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Field evidence supporting monitoring of chemical information on pathways by male

African elephants

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Abstract

When animals move along well-established pathways, sensory cues along the path may provide valuable information concerning other individuals that have used the same route. Yet the extent to which animals use pathways as sources of public social information is poorly understood. Here we quantify wild African savannah elephants (*Loxodonta africana*) responsiveness to olfactory information along natural elephant pathways, habitual routes that link predictable critical resources in the environment. By monitoring the behaviour of elephants travelling on pathways in a predominantly male study population, we found that elephants were highly olfactorily responsive to pathway substrate. Lone travellers were more responsive than elephants travelling in groups, suggesting elephants without social companions may be more dependent on olfactory cues on pathways during navigation. Furthermore, by experimentally presenting olfactory cues on pathways we provide evidence that male African elephants exhibit focused olfactory responses to urine cues of same-sex conspecifics for at least 48 hours from

time of deposition, and that urine from adult elephants was more likely to elicit vomeronasal system responses compared to subadult urine. African elephants may therefore potentially be able to discern the age and maturity of individuals they can expect to encounter in the environment from remote urine cues on pathways. We suggest elephant pathways act as a public information resource, assisting navigating elephants via the deposition of urine and dung by previous travellers on the route. These results could help inform elephant management, which may manipulate olfactory information on pathways in high human-wildlife conflict areas, or could use olfactory urine cues to improve the efficiency of corridors that link protected areas for elephants.

Keywords: chemical communication, conspecific assessment, olfaction, public social information, wildlife corridors

Introduction

Detection and use of olfactory information produced from conspecifics is a widespread and ancient adaptation in mammals (Eisenberg & Kleiman, 1972; Eisthen, 1997). Olfactory products can be classified as signals when they have evolved for the purpose of communication, hence evoking adaptive behavioural changes in the receiver (Maynard Smith & Harper, 2003), or as cues when the products have not evolved for the purpose of communication but are still used by conspecifics to guide behaviour (Thomas, 2011). Such cues can act as public social information, potentially assisting conspecifics in acquiring useful information regarding, for example, habitat selection and foraging decisions (Deutsch & Nefdt, 1992; Galef, 1990; Danchin et al., 2004; Bonnie & Earley, 2007).

In mammals, compounds present in urine can provide conspecifics with information on a range of phenotypic traits including sex, dominance, reproductive state, stress level, and even individual identity (Laska & Hudson, 2010; Rajagopal et al., 2010; Nodari et al., 2008; He et al., 2008; Bates et al., 2008). In many species, both the main olfactory system and vomeronasal system are involved in the detection of pheromones and other odorant molecules present in urine (Verberne, 1976; He et al., 2008; Tirindelli et al., 2009). Similar to visual information left by conspecifics, olfactory cues and signals have a greater potential to be long lasting in comparison to tactile and auditory information, remaining after an individual has departed and long after the information was first created (e.g. habitat copying of nesting sites, Parejo et al., 2005). Furthermore, in comparison to other sensory informing modalities, olfactory information can convey more information concerning individual phenotype in the absence of the depositor, as well as information of the depositor's proximity, due to microbially-mediated modifications to compounds over time, or slow releases of molecules from carrier proteins (Albone et al., 1977; Hurst et al., 1998; Archie & Theis, 2011). However, despite its importance to many mammals, the potential of olfactory information as a tool in wildlife conservation and management remains relatively untapped (Campbell-Palmer & Rosell, 2011), which may reflect both methodological challenges and perhaps researcher bias (Heymann, 2006).

The African savannah elephant (*Loxodonta africana*) has the largest olfactory receptor gene repertoire of any species reported to date (Niimura et al., 2014). Elephant species indisputably have remarkable olfactory abilities, with the Asian elephant (*Elephas maximus*) able to discriminate odours differing in only 1 carbon chain length, and retain information of the identity of trained odours 16 weeks after exposure (Arvidsson et al., 2012). A primary use of the highly developed olfactory sense in elephants is in locating critical ecological resources such as water, as well as for habitat and food selection (Plotnik et al., 2014; Rasmussen &

Krishnamurthy, 2000). Furthermore, behavioural studies both in captivity (Meyer et al., 2008; Rasmussen & Schulte, 1998) and in the wild (Schulte et al., 2012; Bates et al., 2008), provide evidence for an extensive use of chemical cues and signals in elephant social communication. For example the continuous urine dribbling during musth (a temporary heightened sexual state experienced by mature adult male elephants (Poole 1989)) contains compounds signalling sexual status, with males able to discern musth from non-musth urine (Hollister-Smith et al., 2008), and female African elephants monitor the location of family members in relation to themselves from urine deposits (Bates et al., 2008). In the wild, male African elephants are often observed making olfactory assessments of conspecifics genitals, temporal gland secretions and breath (Poole & Granli, 2011). Similarly, focused olfactory responses are often directed toward sources of scent in communal areas, with males investigating dung and urine more than females (Loizi et al., 2009). This may be because for polygynous males such as elephants, both odors from females (to assess reproductive receptibility) and males (to assess potential competitors) may be of interest (Merte et al., 2010). The chemical composition of urine from wild male African elephants is known to change across age and maturity, younger male urine is characterised by high levels of acids and esters, and older males by alcohols and ketones (Rasmussen & Wittemyer, 2002). For male African elephants, olfactory assessment of the urine cues of same-sex conspecifics, may therefore provide important information about the age, reproductive status and location of potential competitors and affiliates (LaDue et al., 2018; Hollister-Smith et al., 2008).

Elephant pathways, regular routes utilised by elephants that connect predictable resources such as feeding sites and waterholes (Von Gerhardt et al., 2014), are multifunctional in elephant ecology, assisting in both improved usage of the environment, and possibly providing an opportunity to monitor conspecifics (Mutinda et al., 2011). In a previous study on African

elephant pathways, Shannon et al. (2009) found that the most heavily used pathways were characterised by the presence of dung piles every 10-50 m. It is also estimated that elephants produce between 45-60 litres of urine daily, in bouts of 5-11 litres at a time (Miller 2006; Benedict, 1936). As a result, elephants are likely to continuously encounter urine and dung deposits from previous users of a pathway, which may provide a valuable source of social information. Indeed, it has been previously proposed that elephant pathways act as sources of public information, potentially allowing for remote communication between individuals or groups concerning age, identity, sexual state, or quality of departed habitat (Mutinda et al., 2011). However, to date no *in situ* experiments have been conducted that investigate the sensory responsiveness of male elephants to olfactory cues on elephant pathways, or quantified their ability to discern the phenotypes of the animals leaving urine deposits.

Here we use an in-situ experiment to examine the importance of olfactory cues as inadvertent public social information on well-established pathways that are a characteristic of many mammal species (Edelstein-Keshet, 1994; Able, 1981; Jamon, 1994). Specifically, we ask if mobilised elephants respond to the olfactory cues of conspecifics on elephant pathways, and if their responses differ depending on the receiver's age and social condition, as well as the age of the conspecifics whose cues they encounter. First, we quantify the general olfactory responsiveness of male African elephants traveling along elephant pathways. We quantify how general olfactory responsiveness is influenced by the age class (adult/ subadult) of the focal individual, predicting that adults and subadults will have divergent levels of olfactory responsiveness. Due to their increased age, adults may be more experienced at utilising elephant pathways effectively and thus more responsive to olfactory information compared to subadults. Alternatively, subadults may be more responsive, in line with previous research on male elephant olfactory investigations of urine at waterholes that found that olfactory

investigation peaked in adolescence and receded in adulthood (Schulte et al., 2012). We also hypothesized that social factors, such as whether the focal elephant is travelling alone or in an all-male group, and spatial position within a travelling group will influence olfactory responsiveness, predicting that elephants traveling in all-male groups, and those not at the front of groups would be less responsive to olfactory cues. Elephants travelling in groups, and following behind other travellers, may have greater dependence on other group members to detect threats, or to navigate pathways using olfactory information, similar to the tendency of many group living vertebrates to reduce vigilance behaviours in larger groups (e.g. Tibetan antelope, *Pantholops hodgsonii*, Lian et al., 2007; ring-tailed coati, *Nasua nasua*, Di Blanco et al., 2006), and for dependence on informed leaders during group movements (resident killer whales, *Orcinus orca*, Brent et al., 2015; whooping cranes, *Grus americana*, Mueller et al., 2013. African elephants, Allen et al., 2020).

Second, we quantify how male elephants monitor fresh urine cues from other males of different age classes (subadult and adult males). We predict olfactory information from elephant urine samples will last longer in the environment, indicated by continuing to evoke focused olfactory responses from passing elephants for longer times since initial deposition, compared to a water control. Whilst elephants may first respond to water controls due to the odour of water, to which elephants are highly sensitive (Plotnik et al., 2014; Ramey et al., 2013), and potentially the visual stimulus of a darkened spot of sand, we predict these responses will diminish as water controls are dried over time – whereas urine samples will continue to emit odours that elicit responses even when dried. We predict responses to adult and subadult urine will be differentially affected by time because the chemical profiles of urine from males of different development stages are expected to be different (Rasmussen & Wittemyer, 2002), which may affect microbial action and the release of odorous chemicals over time (Goodwin et al., 2012).

Moreover, concerning vomeronasal system responses (indicative of heightened interest in samples) we predict age class of the receiver (adult/ subadult) will influence an elephant's likelihood of responding to urine deposits, as the information contained within chemical cues, as well as their detection ability, and relevance, varies with both the condition of depositor and receiver (Schulte et al., 2007). We also hypothesise that urine carries information concerning the depositor's age, and that subadult and adult urine will evoke different levels of vomeronasal system responses in receivers. Urine is likely to be a largely honest indicator of depositor characteristics, such as age (Schulte et al., 2007) as chemical cues in urine are directly linked to phenotype and physiological condition (Gosling & Roberts, 2001). We test these hypotheses through a bio-assay protocol, using readily observable responses to urine samples to discern chemical reception of the receiver to the deposits of varying depositor characteristics in a natural context (Mackintosh, 1985).

Methods

In-situ bioassay design

Data collection was conducted between October 2017 – September 2018, in Makgadikgadi Pans National Park (MPNP), Botswana. The MPNP is as an elephant bull area (Lee et al., 2011) with males representing 98% of elephant sightings (Evans, 2019). Male African elephants are non-territorial, roaming vast and variable distances over their lifetimes (Ngene et al., 2009), and the male population of the Makgadikgadi is largely transitory, with individual bulls staying on average 47 days in the area (Pitfield, 2017).

We exploited the tendency of male elephants to habitually walk along identified elephant pathways in the MPNP (7 pathways used, Figure 1 (a)) to create an in-situ bio-assay, whereby passing elephants were exposed to purposefully placed urine of donor elephants positioned on the main path (Figure 1 (b)). Camera traps (2017 Bushnell Aggressor HD No-glow, set to record video) positioned around the presented samples captured responses. Pathways measured on average 84.8cm in width (Allen et al., 2020, Figure 1 (c)) and were devoid of vegetation, maintained by repeated single file movement of elephants towards and away from the Boteti River, the common terminal point of pathways (Figure 1(a)). Only a minority of elephants on pathways in the MPNP walk off the main path channel (Allen et al., 2020). Elephants that were not in single file on the main pathway were not included for analysis as they did not pass over samples, however their presence was accounted for concerning the grouping condition and position of elephants whose responses were captured.

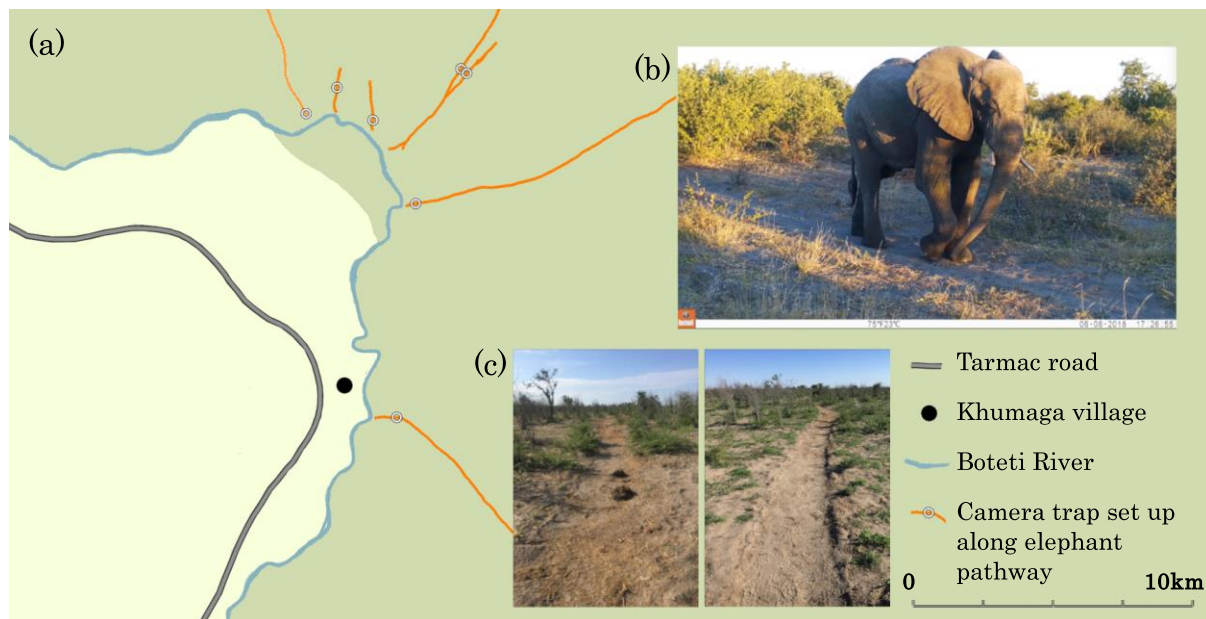


Figure 1: (a) Locations of the 7 highly active elephant pathways (orange lines) used for running in-situ bioassays (camera trap set up at circle point). Dark green represents the MPNP protected area, and light green unprotected land, dominated by human activities, cattle and arable farming

(Stevens, 2018). (b) Example of images from camera traps (set to record video) of elephant investigating a sample. (c) Example elephant pathways in the MPNP.

To ensure uniformity of camera trigger response, we placed cameras on 2m high poles positioned 9m away, at an angle of 45°, on either side of a central “presentation zone” measuring 1m in length along the pathway. During different trials, samples were presented at random points within this presentation zone. By placing a camera facing inwards on either side of the sample, head on footage (essential for aging purposes) was available for both elephants walking towards and away from the river.

Trial type consisted of either no sample, a male elephant urine sample (from an adult or subadult), or a water control sample presented (see sample collection below). For our first hypothesis we wanted to explore the general olfactory responsiveness of elephants to the pathway substrate. To do so, we observed the behaviour of elephants traveling on pathways, exposed to no sample. We monitored the olfactory responses of elephants to a fixed point within the presentation zone, the location of which was assigned randomly per trial. Responses were scored based on an ethogram of olfactory responses (Table 1). For later hypotheses concerning the responses of male elephants to fresh urine cues, we presented male elephant urine samples, or water control samples within the presentation zone, and scored responses to the sample (Table 1).

Table 1

Ethogram of olfactory responses to elephant pathway substrate and presented samples.

	Response	Behaviours included	Description
Non-olfactory	<u>None</u>	Trunk drag	Dragging trunk along pathway substrate
		Trunk suck	Sucking trunk
		Trunk swing	Swinging trunk
		None – other	Trunk held in other postures not indicative to be for the purpose of olfaction. E.g. Two “fingers” of trunk held clenched, trunk rolled up, pulling ear, trunk held floppy etc.
		Periscope	Holding the trunk raised above head in an ‘S’ shape, for detection of airborne scents. Whilst indicative that elephant is using olfactory information – focus is not principally to pathway substrate (Poole & Granli, 2011)
		Hover ahead	Holding trunk ahead in a posture indicating response to olfactory information, but not directed at the pathway substrate in particular. Also includes observed posture to curl trunk over at tip to point ahead
Non-focused olfactory	<u>General olfactory response to pathway substrate.</u>	Tracking	The trunk is held either with the anterior “finger” directed at the ground, or with the entire surface of tip of the trunk (nasal openings) hovering flat over the ground surface. The trunk moves continuously “tracking” the surface of the pathway (no physical contact), with no suspended movement or focused response to any particular point on the pathway (Poole & Granli, 2011)
	(main olfactory system)	J sniff	The trunk is held in a sniff posture just above the ground substrate, with the trunk curled towards the elephant at the tip in a “J” shape, with no suspended movement or focused response to any particular point on the pathway (Poole & Granli, 2011)
Focused olfactory	<u>Sniff</u>	Sniff	Trunk nasal openings deliberately point toward the sample in an extended sniff posture. The trunk is held focused over the sample substrate, rather than moving continuously with the natural gait of the elephant. Elephant may seize movement and continue smelling sample, however no physical contact made (Schulte et al., 2005; Poole & Granli, 2011)
	(main olfactory system)		
	<u>Accessory trunk behaviours</u>	Trunk Shake	Rapid “wriggling” of the trunk up/down or side/side, proposed to be for purpose of clearing nasal pathways to improve olfaction (Schulte et al., 2005)
	(accessory behaviours)	Blow	Forced exhalation through the trunk, often audible with substrate visibly scattered by force. Proposed to be for purpose of clearing nasal pathways to improve olfaction (Schulte et al., 2005)
	<u>Pre-flehmen & flehmen behaviours</u>	Check	Placing the trunk anterior “finger” in physical contact with the sample substrate (Schulte et al., 2005; Poole & Granli, 2011)
	(vomeronasal system)	Pinch	Pinching the sample substrate between the two trunk “fingers” (Schulte et al., 2005)
		Place	Placing the entire tip of trunk nasal opening surface flush with the sample substrate (Schulte et al., 2005; Poole & Granli, 2011)
	Flehmen	Subsequent to physical contact with the sample substrate, curling the trunk into the mouth to touch the vomeronasal organ on the roof of the mouth (Schulte et al., 2005; Poole & Granli, 2011)	

Assembled using information from Schulte et al. (2005), Poole & Granli (2011), as well as our own observations. Elephants detect odorant molecules and pheromones through both the main olfactory and vomeronasal system (Schulte et al., 2005; Lledo et al., 2005), with the trunk acting as the key organ both for detecting airborne chemical compounds (main olfactory

system), as well as for physically transporting less volatile chemicals of heightened interest to the vomeronasal organ (in the dorsal anterior roof of the mouth; vomeronasal system (Rasmussen et al., 1982; Schulte et al., 2005)). See Supplementary video for examples of responses.

Supplementary video available online: Examples of responses to elephant pathway substrate and presented samples.

A total of 665 responses were recorded from male elephants traveling alone or in all-male groups along elephant pathways (123 lone travellers, 542 elephants in 186 groups; See Allen et al., 2020 for group determination methods as well as more information of age demographic and sizes of groups). Within this dataset, all individuals were uniquely identified using distinguishing features such as ear notches, holes and tears, tusk length, girth and shape, skin wrinkles, tail length, and other abnormalities (N individuals=594). Validation of the identification methods showed that elephants could be correctly identified with a 100% success rate (Note A1). Age class was assigned to individuals using a combination of characteristics such as body size and shoulder height (male elephants continue to grow throughout life (Lee & Moss, 1995)), head morphology and size, and tusk girth and splay (Hanks, 1972; Moss, 1996). Males were categorised as subadults, 10-20 years, and adults, 21 years + (Moss, 1996) (Table A1 for summary table of distribution of sample sizes by age, social grouping, and climatic variables). Seven responses were from elephants identified to be in musth (Poole, 1989), but due to their low numbers (1.05% of total responses) we included these responses in analysis. We did however test for any significant effect on olfactory responsiveness of elephants to pathways in the case that a musth male had walked on the pathway before them in a given trial, to account for musth male urine dribbling and elephants' known ability to detect musth males by odour (Hollister-Smith et al., 2008; Poole, 1989).

Sample collection, storage and presentation

Urine samples were collected from age-classed male elephants aggregating along the Boteti River in MPNP. When an elephant was observed urinating, a timer was started, and a sample of urine-soaked sand collected within 20 minutes of urination (mean = 13 min 2 s, SD = 2 min 58 s, range = 7min 25 s - 20min). In attempt to relatively standardise hydration state of elephants, only elephants that had been observed previously drinking at the river < 1 hour prior to urinating were chosen as donors of urine. No urine samples were taken from elephants identified to be in musth. In all cases of sample collection there was no evidence that another elephant had recently urinated at the same site, i.e. no dung boli were immediately present (elephants mostly defecate when they urinate) or hardened flattened substrate indicative of previous urine deposition.

Urine soaked sand was collected using disposable latex gloves, and stored in sterile disposable storage containers, totalling a volume of 2L. An abundance of substrate was always available, but collection focused on the heavily saturated region of sand around where the penis rested just above the ground. For water control samples, we poured water from the river over sand and waited under 20 minutes before storage (mean = 11 min 38 s, SD= 3 min 26 s, range = 7 min 3 s - 19min). River water was used as a control as opposed to distilled water, which was unavailable in the field. Previous research on elephants' olfactory abilities have used vanilla extract as a positive control (Hollister-Smith et al., 2008; Schulte et al., 2007). However for the current study, this approach was deemed inappropriate since the field site is a protected area and we were not permitted to introduce foreign material to the environment. Elephants were observed urinating on both the bank slope, as well as the sand by the river edge. Because the two sand types differed in initial colour, consistency and water saturation level, we collected

water control samples from the two sand types at random (N samples urine: Bank slope = 22, River edge = 26; N water control samples: Bank slope = 11, River edge = 12). All samples were immediately stored at 4°C in a car fridge, to slow microbial metabolic processes involving the compounds found in urine (Goodwin et al., 2012). In 50% of trials, samples were stored overnight and laid on pathways at dawn the next day, and in 50% of trials collected samples were presented the same day before sunset. This allowed us to expose both elephants traveling at night and during the day to the “freshest” samples. When laid on the pathway, the sample was approximately 20 cm in length, 25cm in width.

Because the likelihood of a male exhibiting a focused olfactory response to a sample was not predicted by different treatments of sample prior to presentation (sand type, time spent in fridge, average temperature recording in fridge; Table A2), we could be confident that variation in sample treatment did not affect our results.

Removal of Samples Between Trials

Trials were run for 48 hours from the initial deposit of samples (or the first activation of camera traps in the case where no sample was presented). At the end of trials, samples were carefully removed with a shovel from the presentation zone, collected in a disposable bag and removed from the experimental area. A thin, fresh layer of uncontaminated sand from the area around the pathway was then thrown lightly over the presentation zone, again using a shovel to be sure that no human contact was made with the substrate.

Some elephant pathways routes are thought to persist for decades (Haynes, 2006), and some of the pathways used in this study appear not to have moved since satellite imagery began in the

area in 2004 (personal observation; GoogleEarth, n.d.). It is therefore likely that the stretches of pathway sampled have historically been deposited on with dung or urine from previous utilisers, giving the potential for odour residue. Indeed, a key hypothesis of our study is that trail maintenance and repeated travel on pathways is in part due to such chemical deposition (Blake & Inkamba-Nkulu, 2004; Mutinda et al., 2011). For the current study however, we only chose stretches of pathway for our experimental set up that were free of signs of fresh urine, or fresh, intact dung boli (Hedges et al., 2012). Successive trials were run on the same stretch of pathway (i.e. the number of samples previously presented and removed within presentation zone varied between trials, to a maximum of 9 samples) making it possible that elephants could increase responsiveness due to detection of urine cues from multiple individuals at the same location. However, olfactory responses from elephants were not predicted by the number of previous samples presented at a camera trap set up, nor by a binary effect of whether a sample had or had not been previously laid and removed (Table A3). This suggests that the methods used for removing samples between trials was effective, and olfactory stimuli were not carrying over significantly between trials.

Statistical Analysis

Responses were scored in line with the ethogram (Table 1) in a blind procedure, meaning the identity of the sample was unknown to the scoring researcher. Behaviours were scored by one researcher (CA) to standardise scoring of behaviours. We ran generalized logistic mixed-effects models (GLMMs) where olfactory response was the dependent variable, with elephant ID, and pathway location included as random effects in all models. The level of olfactory responsiveness was scored as elephants passed a sample, with a binary score of 1/0 assigned to each behaviour performed (Table 1). Binary scores were assigned to behaviours (rather than,

for example, duration of sniffs, or number of vomeronasal system responses performed) due to a considerable right-skew in the distribution of the dataset. If an individual elephant passed the same sample multiple times in the 48-hour trial window, only the first response was considered. If an elephant was observed urinating or defecating in the camera frame, the trial was abandoned and camera trap set up re-located due to the potential influence of these fresher deposits on future passing elephants.

Whilst it is theoretically possible that elephants detect chemical information with the trunk held in a variety of seemingly discrete postures, we focused scoring of responses on fixed behaviours and postures known to be indicative of an elephant using the olfactory sense (Table 1). “General olfactory response” behaviours referred to an olfactory responsiveness to the pathway substrate, with no focus on a particular fixed point of interest (Supplementary video available). We first explored the extent to which male elephants showed general olfactory responses to the pathway substrate when no samples were presented, and whether age, grouping and position within groups affected these responses. For these GLMMs, the dependent variable was a binary 1/0 score whereby 1 represented a general olfactory response (“Tracking” and “J-sniff” behaviours, Table 1) to a randomly pre-assigned point within the presentation zone (occupying the same volume as a sample, approx. 0.2m stretch of pathway), and 0 represented a non-olfactory response to this point (Table 1). We ran two GLMMs, (1) with age of receiver (adult/ subadult) and grouping condition (lone traveller/ in all male group) as the fixed effects; and (2) with age of receiver (adult/ subadult) and position within the travelling group (leader/ middle/ rear of groups) as the fixed effects. If an effect was identified as significant predictor in either model, models were rerun to investigate any interaction between age class and grouping factors.

Secondly, we explored how the responses of elephants to the urine of different aged male elephants changed over time since the deposit was laid, owing to potency of cues fading, receivers losing interest, or even new cues emerging due to microbially mediated processes (Goodwin et al., 2012). For these GLMMs, the dependent variable was a binary 1/0 score whereby 1 represented a focused olfactory response to the sample (whereby the sample is the focus of olfactory response in a manner that is distinct from the general olfactory monitoring of the pathway substrate, Table 1), and 0 represented either non-olfactory behaviour or a non-focused (general) olfactory response as the elephant passes over the sample (Table 1). Fixed effects in this model included sample type (adult urine, subadult urine, water control), time since sample laid, and the interaction between these two variables. To explore if the three sample types predicted likelihood of a focused olfactory response to a sample in a manner that diverged over time, we switched reference classes; so that pairwise comparisons could be made between sample types. Trampling on samples by previous passing elephants as well as other passing species may also affect the likelihood of response, and both were recorded and combined into a measure of trample rate. Trample rate also included physical contact made due to accessory trunk behaviour “Blow”, and physical contact with sample due to vomeronasal system responses (Table 1). Trample rate was found to have a weak positive correlation with time since a sample was laid (Spearman’s Rank Correlation, $r_s(454) = 0.190$, $p < 0.001$). Because time since a sample was laid had more relevance to temporal changes in olfactory cues, and therefore our question of interest, we included this variable instead of trampling rate in our model.

Lastly, we investigated whether vomeronasal system responses to urine deposits were influenced by age class interactions. For these GLMM’s, the dependent variable was a binary 1/0 score whereby 1 represented vomeronasal system responses being performed to the sample

(pre-flehmen and flehmen behaviours, Table 1). Activation of the vomeronasal system is indicative of a heightened olfactory response to the sample, as opposed to a more general olfactory investigation (Rasmussen et al., 1982; Schulte et al., 2005). A score of 0 represented all other behaviours in the ethogram being performed to the sample (Table 1). Fixed effects in these models included age of the receiver (adult/ subadult), and sample type (adult urine, subadult urine, water control), and again reference categories were switched to allow for pairwise comparisons between sample types.

Ethical Note

This work received approval from the University of Exeter Research Ethics Committee (application ID: eCLESPsy000545 v3.2) and was conducted with permission of the Botswana Department of Wildlife and National Parks, under research permit EWT 8/36/4 XXXVI (57).

Results

General olfactory response to pathways

With no samples presented on pathways, male elephants were still generally responsive to the pathway substrate, with 61.24% ($N = 128/209$) of passing elephants exhibiting general olfactory responses to the randomly assigned point on the pathway. Focused sniffs were only performed by 1.44% of passing elephants (3/209), accessory trunk behaviours by 0.5% (1/209), and no elephants were observed to perform pre-flehmen or flehmen behaviours when no sample was present. The likelihood of performing a general olfactory response to the pathway substrate was not predicted by season, temperature, wind level, whether the elephant was

traveling in the day or night, or whether a musth male had walked on the pathway ahead of the subject elephant in the trial (Table A4).

There was no significant effect of age on the likelihood of an elephant exhibiting a general olfactory response to the pathway substrate (Age class adjusted odds ratio (aOR) = 0.724, $P = 0.295$, Table 2). There was however, a significant effect of whether the individual was solitary or in a group, with greater probabilities of response in lone travellers compared to elephants traveling in groups (Grouping aOR = 5.039, $P = 0.002$, Table 2). Rerunning models to include interaction terms revealed that there was no interaction between group status and age (output of GLMMs Table 2, Figure 2).

For elephants travelling in all-male groups, position within the group did not predict the likelihood of exhibiting a general olfactory response to the pathway substrate (Table 3).

Table 2

Summary of GLMM's investigating differences in age and social grouping on the likelihood of elephants exhibiting a general olfactory response to pathway substrate.

	Predictor		Coefficient	aOR (+95% CI)	P value
Main effects	Intercept		0.492	1.636 (0.822-3.256)	0.161
model	Age Class	Adult	<i>Ref</i>	<i>Ref</i>	
		Subadult	-0.322	0.724 (0.396-1.324)	0.295
	Grouping	Group Travel	<i>Ref</i>	<i>Ref</i>	
		Lone Travel	1.617	5.039 (1.808-14.046)	0.002 *
Interaction	Intercept		0.520	1.682 (0.853-3.316)	0.133
terms - adult as reference class	Age Class	Adult	<i>Ref</i>	<i>Ref</i>	
		Subadult	-0.379	0.685 (0.363-1.291)	0.242
	Grouping	Group Travel	<i>Ref</i>	<i>Ref</i>	
		Lone Travel	1.420	4.136 (1.258-13.593)	0.019 *
	Age Class *	Adult*Lone	0.704	2.022 (0.177-23.065)	0.571
	Grouping	Travel			
	Intercept		0.142	1.152 (0.598-2.221)	0.673

Interaction terms - subadult as reference class	Age Class	Adult	0.379	1.460 (0.775-2.751)	0.242
		Subadult	<i>Ref</i>	<i>Ref</i>	
	Grouping	Group Travel	<i>Ref</i>	<i>Ref</i>	
		Lone Travel	2.124	8.363 (1.00-69.982)	0.050 *
	Age Class * Grouping	Adult*Lone Travel	-0.704	0.494 (0.043-5.640)	0.571

aOR= adjusted odds ratio, CI = confidence intervals

Lone travellers of all ages were more likely to monitor the pathway with general olfactory responses compared to males travelling in groups.

Table 3

Summary of GLMM investigating effect of age and position within group on the likelihood of elephants exhibiting general olfactory response to pathway substrate.

Predictor		Coefficient	aOR (+95% CI)	P value
Intercept		0.684	1.981 (0.823-4.771)	0.127
Age Class	Adult	<i>Ref</i>	<i>Ref</i>	
	Subadult	-0.360	0.698 (0.362-1.344)	0.282
Position in group	Leader	<i>Ref</i>	<i>Ref</i>	
	Middle	0.107	1.113 (0.513-2.416)	0.786
	Rear	-0.228	0.797 (0.343-1.850)	0.597

aOR= adjusted odds ratio, CI = confidence intervals

Neither age, nor position held within all-male group procession predicted likelihood of elephants exhibiting general olfactory response to pathway substrate.

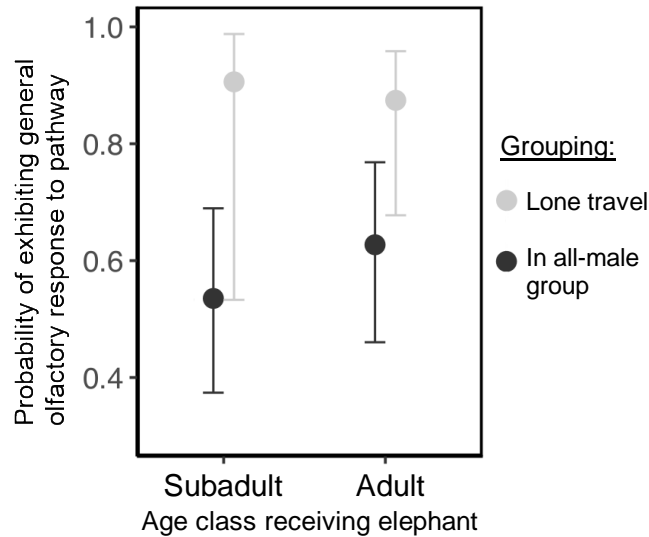


Figure 2: Probability of elephants of different age class and social grouping exhibiting general olfactory responses to pathway substrate when no sample was presented, with 95% confidence intervals indicated.

Responses to experimental urine samples over time

Adult urine had a greater probability of evoking a focused olfactory response than subadult urine (aOR= 0.224, $P = 0.003$, Table A5), but not than the water control (aOR= 1.363, $P = 0.477$, Table A5). Subadult urine had the lowest probability of evoking a focused olfactory response, significantly lower than both adult urine (aOR= 4.463, $P = 0.003$, Table A5) and the water control (aOR = 6.083, $P < 0.001$, Table A5).

The interaction between sample type and time since sample laid was significant in all pairwise comparisons, meaning all sample types were affected differently by time in their influence in predicting focused olfactory response (Figure 3).

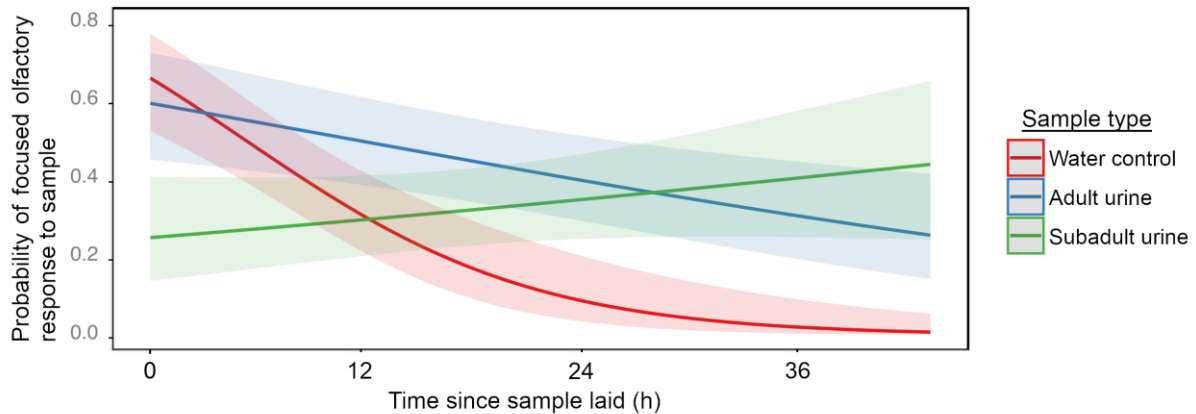


Figure 3: Probabilities of elephant paying a focused olfactory response to each sample type over time since deposit laid, regression coefficients and 95% confidence intervals based on standard errors indicated. Overtime, the water-based control rapidly decreased in its probability of evoking focused olfactory responses in passing elephants, with a significant “time since laid” coefficient of -3.013 , $P < 0.001$. Probability of responding to adult urine remained more constant throughout the 48-hour sample time, with a shallower negative coefficient of -0.806 , $P = 0.012$. Time since deposit was laid had no effect on probability of elephants exhibiting a focused olfactory response to subadult urine, coefficient = 0.473 , $P = 0.284$. The interaction between sample type and time since deposit in predicting focused olfactory response was significant in all combinations of pairwise comparisons between sample types (Table A5 for output of GLMM with full pairwise comparisons of significant interaction regression coefficients, aOR’s, 95% confidence intervals and P values).

Vomeronasal system responses to urine samples

Due to the high observed probabilities of response to water control samples in early hours since deposition (Figure 3), we excluded all responses to all samples in first 8 hours since deposit in the following analyses testing for differences in vomeronasal system responses to samples by males of different age classes.

Age class of the receiving elephant did not predict likelihood of performing a vomeronasal system response to a sample. Whilst overall adults performed these pre-flehmen and flehmen behaviours at a lower probability than subadults did, this difference was not significant (aOR= 2.488, $P = 0.717$, Table 4, Figure 4).

Adult urine elicited pre-flehmen and flehmen behaviours at greater probability than subadult urine (aOR= $1.986 e^{-06}$, $P = 0.010$, Table 5, Figure 4). The water-based control did not evoke pre-flehmen and flehmen behaviours at a different probability than that from either urine sample type (Table 5, Figure 4).

Table 4

GLMM output for likelihood of elephants exhibiting vomeronasal system responses to samples (pre-flehmen and flehmen behaviours) predicted by age class of receiving elephant.

Predictor	% response	Coefficient	aOR (+95% CI)	P value
Intercept		-13.540	$1.317 e^{-06}$ ($1.102 e^{-08} - 1.575 e^{-04}$)	<0.001 *
Age Class of receiving elephant	Adult	3.167	Ref	Ref
	Subadult	7.614	0.912	2.488 ($1.814 e^{-02} - 3.413 e^{02}$)

aOR= adjusted odds ratio, CI = confidence intervals

Age class of receiving elephant did not predict likelihood of exhibiting vomeronasal system response to sample.

Table 5

GLMM output for likelihood of elephants exhibiting vomeronasal system responses to samples (pre-flehmen and flehmen behaviours) predicted by sample type.

Predictor	% response	Coefficient	aOR (+95% CI)	P value	
Water control as reference class	Intercept	-14.110	$7.451 e^{-07}$ ($2.620 e^{-10} - 2.119 e^{-03}$)	<0.001 *	
Sample Type	Water control	4.762	Ref	Ref	
	Adult	10.94	1.085	2.960 ($1.466 e^{-03} - 5.974 e^{03}$)	0.780
	Subadult	1.835	-12.044	$5.877 e^{-06}$ ($1.854 e^{-11} - 1.863$)	0.062
Adult urine as reference class	Intercept	-13.025	$2.205 e^{-06}$ ($3.849 e^{-09} - 1.264 e^{-03}$)	<0.001 *	
Sample Type	Water control	4.762	-1.085	$3.379 e^{-01}$ ($1.667 e^{-04} - 6.848 e^{02}$)	0.780
	Adult	10.94	Ref	Ref	

Subadult 1.835 -13.130 $1.986 e^{-06}$ ($8.784 e^{-11} - 4.488 e^{-02}$) **0.010 ***

aOR= adjusted odds ratio, CI = confidence intervals

Sample type predicted likelihood of vomeronasal system response, modelling the water control as the reference class revealed the control did not evoke pre-flehmen and flehmen behaviours at a different probability than that from either urine sample type. Modelling adult urine as the reference class revealed subadult urine evoked pre-flehmen and flehmen responses significantly less than adult urine.

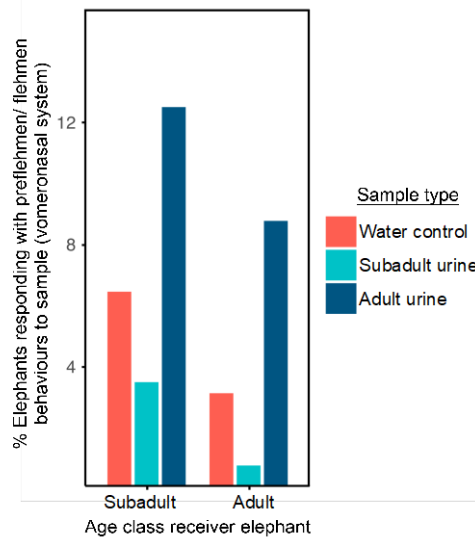


Figure 4: Percentage of subadult and adult elephants exhibiting vomeronasal system responses to different sample types, indicative of heightened response to stimuli.

Since all vomeronasal system responses involved the seizure of movement of the subject elephant moving on the pathway, there is a possibility that responses of elephants to samples could be influenced by their group members' responses. However, the tendency of an elephant to perform a vomeronasal system response to a sample showed no correlation with either the presence of elephants ahead of an elephant in the travelling group performing a vomeronasal system response to the sample (Phi coefficient = 0.0816), or with elephants behind him performing a vomeronasal system response (Phi coefficient = 0.160).

Further exploration of the high probability of response to the water control led us to an additional analysis of the observation that in some cases elephants would throw the sample on

themselves after physical contact and pinching (recorded as a vomeronasal system response, Table 1). Of the 18 occasions where this behaviour was observed, on 13 occasions the sample was a water control (72.22%). A GLMM modelling the probability of throwing sample on oneself following vomeronasal system response by sample type revealed that the water control significantly predicted this response, with greater probability of response compared to both urine sample types (Table A6).

Discussion

Elephant pathways are used by African elephants to improve efficiency when moving between habitats and predictable resources such as waterholes and feeding areas separated in the landscape (Von Gerhardt et al., 2014; Shannon et al., 2009; Mutinda et al., 2011). We provide evidence that substrate-borne olfactory cues inherent to the elephant pathway (accumulations of dung and urine scents from previous travellers) are likely a key stimulus for elephants traveling on pathways, with the majority of elephants observed exhibiting general olfactory responses to the pathway substrate during travel when no samples were presented. Whilst it has been shown that elephants travel in the core of their range using a “Euclidean-cognitive map”, relying on a mental representation of their spatial environment, in the periphery of their range they switch to habitual routes for movement, i.e., pathways (Presotto et al., 2019). The MPNP stands at the fringes of the African elephants’ range in Botswana (Thouless et al., 2016), and bulls are transitory in the area, staying on average for just 47 days (Pitfield, 2017). Many individuals on the pathways are therefore likely to be unfamiliar with the environment and may depend on the pathway as an indicator of movements of other African elephants in the area. In African forest elephants (*Loxodonta cyclotis*) it is suggested that following permanent trails as a search strategy may assist naïve elephants in finding resources connected by trails (Blake &

Inkamba-Nkulu, 2004). This is further supported by our finding that lone travellers were significantly more responsive to the pathway substrate compared to those travelling in all-male groups, potentially because those traveling in groups had more opportunity to rely on conspecifics, rather than environmental olfactory cues for keeping on the scent trail of the pathway (Pettit et al., 2015).

Whilst the pathway is likely also a visual stimulus (Figure 1 (c)), the high probability of elephants exhibiting a general olfactory response to it (61.24% of elephants at a 0.2m of randomly assigned stretch of pathway), suggests the pathway acts as a public information scent trail. Across the animal kingdom, species follow the scent trails of conspecifics for the location of mates, prey, home and forage, as well as for mass migration (snakes, *Thamnophis sirtalis*, Costanzo, 1989; social insects, Edelstein-Keshet, 1994; rats, *Rattus norvegicus domestica*, Khan et al., 2012; wood mice, *Apodemus sylvaticus*. Jamon, 1994), and the olfactory sense is considered a widely utilised modality for spatial orientation and navigation (Lavenex & Schenk, 1998; Buehlmann et al., 2015; Etienne, 2003). We did not find any evidence that age class predicted tendency to exhibit a general olfactory response to the pathway substrate, suggesting olfactory attentiveness to pathways is important to all ages of independent (dispersed from natal herd) male African elephants. We also reject our hypothesis that those leading groups would be more olfactory responsive to pathways, as position held within groups did not predict likelihood of response. This suggests olfactory elements of the pathway that are relevant for purposes other than navigation may be of interest to all mobilised elephants (such as assessment of conspecifics, see below). Or alternatively, that during travel, memory acquisition and active learning of the route (relevant to both leaders and followers) is reinforced and/or encoded by olfactory cues as key informing modalities (Svensson et al., 2014; Piqueret

et al., 2019; Lavenex & Schenk, 1998), i.e., there is olfactory mapping of the environment (Tomlinson & Johnston & 1991).

The tendency of the water control to elicit focused olfactory responses declined rapidly with time since deposit. This differed significantly from the effect of time on adult and subadult urine, suggesting elephants do monitor, and can detect the urine deposits of conspecifics on pathways, as opposed to simply novel sand cues, for at least 48 hours since deposition. The persistence of olfactory responses being performed to urine samples over time is indicative that (1) the odours are still potent enough to be detected by conspecifics, and potentially (2) that the odours are still of interest to passing conspecifics (Schulte & Rasmussen 1999). Greater probability of response to adult compared to subadult urine cues could be due to the different chemical composition of adult and subadult urine (Rasmussen & Wittemyer, 2002), again potentially affecting the potency of odours or their relevance to receiver. The continued focused olfactory response to urine samples over time may be the result of microbial action on the compounds in urine transforming the chemical composition of urine over time (Goodwin et al., 2012). This delayed release of compounds and change in chemical composition over time has been proposed to provide information concerning the age of a deposit, hence an indication to the receiver concerning the proximity of the depositor (Rasmussen & Schulte, 1999; Goodwin et al., 2012). This would be highly adaptive for male elephants monitoring urine deposits on pathways used for travel, as an indication of the age and proximity of same-sex conspecifics.

Concerning vomeronasal system responses to urine cues, we found no evidence that the age class of receiving elephants predicted likelihood of response, despite higher observed probabilities of subadults performing these pre-flehmen and flehmen behaviours. Previous studies have shown that wild male African elephants peak in rate of olfactory investigatory

behaviours performed to urine cues at waterholes in adolescence, but the decline in adulthood is compensated by a pattern of more refined behaviour – i.e., adult elephants only respond to relevant urine cues, such as those that indicate a potential threat (Schulte et al., 2012). Age class of the depositor of urine, did however predict likelihood of response, with adult urine evoking vomeronasal system responses at a greater probability than subadult urine. A urine deposit from an adult male is likely to be of greater relevance than that of subadults to males of all ages. For adults the presence of another adult may indicate a potential threat to mating opportunities, or conversely a potential affiliate for acquisition of knowledge or a sparring partner (Chiyo et al., 2011; Lee & Moss, 2014), whereas subadult males in the environment would be of lesser threat or interest, as the dominance hierarchy of males is strictly related to age and musth state (Lee et al., 2011; Hollister-Smith et al., 2007). Both adults and subadults may also exhibit greater responses to adult urine in an attempt to discern musth state (Hollister-Smith et al., 2008; Rasmussen & Wittemyer, 2002), as an encounter with a musth bull could lead to dangerous physical confrontation for elephants of all ages (Lee et al., 2011; Poole, 1989). Whilst none of the urine samples in the study were from individuals identified to be in musth at the time of sampling, it is possible that urine may carry compounds indicating proximity in time to a transition to musth state, which requires more information to discern than an obvious and potent musth signal. Indeed, in studies supporting that African elephants can determine musth state of remote signallers, it was found that elephants performed more flehmen behaviours to non-musth urine than musth urine (Hollister-Smith et al., 2007).

The lack of significant difference between the probabilities of focused olfactory responses being performed to adult urine and the water control, and for either age class urine in evoking a vomeronasal system response compared to the water control (even after eliminating the first 8 hours since deposit) is notable in the current study. But we suggest the high probability of

responses to the water control may be due to different ecological reasons from that of the responses to urine samples. Elephants were more likely to throw samples of water controls over themselves (subsequent to a physical contact recorded as a vomeronasal response) than urine samples of either age class. This, along with the fact that focused olfactory responses were higher to water controls in the early hours of deposition (Figure 3) than to other sample types, suggests that elephants are in general extremely sensitive to olfactory cues on pathways, and the presentation of the water sample may have evoked “violation of expectation” type response – as an unexpected stimulus to encounter on the pathway (Bates et al., 2008). Indeed, elephants in arid regions such as the MPNP are highly sensitive to the odour of water (Ramey et al., 2013; Ndlovu et al., 2018). We hypothesise that whilst the low vomeronasal system responses to subadult urine may be due to a lack of importance of these cues to passing elephants in relation to adult urine (but no violation of expectation in being an odour cue encountered on pathway); the high probability of response to the water control may be due to the expectations of elephants being violated in encountering this stimulus on the pathway. Subsequent to physical contact using the trunk, elephants in some cases continued to throw the water control sample on themselves – as is common with mud wallowing and dusting behaviours performed by elephants (Mole et al., 2016). Future research conducted in this area is likely to face similar problems in finding an appropriate urine control due to wild elephants’ high sensitivity to water (Ramey et al., 2013; Ndlovu et al., 2018) and novel objects and substances (Poole & Granli, 2011).

Our study highlights the olfactory responsiveness of wild African elephants to elephant pathways during travel. Our results support the hypothesis that the male African elephant olfactory system is extensively used during travel on pathways, but also that male African elephants may monitor urine deposits of conspecifics on pathways, potentially discerning an

indication of the ages' of males they can expect to encounter. We provide support for the hypothesis that a positive feedback loop establishes in the long-term maintenance of elephant pathways, whereby in utilising public information in following the olfactory trails of other elephants, travellers themselves deposit urine and dung cues that enhance pathway persistence (see pheromone trail deposition in foraging ants; Wendt et al., 2020; von Thienen et al., 2015; Frizzi et al., 2018).

Understanding elephant pathway use is essential for land-use planning and reducing wildlife conflict (Songhurst et al., 2016). Following from our results, groups involved in elephant management could explore the use of olfactory cues on elephant pathways as a method for manipulating elephant movements. In Botswana, elephant crop raiding events are significantly more likely in fields close to pathways (Von Gerhardt et al., 2014; Songhurst & Coulson, 2014). Testing whether crop raiding events could be avoided by manipulating the pathway route away from human settlements (i.e. by removing pathway scents and redirecting to an alternate route with purposefully placed deposits of urine and dung) is one suggestion for how our results could be extended to aid in elephant management. In addition, because pathways are known to connect predictable, critical resources as well as dispersal routes (Mutinda et al., 2011; Von Gerhardt et al., 2014; Shannon et al., 2009), elephant managers could experiment with using olfactory cues, elephant urine and dung deposits, to enhance desired corridor routes connecting protected areas (Osborn & Parker, 2003; Baldus et al., 2007; Naidoo et al., 2018; Adams et al., 2016; Lindenmayer & Nix, 1993; Douglas-Hamilton et al., 2005). This method would be of particular interest to the Kavango-Zambezi Transfrontier Conservation Area, that aims to link the elephant populations (encompassing estimated 200,000+ elephants) across 36 protected areas over 5 countries in southern Africa through secure wildlife corridors, whilst

avoiding negative impact on rural communities (Munthali et al., 2018; Metcalfe & Kepe, 2008).

Data Statement

Due to the sensitive nature of reporting on African elephant locations and numbers, the data that support the findings of this study are available on reasonable request from the corresponding author.

Acknowledgements

This work received approval from the University of Exeter Research Ethics Committee (application ID: eCLESPsy000545 v3.2), and was conducted with permission of the Botswana Department of Wildlife and National Parks, under research permit EWT 8/36/4 XXXVI (57).

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Appendix

Note A1: All individuals in the study were uniquely identified by a human observer using distinguishing features such as ear notches, holes and tears, tusk length, girth and morphology, skin wrinkles, tail length, and other abnormalities. Reliability of identification was validated by presenting footage of anonymised elephants to a blind researcher. Using characteristics listed above, the researcher assigned the elephant to an ID in the data base. 100% of elephants were assigned their correct ID number ($N = 30$).

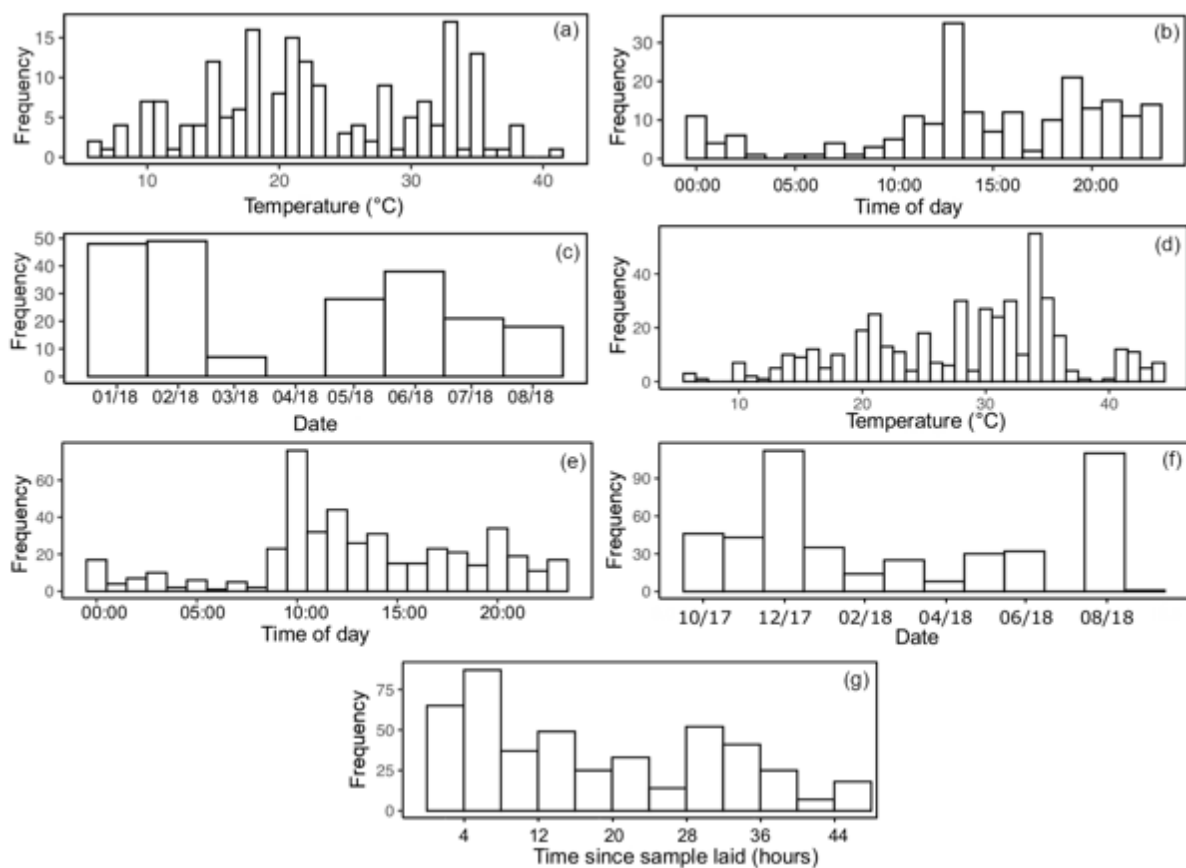


Figure A1: Distributions of responses caught in different trial types under various time and climate conditions. (a) Temperature at time response caught, no sample presented, (b) Time of day response caught, no sample presented, (c) Month of year when response caught, no sample presented, (d) Temperature at time response caught, sample presented, (e) Time of day response caught, sample presented, (f) Month of year when response caught, sample presented, (g) Time since sample laid when response was caught, sample presented. Decrease in responses

caught of passing elephants over time since sample laid largely due to camera traps capturing responses being pulled down by elephants, or camera trap battery dying before 48-hour trial complete.

Table A1

Summary table of sample sizes of responses caught for different trial types in the study.

Trial type	Variable			Sample size / distribution	
No sample presented on pathways	Age class receiver (total)	Subadult		99	
		Adult		110	
	Grouping (total)	Lone traveller		37	
		Group traveller		172	
	Position (total)	Leader		47	
		Middle		73	
		Rear		46	
	Age Grouping	Class*	Subadult	Lone traveller	10
				Group traveller	89
			Adult	Lone traveller	27
				Group traveller	83
	Age Position	Class*	Subadult	Leader	20
				Middle	45
				Rear	20
			Adult	Leader	27
				Middle	28
				Rear	26
	Temperature				See Figure A1
	Time of travel (24-hour clock)				See Figure A1
	Month of year				See Figure A1
Sample presented on pathway	Day/ night travel (infrared footage)	Day		102	
		Night		107	
	Season	Wet		103	
		Dry		106	
	Wind	No wind		185	
		Windy		24	
	Direction of travel	Toward river		159	
		Away from river		50	
	Age class receiver	Subadult		248	
		Adult		208	
	Day/ night travel (infrared footage)	Day		298	
		Night		158	
	Season	Wet		82	
		Dry		374	
Wind	No wind		346		
	Windy		110		
Direction of travel	Toward river		373		
	Away from river		83		
Sample type	Water control		161		

	Subadult urine	119
	Adult urine	176
Sand type sample deposited on	River edge	274
	Bank slope	182
Temperature		See Figure A1
Time of travel (24-hour clock)		See Figure A1
Month of year		See Figure A1
Time since deposit laid		See Figure A1

Distributions and sample sizes of responses caught for ages of receiving elephants, sample types and climatic conditions given for both “No sample presented: General olfactory response” and “Samples presented: Focused olfactory response and vomeronasal system response”. Wet and dry seasons determined following Allen et al., 2020.

Table A2

GLMM output for likelihood of elephant exhibiting focused response to sample predicted by sample treatment prior to presentation

Predictor		Coefficient	aOR (+95% CI)	P value
Intercept		-0.073	0.929 (0.514 - 1.680)	0.808
Sand type	Bank sand	<i>Ref</i>	<i>Ref</i>	
	River edge	-0.143	0.867 (0.607 - 1.238)	0.432
Time in fridge		-0.0002	1 (0.999 – 1)	0.340
Av. temp sample kept at		-0.045	0.956 (0.889 - 1.029)	0.228

aOR= adjusted odds ratio, CI = confidence intervals

Different treatment of sample prior to presentation on pathways had no effect in predicting likelihood of elephants exhibiting a focused olfactory response to samples. Elephant ID and pathway number included as random effects.

Table A3

GLMM outputs for likelihood of elephant exhibiting olfactory responses to pathway by factors relating to samples previously presented at the camera trap set up

GLMM dependent variable	Predictor	Coefficient	aOR (+95% CI)	P value
General olfactory response paid to pathway	Intercept	0.936	2.550 (1.197-5.435)	0.015 *
	Number of previous samples laid at camera trap set up	0.084	1.087 (0.957-1.235)	0.197
	Sample has been presented previous (binary)	No Yes	<i>Ref</i> -0.227	<i>Ref</i> 0.797 (0.332-1.910)
Elephant pauses to smell areas other than presentation zone	Intercept	-2.833	0.059 (0.018-0.188)	<0.001 *
	Number of previous samples laid at camera trap set up	No	<i>Ref</i> <i>Ref</i>	0.342

Sample has been presented previous (binary)	Yes	-0.180	0.835 (0.161-4.331)	0.830
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aOR= adjusted odds ratio, CI = confidence intervals

Previous samples being presented on camera trap set ups was unlikely to have a significant impact on the likelihood of elephants paying general olfactory responses to pathways in our trials. GLMMs of likelihood of elephant paying general olfactory response to pathway, and of likelihood to pause and smell the areas outside the presentation zone within the camera frame were not predicted by factors relating to samples previously presented at the camera trap set up (number of previous samples laid on the pathway, and a binary 1/0 condition of whether samples had or had not previously been laid on pathways in the camera trap set up). Elephant ID and pathway location included as random effects in both models.

Table A4

GLMM output of likelihood of elephant exhibiting general olfactory response to pathway predicted by various environmental factors

Predictor	% response	Coefficient	aOR (+95% CI)	P value
Intercept		1.777	5.914 (0.914-38.276)	0.062
Temperature		-0.052	0.950 (0.889-1.014)	0.124
Season	Dry	61.682	<i>Ref</i>	<i>Ref</i>
	Wet	62.025	0.152	1.165 (0.559-2.426)
Wind	No wind	62.722	<i>Ref</i>	<i>Ref</i>
	Windy	58.824	0.084	1.088 (0.363-3.265)
Time of travel	Day	56.471	<i>Ref</i>	<i>Ref</i>
	Night	66.337	-0.274	0.760 (0.269-2.146)
Musth male has passed in trial	No	63.218	<i>Ref</i>	<i>Ref</i>
	Yes	41.667	-0.492	0.611 (0.163-2.291)

aOR= adjusted odds ratio, CI = confidence intervals

Likelihood of paying general olfactory response to the pathway was not predicted by environmental conditions of season, temperature, wind level, day or night travel, or whether a musth male had passed the experimental set up previously in the trial. Elephant ID and pathway location included as random effects.

Table A5

GLMM output of likelihood of exhibiting focused olfactory responses to samples predicted by sample type and time since sample laid, and interaction between both factors.

Reference class (sample type)	Predictor	Coefficient	aOR (+95% CI)	P value	
Water control	Intercept	0.701	2.016 (1.015-4.002)	0.045 *	
	Sample type	Water control	<i>Ref</i>	<i>Ref</i>	
		Adult Urine	-0.310	0.734 (0.312-1.724)	0.477
		Subadult Urine	-1.806	0.164 (0.063-0.428)	< 0.001 *
		Time since sample laid	-3.013	0.049 (0.011-0.217)	< 0.001 *
		Sample type * Subadult urine *	3.486	32.644 (6.420-165.978)	< 0.001 *
		Time since sample laid	Time since sample laid		

		Adult urine * Time since sample laid	2.207	9.089 (1.929-42.830)	0.005 *
Adult urine	Intercept		0.391	1.478 (0.737-2.969)	0.271
	Sample type	Water control	0.310	1.363 (0.580-3.203)	0.477
		Adult Urine	<i>Ref</i>	<i>Ref</i>	
		Subadult Urine	-1.496	0.224 (0.084-0.595)	0.003 *
	Time since sample laid		-0.806	0.447 (0.238-0.836)	0.012 *
	Sample type * Time since sample laid	Subadult urine *	1.279	3.592 (1.271-10.150)	0.016 *
	Subadult urine	Sample type	Water control	1.806	6.083 (2.337-15.829)
Adult Urine			1.496	4.463 (1.681-11.848)	0.003 *
Subadult Urine			<i>Ref</i>	<i>Ref</i>	
Time since sample laid			0.473	1.604 (0.676-3.806)	0.284
Sample type * Time since sample laid		Adult urine * Time since sample laid	-1.279	0.278 (0.099-0.156)	0.016 *
Time since sample laid		Water control *	-3.486	0.031 (0.006-0.787)	< 0.001 *
		Time since sample laid			

aOR= adjusted odds ratio, CI = confidence intervals

The differential effect of time on different sample types all significantly predicted whether an elephant exhibited a focused olfactory response to samples.

Table A6

GLMM output of likelihood of throwing sample on self predicted by sample type

Reference class	Predictor	Coefficient	aOR (+95% CI)	P value	
Water control	Intercept	-2.432	0.088 (0.050-0.155)	<0.001 *	
	Sample type	Water control	<i>Ref</i>	<i>Ref</i>	
		Adult	-1.329	0.265 (0.085-0.829)	0.023 *
		Subadult	-2.338	0.096 (0.012-0.748)	0.025 *
Adult urine	Intercept	-3.761	0.023 (0.009-0.063)	<0.001 *	
	Sample type	Water control	1.329	3.777 (1.206-11.833)	0.023 *
		Adult	<i>Ref</i>	<i>Ref</i>	
		Subadult	-1.010	0.364 (0.040-3.302)	0.369

aOR= adjusted odds ratio, CI = confidence intervals

Likelihood of throwing sample over self was predicted by sample type, with the water control evoking this behaviour significantly more than subadult and adult urine. Elephant ID and pathway location included as random effects.