discriminating unfamiliar conspecifics. We predicted that wild mongooses tested on their natural territories would (i) show greater interest in mongoose odors than blank controls, (ii) show reduced interest in odors from genetically related or MHC-similar individuals, and (iii) respond more strongly to odors from MHC-diverse individuals in both mating (opposite-sex) and competitive (same-sex) contexts.

MATERIAL AND METHODS

Study site

Data used in this study were collected from a wild population of banded mongooses in Queen Elizabeth National Park in Uganda (0°12'S, 27°54E'). The study area consists of approximately 10 km² savannah and includes the Mweya peninsula and the surrounding mainland area. Behavioral, life-history and genetic data as well as information on group composition and territorial structures have been collected regularly and systematically for over 25-years. The population consists of 10-12 packs at any one time, corresponding to approximately 250 individuals. Individuals are identifiable in the field by sight based on (1) dye patterns in the fur that were applied using commercial hair dye (L'Oreal, UK) for individuals up to 6 months old, and (2) shaved fur patterns or (3) color-coded plastic collars for adults that had stopped growing. Shave patterns and collars were maintained during trapping events that took place every 3-6 months as described by Cant (2000), Hodge (2007), and Jordan et al. (2010). Upon first capture, individuals were given either an individual tattoo or a subcutaneous pit tag under anesthetic (TAG-P-122IJ, Wyre Micro Design Ltd., UK) to allow permanent identification, and a 2mm tissue sample was taken from the tip of the tail for genetic analysis.

Odor collection

Banded mongooses scent mark frequently and use it for communication between packs, for example to mark their territory (Jordan, 2009), and also to convey information within packs regarding reproductive state (Mitchell et al., 2017a), and relatedness (Mitchell et al., 2018). Thus, we used anal gland secretion (AGS) as the source of odor. We collected AGS from the only two well-habituated social groups (known as 1B and 1H) that were inhabiting non-neighboring territories and thus were unfamiliar with each other. Samples were collected between May and July of 2022 from 37 adults (≥12 months of age), comprising 8 females and 29 males. These individuals represent all adult individuals from pack 1B (females=8, males=24) and five males from pack 1H. The second group was just recently formed through a fusion of males from a habituated group and unhabituated females, which is why no scent collections or presentations to those females were possible. These circumstances together with the higher longevity of males (Cant et al., 2016) caused a strong male bias in our sample. Animals were trapped according to the protocol described in Jordan et al. (2011b). In short, animals were trapped using Tomahawk traps equipped with bait and anaesthetized using isoflurane. Before extraction of AGS, the skin surrounding the exit of the gland was cleaned using clean cotton wool and Nitrile gloves were worn by the handler during the procedure. Without touching the gloves, the AGS was then collected in 2.5 ml screw-cap glass vials. Distribution between sample vials was solely performed using glass pipettes or metal spoons to avoid altering the odor. The AGS was then immediately frozen in liquid nitrogen until further usage. Groups used for scent presentations were not in estrus and females were not pregnant during sample collection.

Odor presentations

A total of 361 odor presentations (323 experimental and 38 control) were conducted using samples from 37 donors and presented to 38 recipient (Pack IB: females = 10, males = 23; Pack 1H: males = 5). Each individual received one control and an average of nine experimental odor presentations (range = 4-32). Odor samples were removed from the liquid nitrogen and were put on ice in a thermos flask (for a maximum of 90 minutes) until usage in the field. Once a pack was located and individuals resumed foraging, the sample was defrosted and applied to a clean tile using a glass stick or metal spatula. Presentations followed the methods of Mitchell et al. (2017b). Briefly, the tile was placed on the ground within 2 m of the focal individual (depending on habituation) while it foraged at least 1 m from conspecifics. Responses were filmed with a handheld camera and recording stopped when the individual resumed foraging, moved more than one body-tail length from the tile, or began resting or grooming. The tile was cleaned with hot water and baking soda using a brush after every presentation. A control was conducted for each individual to make sure individuals were not responding to the novelty of the tile itself, but to the odor presented. For

this reason, individuals were presented with a clean tile that contained no odor sample. Each individual was only presented one odor per day and after two days of presentations the pack was given one day without presentations. Moreover, if a presentation was interrupted, e.g. an individual inspecting the odor was startled by a warning call or pushed away from the odor by another individual, repetition of the presentation of this odor was shifted as far to the end of the field season as possible. Both measures were implemented to avoid habituation to the odor and thus changes in the response to it.

Video analysis of responses

Videos were analyzed independently by two people using BORIS software (Friard and Gamba, 2016). Responses to the odor presented were categorized as (i) time spent in proximity (one body-tail length) to the odor (= duration in seconds) (ii) time spent directly above or touching the tile containing the odor (= contact in seconds), and (iii) marking behaviors. Duration and contact both started once the nose of the individual was above the tile. Contact time was measured until the individual either stopped touching the tile with a body part or until it stopped holding its head above the tile. 'Duration time' continued until the individual resumed foraging, laid down, groomed other individuals, or moved away from the tile with a distance of at least one body-tail length. Contact behaviors could be split further into sniffing, licking and rolling. Marking behavior included overmarking as well as markings in the vicinity of the tile (one body-tail length) and could either be urine, feces or AGS markings. As concluded by Mitchell et al. (2017a), these measures are not independent of one another, as an increased number of vicinity marks increases the duration spent in the vicinity of the odor and thus need to be interpreted accordingly. Moreover, since we didn't know in which context MHC diversity might influence behavior, we included both overmarking, which may have a competitive function (Jordan et al., 2011a; Rich and Hurst, 1999; Wolff et al., 2002), as well as vicinity marks, which are thought to be important in mate-choice decisions (Rich and Hurst, 1999), in the analysis.

Neutral genetic analyses

We extracted DNA from all individuals present during the experiments, including 37 from whom we collected AGS and 38 to whom we presented the odors (36 individuals overlapped between AGS and recipient groups) using the Qiagen® DNeasy blood and tissue kit according to the manufacturers protocol. These individuals were genotyped at 35-43 neutral microsatellite loci based on the methods described in detail by Sanderson et al. (2015). Individual standardized multilocus heterozygosity (sMLH) was calculated using the R package InbreedR (Stoffel et al., 2016). Genetic marker-based relatedness (Queller and Goodnight, 1989) was estimated using GENALEX (Peakall and Smouse, 2006).

MHC genetic analyses

We genotyped banded mongooses at MHC loci using a custom Twist hybridization panel and PacBio HiFi long-read sequencing (Winternitz, J.C., Schubert, N., Heitlinger, E., Foster, R. G., Cant, M.A., Mwanguhya, F., Businge, R., Kyambulima, S., Mwesige, K., Nichols H.J., unpublished). 32 samples used in this study were prepared for HiFi sequencing. Due to low DNA quality and off-target read amplification, 20 samples yielded sufficient data, producing a total of 107,091 unique HiFi reads (mean ± s.d. = 5,345 ± 3,368 per individual). Reads were assembled, mapped, and variants called with standard pipelines, and individuals were successfully genotyped at seven MHC-I and seven MHC-II loci. MHC diversity was quantified as the number of alleles and functional supertypes per individual, while similarity between individuals was estimated from allele and supertype sharing (Wetton et al., 1987). As the number of alleles per individual increased with HiFi read count (Pearson's cor = 0.532, p-value = 0.028), the number of unique HiFi reads was included in downstream analyses. Supertypes were defined by clustering amino acid physio-chemical descriptors of peptide-binding residues, with clustering repeatability statistically validated. Full laboratory protocols, bioinformatic workflows, and clustering procedures are detailed in the Supplementary Materials.

Ethical note

Research was conducted under approval of the Uganda National Council for Science and Technology with the research registration number NS273ES, the Uganda Wildlife Authority and the corresponding reference COD/96/05 and the Ethical Review Committee of the University of Exeter. All research procedures adhered to the ASAB Guidelines for the Treatment of Animals in Behavioral Research and Teaching (ASAB Ethical Committee and ABS Animal Care Committee, 2022).

Statistical analysis

Preliminary analyses

We first tested whether banded mongooses responded to anal gland odors rather than to a novel object. To do so, we fitted six linear mixed effect models (LMMs) comparing behavioral responses between control and experimental presentations using 38 recipient individuals (10 female, 28 male) from 2 packs presented with odor from 37 unfamiliar individuals. This initial step established which responses differed significantly between treatments and were therefore appropriate for inclusion in subsequent analyses. Full details of LMM fitting can be found in the Supplementary methods.

Correlational analysis

Strong collinearity among variables included in statistical models can impede model interpretation (Harrison et al., 2018). Therefore we used Pearson's product-moment correlation to investigate the degree of correlation between (1) MHC diversity (the number of distinct alleles per individual) and genomic diversity (sMLH), (2) MHC allele similarity and relatedness, and (3) MHC supertype similarity and relatedness. Since response measures are not independent of one another, as the time spent sniffing an odor or marking should correlate with the time spent in proximity to an odor (Mitchell et al., 2017a), we also investigated potential collinearities between all behavioral response variables (Contact, Sniffing, Duration, Licking, Marking and Rolling).

Correlations between the MHC diversity measures showed highly significant and strong correlations between MHC allele number and supertype number ($r = 0.897$, $p < 1.04E-6$) and MHC allele similarity and MHC supertype similarity ($r = 0.788$, $p = 1.73E-4$). Since both allele number and supertype number contain different levels of information on functional diversity of the MHC, we decided to include all measures in our analyses but in separate models. Among behavioral responses, contact and rolling were highly correlated ($r = 0.737$, $p < 2.2E-16$), but only contact, sniffing, and duration differed between control and experimental presentations; these were therefore carried forward. Contact and duration also correlated strongly ($r = 0.698$, $p < 2.2E-16$), yet we kept both in separate models to capture potentially distinct behavioral information. Full correlation results are presented in Supplementary Tables S1 and S2.

Linear mixed models

Following preliminary analyses and variable selection, we investigated whether banded mongooses responded to the sex and genetic diversity of the odor donor, and to the relatedness between the donor and recipient. MHC measures were not included in this model to maximize the size of the dataset (only 20 of 39 individuals had MHC data available for them). One individual (BF931) was removed from the analyses as it had only been genotyped at 5 microsatellite loci, so relatedness and heterozygosity estimates were potentially unreliable (all other individuals had been genotyped at a minimum of 18 loci). Another (BM952) was removed from the odor donors because it lacked microsatellite data.

In three separate LMMs, we modeled our response variables (contact (log), sniffing (log +1) or duration (log)) predicted by the sex of the donor and recipient, genetic relatedness and sMLH. The identity of the odor donor and recipient were included as random effects. The packs of the odor donor and recipients were not included as random effects because the variance explained by them was low, including them usually led to a singular fit, and the effect of the pack should be subsumed within the individual identities of the pack members.

This analysis included 308 odor presentations: male to male (N=228), male to female (N=45), and female to male (N=35). No female odor was presented to females, as the females of one of the packs were not sufficiently habituated to perform presentations. Odor presentations involved 37 individual recipients (Pack IB: female = 9, male = 23; Pack 1H: male = 5) and 35 individual odor donors (Pack IB: female = 7, male = 23; Pack 1H: male = 5).

To investigate the effect of MHC diversity on behavioral responses, we fitted six LMMs, each using one of the following response variables: (contact (log), sniffing (log +1) or duration (log)). Explanatory variables included the number of unique MHC alleles and, in separate models, the number of distinct supertypes. We also included sMLH to control for background genomic diversity, the number of HiFi reads to control for sequencing effort, and the sex of both the odor donor and recipient. Odor recipient identity was included as a random effect. Odor donor identity was not included as a random effect because it explained zero variance and caused a singular fit, likely because the variance associated with the odor donor was related to its sex. One individual (BM867) was excluded from odor donors because it had an anomalously low number of MHC alleles despite an extremely high number of PCR duplicate reads, indicating sequencing artifacts and yielding an outlier in unique HiFi read counts. After this exclusion, the dataset comprised 111 experimental odor presentations (male to female: N = 9, female to male: N = 20, male to male: N = 82, no female odor was presented to females) involving 17 odor donors (Pack IB: female = 4, male = 12; Pack 1H: male = 1) and 36 recipients (Pack IB: female = 9, male = 22; Pack 1H: male = 5). This means that all 31 individuals from Pack IB were presented with odor from a single male from Pack 1H, and results should be interpreted cautiously.

Finally, we investigated whether banded mongooses responded differently to odors based on MHC similarity between odor donor and recipient. Since MHC similarity data requires MHC genotypes to be available for both odor donor and recipient, there was a very limited number of data points available for this analysis. Individual BM867 was now excluded from recipients because his MHC similarity depended on his MHC genotype. This dataset comprised 33 odor presentations (male to female: N = 6, female to male: N = 4, male to male: N = 23) involving 17 odor donors (Pack IB: female = 4, male = 12; Pack 1H: male = 1) and 18 recipients (Pack IB: female = 6, male = 11; Pack 1H: male = 1). Again, all 17 recipients from Pack IB were presented with odor from a single male from Pack 1H. As with previous analyses, we fitted six models with the following response variables: (contact (log), sniffing (log +1) and duration (log)). MHC allelic and supertype similarity between donor and recipient were fitted as separate predictor variables, and recipient ID was fitted as a random effect. Due to the small

dataset, the model was reduced to a single predictor and random term to retain sufficient statistical power and avoid overfitting.

All LMMs were constructed in R version 4.4.0 (Team, 2023) using the lme4 package (Bates et al., 2017) and were fitted with a Gaussian family. Significant fixed effects were detected using the R package *afex* version 1.4-1 (Singmann et al., 2018) with Type III Analysis of Variance with Satterthwaite's method. Full details of donors and recipients, controls, and number of trials across the four different analyses (control vs experimental analyses, neutral diversity analyses, MHC diversity analyses, and MHC similarity analyses) are summarized in Table S5.

RESULTS

Influence of sex, relatedness and sMLH

Banded mongooses varied their responses towards the presentation of an unfamiliar AGS odor depending on the sex of the recipient, the sex of the odor donor, and their genetic relatedness (Tab. 1), whereas donor genomic diversity (sMLH) had no effect. Specifically, males consistently showed stronger responses than females, spending almost twice as long in contact with the presentations (predicted means on the original (seconds) scale: 7.0 ± 1.5 s vs. 3.4 ± 0.7 s, $p = 0.0002$), 50% longer sniffing them (4.6 ± 0.8 s vs. 3.0 ± 0.5 s, $p = 0.0029$), and nearly twice as long in total response duration (17.6 ± 3.4 s vs. 9.4 ± 1.8 s, $p < 0.0001$). Donor sex also influenced responses: odors from females elicited almost twice the contact time (3.4 ± 0.6 s vs. 1.9 ± 0.3 s, $p = 0.0029$) and longer total response durations (9.4 ± 1.6 s vs. 5.5 ± 1.0 s, $p = 0.0026$) compared to odors from males (Fig. 1). Finally, the duration of the response to odors decreased as relatedness between the donor and recipient increased, with closely related pairs showing roughly half the response duration observed between unrelated individuals (Fig. 2).

Table 1 Model output for models investigating influences affecting responses. Model output for effects of genetic relatedness between recipient and odor donor on contact, sniffing and duration responses (all on a log scale). P-values were calculated based on Satterthwaite's method. Significant p-values are in bold. Sample sizes were the same for all models. Observations: 308, Recipients: 37 (Pack IB: 9 F, 23 M; Pack 1H: 5 M), Odour donors: 35 (Pack IB: 7 F, 23 M; Pack 1H: 5 M).

Response variable	Fixed effect	Estimate	SE	p-value
Contact	Recipient sex	**0.725**	**0.182**	**0.0002**
	Odor sex	**-0.554**	**0.176**	**0.0029**
	sMLH	0.271	0.485	0.5828
	relatedness	-0.322	0.316	0.3093
Sniffing	Recipient sex	**0.411**	**0.128**	**0.0029**
	Odor sex	0.168	0.108	0.1230
	sMLH	-0.045	0.299	0.8822
	relatedness	0.070	0.192	0.7149
Duration	Recipient sex	0.626	0.139	**3.02E-05**
	Odor sex	-0.531	0.164	**0.0026**
	sMLH	0.124	0.468	0.7935
	relatedness	-0.634	0.260	**0.0151**

Influence of MHC diversity

MHC diversity of the odor donor, measured as the number of distinct alleles, had no effect on contact or sniffing. For duration, the effect was borderline (estimate = -0.045, SE = 0.023, p = 0.059; Fig. 3a, Tab. 2), with response times decreasing by ~4% for each additional allele in the donor's MHC repertoire. In these reduced datasets, male recipients still spent more than twice as long in contact with odors and nearly three times longer overall compared to females.

Similar to the allelic diversity effect, the number of distinct supertypes per individual, representing the amount of functional diversity of an individual rather than allelic diversity, showed a 9% reduction in duration response for every additional supertype (Fig. 3b, Tab. 2). This indicates that individuals spent longer time in the vicinity of odors that had fewer supertypes before resuming foraging or resting behaviors. In this model we again found that male recipients had approximately 2.5 times higher contact responses and nearly 3 times longer duration spent near the odor than females (Fig. 3, Tab. 2). Odor sex, genome-wide heterozygosity (sMLH), and the number of distinct supertypes had no detectable influence on any of the behavioral responses in these analyses.

Table 2 Model outputs for relationships with MHC diversity. Model output for effects of MHC diversity of the odor donor measured as the number of distinct alleles or supertypes respectively on contact, sniffing and duration responses. Significant p-values are in bold. Sample sizes were the same for all models. Observations: 111, Recipients: 36 (Pack IB: 9 F, 22 M; Pack 1H: 5 M), Odour donors: 17 (Pack IB: 4 F, 12 M; Pack 1H: 1 M).

MHC measure	Fixed effect	Response variable	Estimate	SE	p-value
Alleles	Recipient sex	Contact	0.893	0.357	**0.0145**
	Odor sex		-0.331	0.287	0.2516
	sMLH		-0.234	0.850	0.7841
	MHC diversity		-0.012	0.028	0.6550
	Unique reads (log)		0.189	0.202	0.3518
	Recipient sex	Sniffing	0.385	0.255	0.1390
	Odor sex		0.156	0.177	0.3800
	sMLH		0.018	0.527	0.9720
	MHC diversity		0.017	0.017	0.3110
	Unique reads (log)		-0.046	0.125	0.7150
	Recipient sex	Duration	0.982	0.288	**0.0010**
	Odor sex		-0.368	0.242	0.1319
	sMLH		-0.284	0.715	0.6919
	MHC diversity		-0.045	0.023	0.0593
	Unique reads (log)		0.297	0.170	0.0837
Supertype	Recipient sex	Contact	0.919	0.358	**0.0123**
	Odor sex		-0.333	0.284	0.2440
	sMLH		-0.057	0.890	0.9491
	MHC diversity		-0.037	0.047	0.4319
	Unique reads (log)		0.237	0.214	0.2701
	Recipient sex	Sniffing	0.383	0.255	0.1410
	Odor sex		0.129	0.177	0.4690
	sMLH		0.098	0.555	0.8610
	MHC diversity		0.001	0.029	0.9850
	Unique reads (log)		-0.002	0.133	0.9910
	Recipient sex	Duration	1.054	0.287	**0.0004**
	Odor sex		-0.354	0.237	0.1392
	sMLH		0.121	0.744	0.8716
	MHC diversity		-0.095	0.039	**0.0166**
	Unique reads (log)		0.391	0.179	**0.0315**

Influence of MHC similarity

Neither MHC allele similarity, supertype similarity, nor any other fitted variable had a significant effect on contact, sniffing or response duration (Table S4).

DISCUSSION

We found that banded mongooses varied in their duration investigating unfamiliar odors based on genetic relatedness to the odor donor and MHC diversity but not on MHC similarity or overall genetic diversity. Responses also differed depending on the sex of the odor donor

and recipient, with males spending 0.5 to 3 times longer in contact, sniffing, and in proximity to odors than females, particularly for female odors. These findings provide evidence for discrimination of genetic relatedness and MHC diversity in unfamiliar individuals' odors, suggesting that banded mongooses may employ kin recognition mechanisms like phenotype matching (Hepper, 1991; Holmes and Sherman, 1982; Lacy and Sherman, 1983) to assess genetic information in conspecifics.

Banded mongooses face a high risk of inbreeding due to limited dispersal, with over 80% of individuals remaining in their natal pack (Cant et al., 2016). In our study population, 64% of pups are born to females mating with resident males (Nichols et al., 2014), resulting in more than 7% of pups being offspring of first-order inbreeding, such as parent-offspring or full-sibling matings (Wells et al., 2018). This inbreeding has significant fitness costs, including increased parasite load (Mitchell et al., 2017c), reduced yearling body mass, and lower reproductive success in males (Wells et al., 2018). Identifying kin during mate selection could help mitigate these risks. Supporting this, inbreeding occurs less often than expected by chance, and males preferentially mate-guard less related females (Sanderson et al., 2015). As these patterns cannot be explained by familiarity-based cues (Khera et al., 2021), other mechanisms, such as phenotype matching, may be involved. Banded mongooses also appear to discriminate kin in other contexts, including cooperative behaviors (Vitikainen et al., 2017) and competitive interactions (Thompson et al., 2017).

Mitchell et al. (2018) demonstrated that banded mongooses can differentiate odors based on relatedness among familiar group members, but it was unclear whether this discrimination was due to the odors themselves or prior knowledge of the individuals. They found no evidence of relatedness discrimination in unfamiliar individuals, though their relatively small sample size (N = 121 presentations) may have limited the analysis. In contrast, our study used a larger sample (N = 308 presentations) of unfamiliar individuals and found that relatedness significantly decreased the duration of responses to odors. By exclusively testing unfamiliar individuals, we eliminate the confounding effect of familiarity, providing strong evidence that banded mongooses use odor-based cues to assess relatedness via phenotype matching.

While familiarity is a common proxy for relatedness (Pusey and Wolf, 1996), it may be insufficient in species where reproductive and social dynamics complicate the use of associative learning. In cooperative species with high reproductive skew—such as meerkats, where dominant pairs monopolize reproduction (Sharp and Clutton-Brock, 2010)—familiarity might suffice for kin discrimination within packs. Yet if full-sibling cohorts disperse before later litters are born, familiarity alone cannot prevent inbreeding, and phenotype matching via odor has been suggested as a complementary mechanism (Leclaire et al., 2013). In banded

mongooses, which exhibit low reproductive skew and highly synchronized breeding among both dominant and subordinate individuals (Gilchrist, 2006), familiarity is an even less reliable cue. Their communal litters, formed by multiple females giving birth simultaneously, often contain mixed paternities, making a mechanism like phenotype matching essential for assessing relatedness independently of familiarity.

Other cooperative species also demonstrate phenotype matching for kin discrimination. African cichlids use visual and chemical cues to assess relatedness among separately reared individuals (Le Vin et al., 2010), and African clawed frog tadpoles apply MHC-based self-referencing to distinguish kin (Villinger and Waldman, 2008). However, disentangling phenotype matching from learned familiarity remains challenging. For example, while baboons exhibit preferential treatment of genetic offspring over unrelated offspring from consorts, it remains unclear whether this is due to genetic recognition or behavioral cues, such as perceived mating effort with the mother (Buchan et al., 2003). Studies must carefully account for these confounding factors, recognizing that familiarity and phenotype matching are not mutually exclusive and may operate in tandem (Porter, 1988; Tang-Martinez, 2001).

In banded mongooses, existing evidence from other studies suggests that phenotype matching may not provide precise relatedness assessment. This imprecision could explain the persistence of inbreeding (Wells et al., 2018), even though mongooses tend to mate with less closely related individuals compared to random mating (Sanderson et al., 2015). Interestingly, this uncertainty in phenotype matching may also support synchronized breeding, which facilitates cooperative behavior. For instance, while banded mongoose females cannot distinguish their own offspring within communal litters, nor can pups identify their mothers (Marshall et al., 2021), breeding asynchrony can lead to infanticide (Hodge et al., 2011). Non-breeding females, having no offspring to risk, are more likely to commit infanticide, causing litters to fail within the first week (Cant et al., 2014). Conversely, synchrony in breeding results in mixed-parentage litters that rarely fail early, likely because imprecise relatedness cues prevent females from risking harm to their own pups.

This inability to assess relatedness precisely may also facilitate a “veil of ignorance,” which promotes equal contributions in cooperative behaviors such as communal offspring care (Marshall et al., 2021). Such mechanisms are thought to enhance cooperation by minimizing kin discrimination, as seen in other species (Queller and Strassmann, 2013). For example, social insects could theoretically discriminate between patrilines using self-referencing for phenotype matching but instead use colony-wise phenotypes as a template, preventing patriline-specific discrimination (Keller, 1997; Queller and Strassmann, 2002). Similarly, male birds can differentiate between broods sired from other males and avoid raising them, yet they do not favor their own offspring within mixed broods (Keller, 1997). In banded

mongooses, the ability to detect relatedness without consistently applying this information aligns with theoretical predictions that uncertainty in kin recognition can promote cooperative behavior (Frank, 2003; Okasha, 2012; Queller and Strassmann, 2013).

The MHC plays a key role in immune response and has the potential to generate odor cues, directly or indirectly (Schubert et al., 2021), providing information about an individual's genetic makeup. Kin discrimination based on MHC-derived odor cues has been observed in various species and contexts. For instance, house mice exhibit a preference for communal nesting with relatives to reduce infanticide and exploitative risks when caring for pups, using MHC similarity as a cue for relatedness (Manning et al., 1992). Similarly, African clawed frog tadpoles prefer half-siblings sharing MHC alleles, likely employing a self-referencing mechanism (Villinger and Waldman, 2008). In mate selection, animals may make use of MHC-related odor cues to increase offspring MHC diversity (Schwensow et al., 2008), potentially enhancing genomic diversity and reducing inbreeding risks (Jennions, 1997; Mays and Hill, 2004; Tregenza and Wedell, 2000).

In our study, banded mongooses responded to genomic relatedness in odors but showed no evidence that MHC similarity influenced these responses, and MHC similarity was not correlated with genomic relatedness. This may reflect the limited sample size (18 recipients, 33 presentations), the fact that half of the subjects were presented with the same odor, or subtle effects requiring larger datasets to detect (Gaigher et al., 2019). Nonetheless, mongooses adjusted their responses according to donor MHC diversity (alleles and supertypes), independent of genomic diversity. These findings suggest that mongooses can directly detect MHC diversity in odors. Males, in particular, showed reduced interest in odors from more MHC-diverse individuals, perhaps because highly diverse males represent stronger competitors while highly diverse females may be less fit. This interpretation aligns with prior work in banded mongooses showing that females with higher diversity have lower reproductive success, whereas males with higher diversity reproduce more successfully (Schubert et al., 2025). This pattern is consistent with broader comparative evidence: phylogenetic meta-analyses and meta-regressions have found female preference for MHC-diverse males across 27 vertebrate species, including mammals, birds, reptiles, and fishes (Kamiya et al., 2014), as well as similar trends in primates, with statistically significant effects in humans (Winternitz et al., 2017).

These results also fit within the broader behavioral ecology of the species. Male banded mongooses responded more strongly to unfamiliar odors than females (Mitchell et al., 2018), reflecting their greater role in territorial defense (Cant et al., 2016). Subordinate males are often the first to confront intruders, showing heightened aggression and inspection (Cant et al., 2002), while extra-group paternity, accounting for approximately 18% of offspring (Nichols

et al., 2015), offers them rare reproductive opportunities during inter-pack encounters (Green et al., 2024). Males may therefore have a dual motivation to assess unfamiliar individuals for sex, genetic quality, and compatibility, as such encounters can both threaten and enhance fitness. In this context, banded mongooses may use genomic relatedness to gauge mate compatibility and MHC diversity-linked odor cues to assess the competitive threat posed by intruders, paralleling MHC-based discrimination observed across species.

OUTLOOK

Our study provides first evidence that odor cues might be used to discriminate relatedness levels and MHC diversity in unfamiliar conspecifics in banded mongooses. Given the high risk of inbreeding in banded mongoose groups, phenotype matching is a plausible mechanism for relatedness assessment and may have evolved as an inbreeding avoidance strategy. MHC diversity, in contrast, is more likely assessed through direct detection of odor signatures linked to MHC genotype. Such information could also be used to evaluate intruders and potential competitors for mates. Future studies should be planned strategically, with genotyping of each individual as the first step to allow for ideal MHC combinations in odor recipient and donor, and use sample sizes large enough to allow investigating same- and opposite-sex contexts separately. Habituation-dishabituation trials using odors that vary in genomic relatedness and MHC diversity could help pinpoint the threshold at which banded mongooses can discriminate.

Competing interests

The authors declare that there are no competing interests.

Availability of data and materials

Analyses reported in this article can be reproduced using the data provided by Winternitz (2025).

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used ChatGPT for AI-assisted copy-editing. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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FIGURE LEGENDS

Figure 1 Sex-dependent responses. Predicted contact (a), sniffing (b), and duration (c) times (s) towards male and female odors are shown separately for male and female recipients and colored by the sex of the odor donor. Larger points show model-predicted values, with the effects of other variables averaged over their observed values in the dataset. Error bars indicate 95% confidence intervals. Smaller points show empirical data, jittered slightly for clarity. Significance levels: *** $p < 0.001$; ** $p < 0.01$.

Figure 2 Duration response declines with increasing relatedness. Model predictions are shown while controlling for other predictors and including recipient ID and donor ID as random effects. Raw data points are overlaid for visualization. Relatedness values are expressed relative to the population mean, with negative values indicating below-average relatedness. Shaded areas represent 95% confidence intervals around the prediction line.

Figure 3 Duration response in relation to MHC diversity. Predicted response duration is shown in relation to the number of distinct MHC alleles (a) and supertypes (b), while controlling for the effect of sex. Raw data points are overlaid for visualization. Shaded areas represent 95% confidence intervals around the prediction line.

Fig. 1.

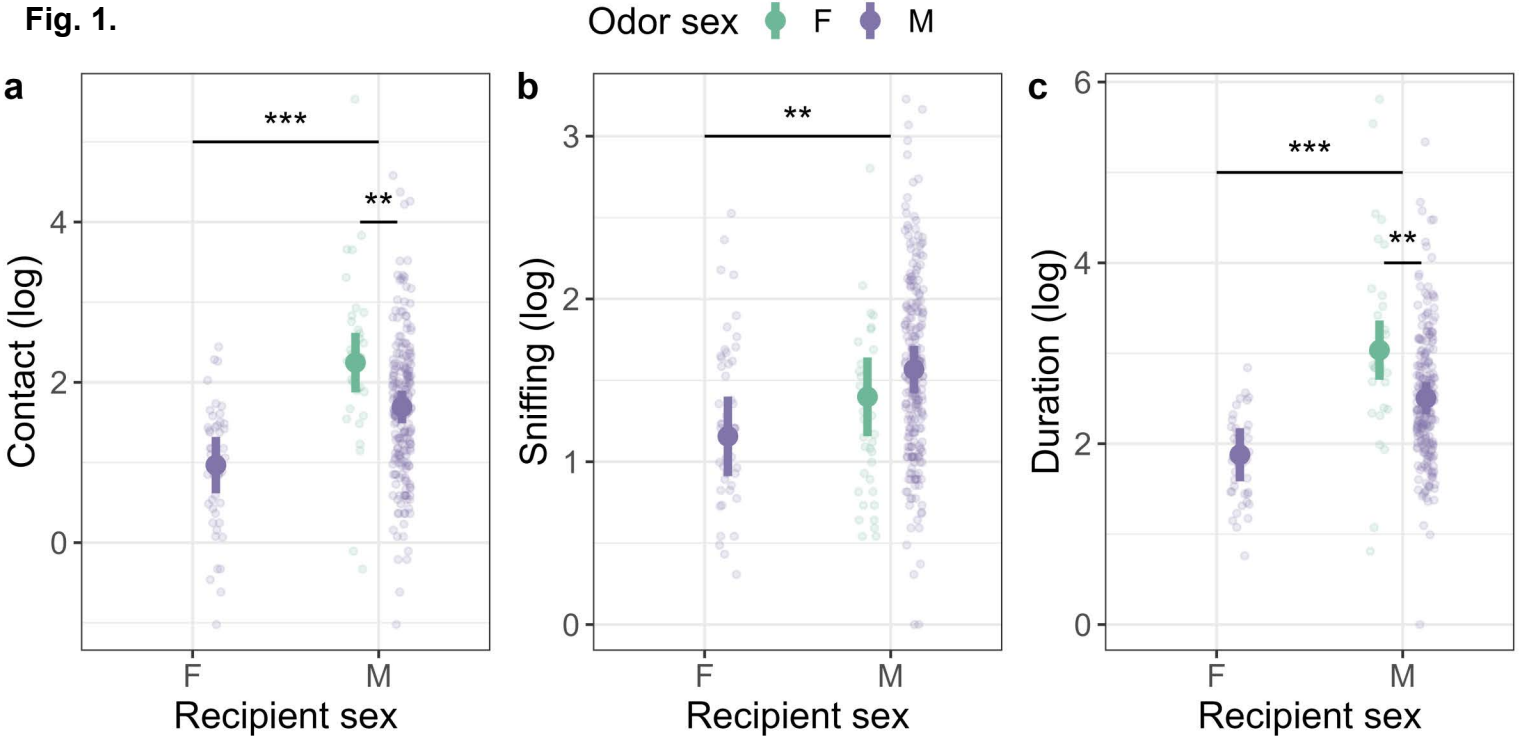

Odor sex
F
M
a
b
c
***
**
Contact (log)
Sniffing (log)
Duration (log)
0
1
2
3
4
6
F
M
Recipient sex

Fig. 2.

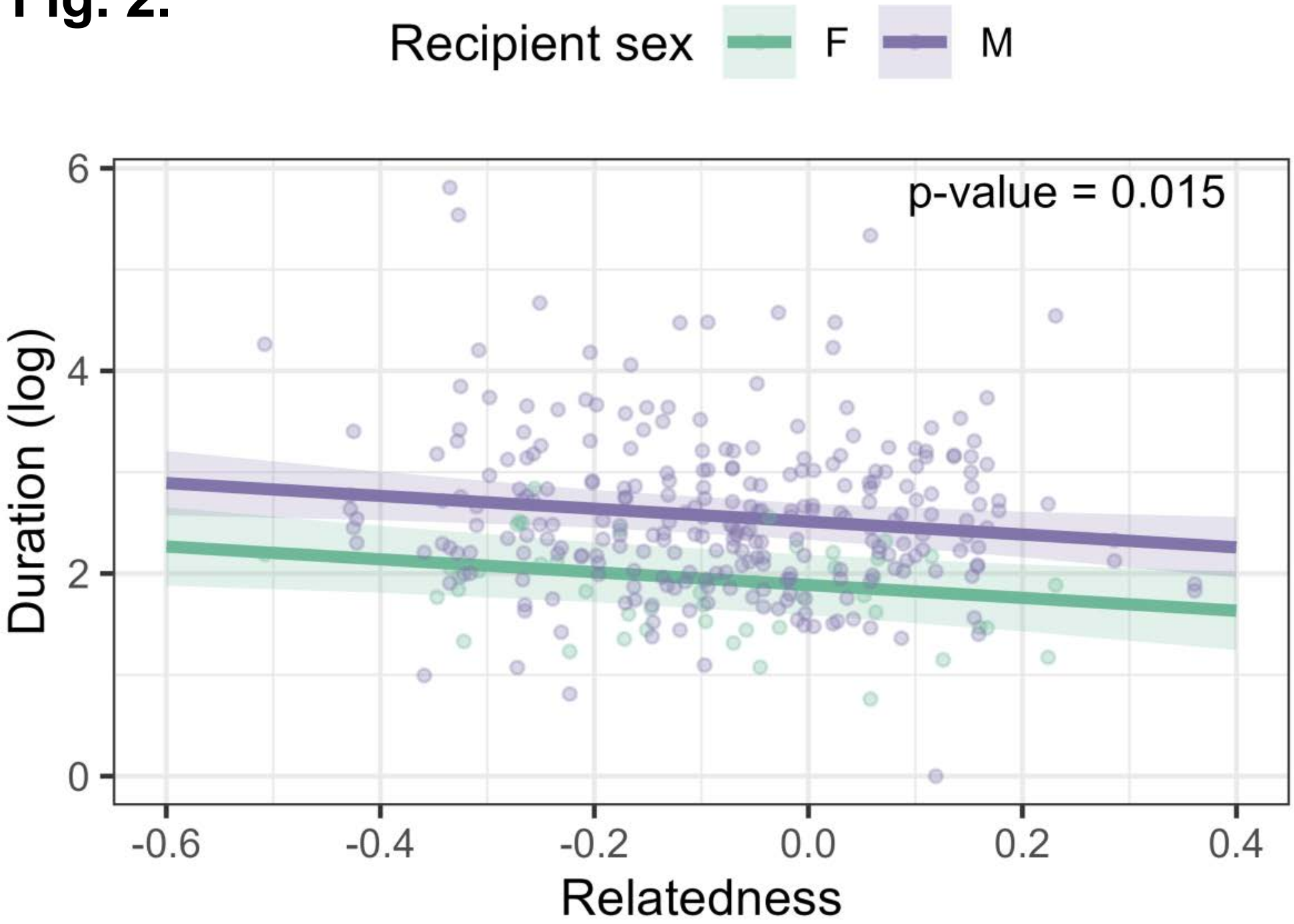
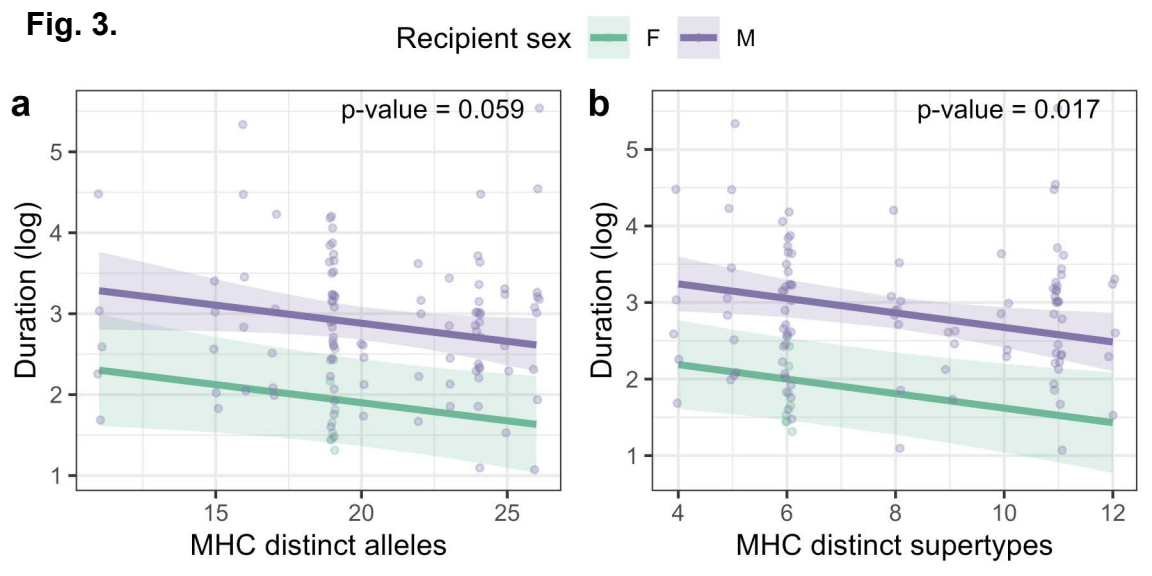

Recipient sex
F
M
p-value = 0.015
Duration (log)
6
4
2
0
-0.6
-0.4
-0.2
0.0
0.2
0.4
Relatedness

Fig. 3.


Recipient sex
F
M
a
p-value = 0.059
Duration (log)
MHC distinct alleles
b
p-value = 0.017
Duration (log)
MHC distinct supertypes

SUPPLEMENTARY MATERIAL for:

Banded mongooses discriminate relatedness and MHC diversity in unfamiliar conspecifics

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SUPPLEMENTARY METHODS

MHC genetic analyses

MHC genotyping was carried out using target enrichment and PacBio long-read sequencing described in Winternitz, J.C., Schubert, N., Heitlinger, E., Foster, R. G., Cant, M.A., Mwanguhya, F., Businge, R., Kyambulima, S., Mwesige, K., Nichols H.J. (unpublished data). First, a custom hybridization panel was designed by Twist Bioscience to be compatible with PacBio HiFi long reads. Briefly, 68 banded mongoose MHC partial exon sequences (GenBank Accession numbers PQ137681 - PQ137748) were blast searched against the banded mongoose genome NCBI GenBank HiC chromosomal assembly GCA_028533875.1 (Megablast, max e-value = 1e-50, maximum hits =1 per sequence). These exon sequences matched 17 unique scaffolds. We restricted potential hybridization targets to those from scaffolds at least 2000 bp long, leading to 14 genomic regions across 12 scaffolds (3 on scaffold/chromosome 8) that included 9 putative class I loci and 5 putative DRB loci.

Next, hybridization, library preparation and sequencing were carried out at Edinburgh Genomics according to the manufacturers' protocols. Briefly, 30 banded mongoose samples with both microsatellite and AGS data and with average DNA concentration of 7.1 ng/ul (range = 0.2-29.0, SD = 7.5) were used to create PacBio libraries. After size selection, post-PCR quantification, and malfunction in the PacBio Sequel IIe system, only 27 banded mongoose DNA samples had high enough concentration and quality to create PacBio libraries for sequencing. Hybridization was carried out using version 'REV 2' of the Twist library preparation and enrichment protocol and a Twist custom panel of mongoose probes. The final sequencing library was loaded on a PacBio Sequel IIe system and produced 173,486 total HiFi reads. Samples were demultiplexed and PCR duplicate reads were removed prior to downstream processing using pbmarkdup v1.0.3 (https://github.com/PacificBiosciences/pbmarkdup), leaving 127,716 unique reads, mean 4912 (SD = 3151) per sample. Genotyping was carried out as described in Winternitz, J.C., Schubert, N., Heitlinger, E., Foster, R. G., Cant, M.A., Mwanguhya, F., Businge, R., Kyambulima, S., Mwesige, K., Nichols H.J. (unpublished data). For each sample, HiFi reads were assembled *de novo* into diploid-aware contigs using hifiasm (Cheng et al., 2021) and blast searched against the custom Twist target panel. Contigs of interest were then aligned using MAFFT (Katoh et al., 2002; Katoh and Standley, 2013) and a maximum likelihood phylogenetic tree was created using IQTree (Kalyaanamoorthy et al., 2017; Nguyen et al., 2015) to identify monophyletic putative loci. Consensus reference loci sequences were created using custom R code and for each individual raw HiFi reads were mapped to these references using pbmm2 v1.0.3 (https://github.com/PacificBiosciences/pbmarkdup). Variants were called using DeepVariant (Poplin et al., 2018) and haplotypes phased using WhatsHap (Martin et al., 2016). Consensus reference loci were annotated using carnivore NCBI reference sequence MHC annotations and Exonerate v. 2.4.0 (Slater and Birney, 2005) and these gene annotations were transferred to individuals' haplotypes using liftoff (Shumate and Salzberg, 2021). In total, individuals were genotyped at 7 MHC-I loci and 7 MHC-II loci. As the number of alleles per individual increased with HiFi read count (Pearson's cor = 0.532, p-value = 0.028), the number of unique HiFi reads was included in downstream analyses.

MHC similarity between individuals was estimated as allele and supertype sharing calculated as twice the sum of alleles (supertypes) the individuals shared divided by the sum of alleles (supertypes) of both individuals: D=2Fab/(Fa+Fb) (Wetton et al., 1987). MHC diversity was estimated as the total number of alleles and supertypes in an individual. Supertypes were

estimated using amino acid distances between sequences and then grouped based on functional similarity. The Sandberg distance (Sandberg et al., 1998), 5 physio-chemical z-descriptor values, was calculated for each MHC peptide binding residue (MHC-I (Saper et al., 1991); MHC-II (Brown et al., 1993)) using the R package 'Peptides' (Osorio et al., 2015), and transcribed into a similarity matrix. To these matrices we applied find.cluster() using the criterion "goesup" and method = "kmeans" for MHC-I and criterion "diffNgroup" and method = "ward" for MHC-II. This method was repeated 1000 times and the mean, mode, and median number of clusters calculated to arrive at 11 and 10 clusters, respectively. We assigned alleles to groups using the dapc() function (i.e., discriminant analysis of principal components) from the R package 'adegenet' (Jombart, 2008) and repeated this process 1000 times to estimate repeatability with light's kappa value in 'irr' R package (Gamer et al., 2019) For MHC-I, repeatability Kappa = 1 and the mean assignment proportion was 0.988. For MHC-II repeatability was perfect, with Kappa = 1 and the mean assignment proportion = 1.

Control vs experimental presentations

Linear mixed effect models (LMMs) were established to test for a difference between control and experimental presentations for the different response measures. Each model included one of the six response variables: licking, marking, contact (log), sniffing (log +1), duration (log), and rolling (log +1). Some response variables were log transformed to avoid heteroscedasticity issues, and for variables with many zero values, 1 was added to include these in the log transformation. Each model also included the type of presentation (control or experimental) as an explanatory variable, and the identity of the odor recipient and the pack they reside in as random effects.

RESULTS

Correlational analyses

We did not find a strong correlation ($r < 0.3$ in all cases) between microsatellite-derived measures and MHC-derived measures (Tab. S1). However, strong significant correlations ($r > 0.7$) were detected between MHC diversity measured as distinct alleles and distinct supertypes as well as MHC similarity of alleles and supertypes (Tab. S1). Nonetheless these MHC measures were used for further investigation, as they were not fitted simultaneously in a model and they contain information on MHC functional diversity on different scales.

We did not find strong correlations between the three behavioral response variables that we included in our LMMs; Contact, Sniffing and Duration ($r < 0.3$ in all cases) with the exception of Contact and Duration ($r = 0.698$, Tab. S2).

Control vs experimental presentations

Contact (estimate = 0.6861, SE = 0.1510, t-value = 4.542, p-value = 7.87E-06), sniffing (estimate =0.4575, SE = 0.0884, t-value = 5.178, p-value = 3.97E-07), and duration (estimate = 0.5017, SE = 0.1255, t-value = 3.997, p-value = 7.96E-05) differed significantly between control and experimental presentations (Fig. S1, Tab. S3). For licking (estimate = 0.0705, SE = 0.0843, t-value = 0.835, p-value = 0.404), marking (estimate = 0.1650, SE = 0.1223, t-value = 1.348, p-value = 0.178) and rolling (estimate = -0.0518, SE = 0.4351, t-value = -

0.119, p-value = 0.905) there was no significant difference between control and experimental treatments detectable, likely because these behaviors were relatively rare (Figure S1).

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TABLES

Table S1 Correlational analysis for standardized multi-locus heterozygosity (sMLH), relatedness and MHC diversity measures Correlation estimated using Pearson's product-moment correlation analysis. Shown are the corresponding p-values and the 95% confidence intervals. Significant p-values are in bold.

Variables investigated	r	p	Lower CI	Upper CI	df
sMLH & MHC allele number	0.071	0.786	-0.424	0.533	15
sMLH & MHC supertype number	0.194	0.457	-0.317	0.617	15
MHC allele number & MHC supertype number	**0.897**	**1.04E-06**	0.733	0.963	15
Relatedness & sMLH	0.369	0.145	-0.136	0.722	15
Relatedness & MHC allele similarity	0.191	0.462	-0.319	0.615	15
Relatedness & MHC supertype similarity	0.299	0.244	-0.213	0.681	15
MHC allele similarity & MHC supertype similarity	**0.788**	**1.73E-04**	0.495	0.92	15

Table S2 Correlational analysis for response measures Correlation estimated using Pearson's product-moment correlation analysis. Shown are the corresponding p-values and the 95% confidence intervals. Sample sizes were the same for all models. Observations: 361, Recipients: 38 (Pack IB: 10 F, 23 M; Pack 1H: 5 M), Odor donors: 37 (Pack IB: 8 F, 24 M; Pack 1H: 5 M). Significant p-values are in bold.

Variables investigated	r	p	Lower CI	Upper CI
Contact & Duration	**0.698**	**<2.2E-16**	**0.636**	**0.751**
Contact & Sniffing	0.180	**0.002**	0.070	0.286
Contact & Licking	0.014	0.811	-0.098	0.125
Contact & Rolling	**0.737**	**<2.2E-16**	**0.681**	**0.784**
Contact & Licking	0.032	0.575	-0.080	0.143
Duration & Sniffing	0.136	**0.017**	0.024	0.244
Duration & Licking	-0.013	0.827	-0.124	0.099
Duration & Rolling	**0.635**	**<2.2E-16**	**0.563**	**0.697**
Duration & Marking	**0.122**	**0.033**	**0.010**	**0.230**
Sniffing & Licking	-0.030	0.599	-0.141	0.082
Sniffing & Rolling	-0.082	0.152	-0192	0.030
Sniffing & Marking	**0.139**	**0.015**	**0.027**	**0.246**
Licking & Rolling	0.027	0.636	-0.085	0.138
Licking & Marking	-0.070	0.221	-0.180	0.042
Rolling & Marking	-0.088	0.125	-0.198	0.024

Table S3 Model output for relationship between response variable and presentation type The table shows the model output investigating differences between control and experimental presentations using an LMM. P-values were calculated based on Satterthwaite's method. Sample sizes were the same for all models. Observations: 361, Recipients: 38 (Pack IB: 10 F, 23 M; Pack 1H: 5 M), Odor donors: 37 (Pack IB: 8 F, 24 M; Pack 1H: 5 M). Significant p-values are in bold.

Response variable	Estimate	SE	t-value	p-value
Contact	**0.686**	**0.151**	**4.542**	**7.87E-06**
Sniffing	**0.458**	**0.088**	**5.178**	**3.97E-07**
Duration	**0.502**	**0.126**	**3.997**	**7.96E-05**
Licking	0.071	0.084	0.835	0.404
Rolling	0.165	0.122	1.348	0.178
Marking	-0.052	0.435	-0.119	0.905

Table S4 Model output for models on MHC similarity LMM output for effects of MHC similarity between recipient and odor donor on contact, sniffing and duration responses. Sample sizes were the same for all models. Observations: 33, Recipients: 18 (Pack IB: 6 F, 11 M; Pack 1H: 1 M), Odor donors: 17 (Pack IB: 4 F, 12 M; Pack 1H: 1 M).

MHC measure	Fixed effect	Response variable	Estimate	SE	p-value
Alleles	MHC similarity	Contact	0.739	1.330	0.584
	MHC similarity	Sniffing	-0.303	0.844	0.723
	MHC similarity	Duration	-1.042	1.188	0.387
Supertype	MHC similarity	Contact	1.328	1.089	0.234
	MHC similarity	Sniffing	0.213	0.702	0.764
	MHC similarity	Duration	-0.420	0.989	0.674

Table S5 Summary Table of individual trials across analyses Details of donors and recipients, controls, and number of trials across the four different analyses (and their datasets).

Category	Total N	Used in control vs experimental analysis (dataset 1)[1]	Used in neutral diversity analysis (dataset 2)	Used in MHC diversity analysis (dataset 3)	Used in MHC similarity analysis (dataset 4)
Unique donors (D)	37	37	35	17	17
Unique recipients (R)	38	38	37	36	18
Male D	29	29	28	13	13
Female D	8	8	7	4	4
Male R	28	28	28	27	12
Female R	10	10	9	9	6
Male D, Female R	50	50	45	9	6
Female D, Male R	40	40	35	20	4
Male D, Male R	233	233	228	82	23
Experimental (E)	323	323	228	111	33
Control (C)	38	38	–	–	–
Total trials	361	361	308	111	33

1. 10 unique females recipients and 20 unique male recipients were each presented a control tile (N = 38).

FIGURES

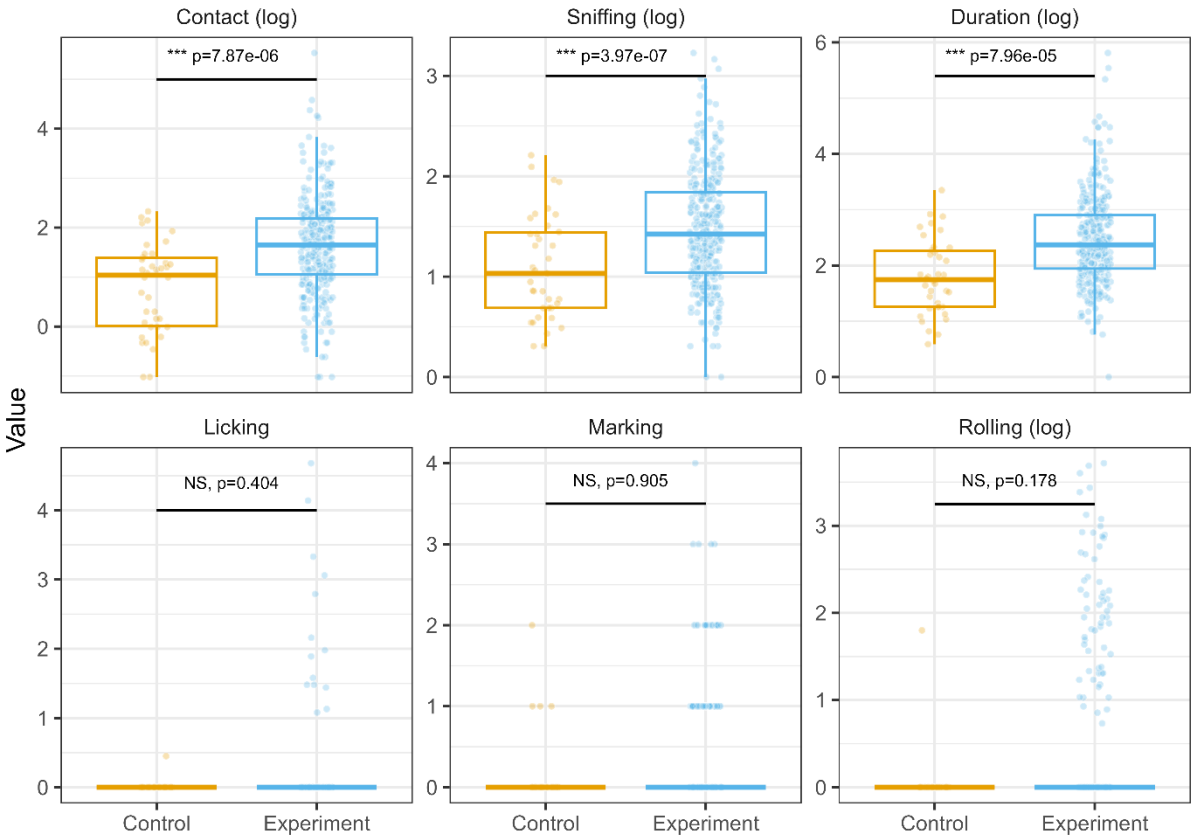

Figure S1 Differences between control and experimental presentations Difference in the response values for the control and experimental presentations are shown. Boxplot whiskers show the 25th and 75th percentiles, the box shows the inner 50th percentile, and the line shows the median. Raw data is superimposed and "jittered" horizontally for visualization. P-values were calculated based on Satterthwaite's method.