PLOS ONE

Meat in a carnivore's diet allows a group of captive meerkats to discriminate between the faeces of predators and non-predators

--Manuscript Draft--

Manuscript Number:	PONE-D-13-17133R1
Article Type:	Research Article
Full Title:	Meat in a carnivore's diet allows a group of captive meerkats to discriminate between the faeces of predators and non-predators
Short Title:	Category discrimination in meerkats
Corresponding Author:	Sean O'Hara, Ph.D University of Salford Salford, Greater manchester UNITED KINGDOM
Keywords:	carnivore; herbivore; predators; meat; odour; Suricata suricatta
Abstract:	The ways in which animals form categories and solve discrimination problems have long been subjects of scientific interest. Here, we measured meerkats' investigation time of stimuli that indicated the presence of a predator, but was not the actual predator. We also asked, if the meerkats do discriminate between different stimuli, on the basis of what feature(s) are they able to make that discrimination? We conducted trials on a large group of predator-naïve captive meerkats at Knowsley Safari Park, Merseyside. In phase 1, meerkats were presented with fresh faeces of potential predators (dog, serval), non-predators (giraffe, elephant) and a control (rubber ball) to record interaction times according to stimulus type. Meerkats interacted for longer with predator faeces. In phase 2 we used one of our predator species (dog) and presented faecal samples to the group that differed by diet (meat versus vegan-fed). Meerkats spent significantly longer inspecting the faeces of meat-fed dogs. Finally, we re-ran phase 2 using aged faeces, predicting that interest would dissipate now that the scents provided no temporally salient information. Interest in both faecal types diminished. Taken together, our results suggest that these predator-naive meerkats might be able to infer the presence of an absent predator on the basis of a secondary cue alone, and that detection is being achieved as a consequence of predators' meat-eating activities.
Order of Authors:	Sean O'Hara, Ph.D
	Heather Williams
	Catherine O'Hara
Suggested Reviewers:	Alex Thornton, PhD University of Exeter alex.thornton@exeter.ac.uk meerkat cognition researcher
	Simon Townsend, PhD University of Zurich simon.townsend@ieu.uzh.ch meerkat researcher, experimental trials.
	Anna Wilkinson, PhD University of Lincoln awilkinson@lincoln.ac.uk animal cognition expert
Opposed Reviewers:	
Response to Reviewers:	Response to reviewers: rebuttal statement PONE-D-13-17133 How meerkats detect predators via a stimulus that signals presence, but is not the actual predator PLOS ONE

Dear Dr O'Hara,

Thank you for submitting your manuscript to PLOS ONE. After careful consideration, we feel that it has merit, but is not suitable for publication as it currently stands. Therefore, my decision is "Major Revision."

We invite you to submit a revised version of the manuscript that addresses the points below:

Both reviewers make many important and helpful comments - I would like all these points addressed, but in particular:

Both reviewers are unconvinced by the statistical analysis. I share their concern that in a comparison of 2 variables it is unclear which mean and SD you should consider - if you ask if the mean of herbivore investigation is more than 2SD less than the mean of the carnivore the answer is no - yet the other way around the answer is yes. Insufficient explanation and justification for these results is given - reviewers encourage you to explore alternatives.
We agree with reviewer one that if we write the paper specifically about this group of meerkats a repeated-measures approach would be justified and appropriate. At your suggestion we have made major revisions: the data have been re-analysed and the paper re-written.

The nature of the control trials - neither reviewer was convinced that a rubber ball was an appropriate control - the plastic 'fake' poo mentioned in pilot work would seem much more appropriate. Given most of your analyses hinge on the control results it is vital that this an appropriate control and I am not convinced of this currently.
We believe the reviewers adopted that view because it was not clear that the ball provided an olfactory cue. We have made changes to our wording to now make it clear that it did. We would disagree that the fake poo would have served as a better control as it is visually related to the test stimuli. As such, it would distinguish less well between a meerkat response towards our experimental stimuli and a control than an entirely novel item such as a rubber ball. Please see further justification of our control choice below in response to referees' specific comments.

Given the only DV is time spent investigating all claims of 'inferring an absent predator' should be removed. If appropriate alarm calling or anti predator behaviour was elicited by these stimuli this may be a justified speculation, but without this, this is not justified by the data presented.
Careful re-write undertaken.

I think the simple explanation that meerkats may be more interested in stimuli containing fresh meat, given its similarity to their food items, has not been sufficiently addressed. Meerkats are not scavengers, so old meat may not be considered a potential food item. Faeces containing fresh meat, may be interesting due to its similarity to their natural fresh meat diet - Just because no meerkat was seen eating the faeces, does not rule this out in a convincing manner. I don't see how you can address this experimentally, but ackowledgement of this alternative explanation for these results should be clearly made in the discussion.
Inclusion of additional wording to address this has been placed in the discussion. We agree that ruling this out convincingly is difficult. And, although we realise that phase 3 trials do not do rule out this explanation entirely, the reason we implemented phase 3 was to go so way to addressing that problem.

We encourage you to submit your revision within forty-five days of the date of this decision.

When your files are ready, please submit your revision by logging on to http://pone.edmgr.com/ and following the Submissions Needing Revision link. Do not submit a revised manuscript as a new submission. Before uploading, you should proofread your manuscript very closely for mistakes and grammatical errors. Should your manuscript be accepted for publication, you may not have another chance to

make corrections as we do not offer pre-publication proofs.

If you would like to make changes to your financial disclosure, please include your updated statement in your cover letter.

Please also include a rebuttal letter that responds to each point brought up by the academic editor and reviewer(s). This letter should be uploaded as a Response to Reviewers file.

In addition, please provide a marked-up copy of the changes made from the previous article file as a Manuscript with Tracked Changes file. This can be done using 'track changes' in programs such as MS Word and/or highlighting any changes in the new document.

If you choose not to submit a revision, please notify us.

Yours sincerely,

Katie Slocombe, Ph.D
Academic Editor
PLOS ONE

Journal requirements:

When submitting your revision, we need you to address these additional requirements.

1) Thank you for including the following details of field study permits and/or approvals in the Acknowledgments section of your manuscript: [Knowsley Safari Park for granting research permission; and especially the keepers for their patience and providing access]. To comply with PLOS ONE submissions requirements for field studies, please include this information in the Methods section of the manuscript and in the "Ethics Statement" field of the submission form (via "Edit Submission"). Your submission will then be able to progress towards peer review. If you have any questions or concerns about this request, please let us know. Additional information about PLOS ONE guidelines for reporting observational and field studies can be found at http://www.plosone.org/static/guidelines#observational

[Note: HTML markup is below. Please do not edit.]

Reviewers' comments:

Reviewer's Responses to Questions

Comments to the Author

1. Is the manuscript technically sound, and do the data support the conclusions?

The manuscript must describe a technically sound piece of scientific research with data that supports the conclusions. Experiments must have been conducted rigorously, with appropriate controls, replication, and sample sizes. The conclusions must be drawn appropriately based on the data presented.

Reviewer #1: No

Reviewer #2: Partly

Please explain (optional).

Reviewer #1: I think this is an interesting piece of work, and I agree (from eyeballing the raw data) that there does seem to be a greater reaction to carnivores. Nevertheless, there is insufficient information available to judge whether the data

presented really support this conclusion.

As the experiment is based on a single study population, presentation order could be a significant factor. Although the authors state that presentation order was "randomised and counter-balanced", they should still check for decline in interest across successive trials. This is particularly important as trials seem to have conducted on successive days (in blocks of 6). How much time elapsed between these blocks? Why were dogs considered a "successful" predator? Was the Serval unsuccessful? Meerkats did have previous exposure to dogs, but not to Servals - how might this effect the results? Why was a ball, and not the fake poo, used as the control? As your hypotheses are based on differences in diet and smell, a fake (and presumably scent-free) poo would better control. In phase 1, there seems to be little difference between responses to herbivores and carnivores.

With further clarification and more information, this research has potential.
Figures for all trials have now been included so as to provide clear visual information concerning successive trials. They show a waning response wasn't apparent. (We were happy to proceed with the study trials as we did, incidentally, test for habituation to stimuli in our earlier pilot study and found no evidence for temporal patterns.)
"Successful" has been removed.
Our hypotheses were based on diet, smell and vision. We chose a rubber ball as an arbitrary object control as we wanted to be sure that the meerkat response was a response to our experimental stimuli and not to any novel stimuli that entered their enclosure. The ball was novel and distinctive, bright orange in colour and smelled of rubber. Thus, we feel it had all of the necessary components to make an appropriate control here. It appeared to elicit the desired response as it received immediate and thorough investigation by the group. Meat-containing stimuli, however, proved to elicit more concerted attention.

Reviewer #2: Although the study is a nice attempt to study the way meerkats investigate (and potentially interpretation), however setup has some problems and manuscripts could be clearer and better written. The used control (with plays a major role in analysis) is questionable for phase 1 and unclear or absent for phase 2 and 3.

see comments to authors for details

---

2. Has the statistical analysis been performed appropriately and rigorously?

Reviewer #1: No

Reviewer #2: No

Please explain (optional).

Reviewer #1: As you are testing the hypothesis that "meat in one carnivore sample, but not the other, leads meerkats to making the discrimination", I think in phase 2 you should be comparing meat-fed and vegan-fed dogs, not comparing them to the baseline (it's not clear whether you have done this, but Table 1 seems to imply this).

In phase 1, although I appreciate there is greater difference between the control and carnivore than control and herbivores, I also think you should be comparing the carnivore and herbivore presentations - there's little evidence the meerkats react differently to the two feces types.

I'm not convinced by the "2 standard deviations method". Previous research in animal behaviour has relied on single subjects, and they have not used this method. To determine if THIS GROUP can distinguish between the presentations, then standard non-parametric tests (e.g. kruskal wallis) would be fine, as long as the presentations are independent (and I'm not convinced they are if they are on subsequent days). A longitudinal analysis approach could control for this by quantifying the extent to which the trial from the previous day influenced the result.

The results section has had a complete re-write. The statistical analysis has been re-done using a repeated-measures approach and the discussion has been re-written referring only to this group of meerkats when discussing our specific results. We have included figures for all the tests in all three phases to provide better clarity to the reader.

Reviewer #2: The choosen statistical analysis seems to be ok, although it is unclear how "post-hoc" difference can be calculated with this method. It clear how one can test if the observed response for a variable is different from the mean of the observed (e.g. larger of smaller than mean +/- 2SD), but it is unclear how this works to test for difference between herbivore and carnivore. Also it is unclear how the SD of the tested variable influences the test. Some clarifications in the statistical analysis section are needed.

Taking the analysis of the second trial as an example
vegan mean = 306 sd= 176 --> value with needs to be passed to reach sig.
306+2*176=648
meat mean = 732 sd = 279, just looking at the mean one would conclude that there is a significant difference, however if one includes the sd of this then 732-2*279=174 with is no where close to significance. More details are needed to be able to conclude if used analysis is appropriated.
Statistical approach addressed above.

A more general point is the question if the used control is the appropriated control. The inspection of a ball may be driven by different factors as the inspection of a feaces. A ball bay be inspected because it is novel or unexpected, feaces potentially is rather inspected to establish the presents of predators. More justification for the use as the ball as control is needed.
Please see above.

---

3. Does the manuscript adhere to standards in this field for data availability?

Authors must follow field-specific standards for data deposition in publicly available resources and should include accession numbers in the manuscript when relevant. The manuscript should explain what steps have been taken to make data available, particularly in cases where the data cannot be publicly deposited.

Reviewer #1: Yes

Reviewer #2: No

Please explain (optional).

Reviewer #1: (No Response)

Reviewer #2: At present it is unclear if data is available and it seems no video data was recorded with makes validation tricky.

---

4. Is the manuscript presented in an intelligible fashion and written in standard English?

PLOS ONE does not copyedit accepted manuscripts, so the language in submitted articles must be clear, correct, and unambiguous. Any typographical or grammatical errors should be corrected at revision, so please note any specific errors below.

Reviewer #1: No

Reviewer #2: No

Please explain (optional).

Reviewer #1: Greater care on English use should be taken - the manuscript has many sentences which would be clearer if tidied up. As an example:
"We next tested the hypothesis that meat in one carnivore sample, but not the other, leads meerkats to making the discrimination" (line 178), would read better as: "We tested the hypothesis that meerkat discrimination of feces is due to the meat diet of carnivores"
There are many sentences like this which could be improved.

Reviewer #2: Manuscript contains several long and poorly structured sentences and needs revision.
Detailed comments are given below in comments to authors

---

5. Additional Comments to the Author (optional)

Please offer any additional comments here, including concerns about dual publication or research or publication ethics.

Reviewer #1: (No Response)

Reviewer #2: Title is confusing and should potentially include reference to secondary predator cues.
New title

We've provided some responses to reviewer 2's line-specific comments below; however, the paper has since undergone a significant re-write which we hope addresses the points raised.

Numbers below refer to line numbers in manuscript.

15 When animals detect secondary predator cues they don't detect the predator, they rather potentially establish the possible presence of a predator
Sentence corrected.

24 what are baseline levels? How where these established?

32-35 very long sentence. please rephrase

36-37 only examples of harmful are given rather present a situation of harmful versus non harmful that animals face.
Wording changed to include non-harmful.

37-38 confusing sentence. Needs to be rephased.

40. try to avoid the use of uncommon words such as frugally, it makes the text hard to understand and unclear.
Changed.

40-42 sentence is incomplete and needs to be restructured.

49 Olfactory signatures rather refer to these as Olfactory cues and in same sentence make reference to the commonly used terminology of secondary predator cues
Changed to Olfactory cues.

63-69 authors are critical towards the work done by Holken and Manser, but fail to provide details of the potential concessions and constrains.. ...more details are needed to justify the critique and to clarify what the present study does to avoid these.
We are extremely reluctant to expand on this. It is definitely not our intention to have the paper read as any type of assault on their work; expansion could lead to that impression (We scaled back earlier edits of the ms for that reason). We make reference to specific points in the introduction in two paragraphs and further allude to more in the Methods. We directly refer to and cite their paper and feel that any reader

	interested in the exact details of their study and how they differ from ours could easily access that information if they wished to do so. 74 the word assertion seems to be out of place here as it refers to "Something declared or stated positively, often with no support or attempt at proof"... ...as conclusions here are based on experimental testing a different word seems needed. 76-78 again long sentence that needs to be restructured. 78-80 reference is made to phases of experiment before general experiment is introduced. An additional sentence is needed to introduce the general experiment and that it consisted of multiple phases. 95-99 needs restucturing e.g. ... i) ones that cannot be held constant between natural groups (e.g. group age, composition, experience, intra-group relationships); ii) ones that are present in the background (add example); iii) are unknown to the researchers (e.g. avoidance of an area of the habitat or presence of an undetected behaviour, suppressing noise or odour); and/or iv) are untested by them (e.g. weather). 100-103 The testing of only one group will only control for a part of the problems mentioned in the sentences between 95-99. Things like weather or unknown background factors could still vary between trials and days within the same test group. Wording revised to take away that (non-intended) insinuation. 103-105 This problem could have partly have been avoided by testing individual animals within the group rather than using group responses. We agree. But not an option for us as these were not our animals. Wording addressing issue expanded and added to. 107-109 why was inspection time the only response measured? One could imagine that factors such as vigilance or guarding behaviour would also be affected depending on exposure to different cues. We agree. Had these trials been conducted on a small number of study individuals that would have been done. But, as we were working with a large group we did not have the resources available for this study to undertake that type of observational approach in a sufficiently robust way. We have adjusted wording accordingly to not make claims about inferring presence of a predator to not claims beyond our data. Further explanation has been added in to the discussion. 110-112 How where stimuli presented? Where the presented in sight of meerkats or rather hidden. If cues were presented in sight of meerkats, meerkats could have associated cues with researchers. Presentations were made in full view of the meerkats. All introductions to them are e.g. feeding by the keepers. Additional wording to clarify added. The point, however, is a valid one; but we were interested in differences in response between stimuli – and all stimuli were introduced in exactly the manner and to the same location. 115-117 Was the response also video recorded? Or are where only times recorded? Timings were made using a digital stopwatch. 119 morning/afternoon effect what was this effect? and could it affect the interpretation of the results We don’t think so. We simply wished to hold constant the time of day (cf. Hollen & Manser). Our pilot study suggested meerkats were more active in the morning than in the afternoon; hence it was chosen. 122-126 why the in-balance in number of trials per stimuli? All trials categories are n=6 presentations. The additional information given is to provide full information on how many individuals contributed to the classes of presentations. For example, three different elephants each contributed once to the three elephant samples that were presented; two vegan dogs provided the six samples that were used etc.

126-127 randomised and counterbalanced presentation order seem to be incompatible. More details should be provided
Wording revised.

143-147 (see comment line 110-112).

General comments statistics and results. see remarks in statistical section (2)

178-181 what was the control here as this is needed for the used statistical analysis

186-187 it is again unclear what control is for the phase 3. is it the control of phase 1? If this is the case than one can really ask the question of the control using a ball (with in itself is a questionable control) in 2010 can be used as a control for experiments in 2011 and 2012. If control with ball was repeated in other years data needs to be presented. Maybe these results would be clearer if figure 2 was repeated or included phase 2 and 3.

193 our meerkats??
Text revised throughout.

194-196 again a unclear long sentence try the rephrase

198 "informs meerkats" of what?

205 "Olfactory-mediated examination appears to be prioritised in meerkat close quarter investigation." On what is this statement based? and what is meant with close quarter investigation?

206 non-present predators rather non-visible predators as predator may be present as it deposit feaces

208-212 The lack of interest in the faeces that lacked the appropriate olfactory component re-emphasises the question if the used control is the correct one. I assume the ball used also lacked these olfactory cues and therefore potential interest by meerkats. These observations suggest that a olfactory cues that is not related to either herbivores or carnivores should have been used as control.
We agree. Indeed we used a stimulus with an olfactory cue that was neither herbivore nor carnivore: rubber. The rubber ball was chosen for its potential to provoke a response on three levels: 1) novelty, 2) visibility, 3) rubbery smell.

212 add reference

243-245 not dissimilar to that which they displayed towards herbivore faeces in phase 1. Was this tested?
Test result now included.

251-255 Why where these observations not made during the current study?
See above.

257-258 Although I generally agree with this statement in would be more confincing if data on the diet of the meerkats would be provided. Whilst wild meerkats are indeed small carnivores, captive groups are often fed with fruit and vegetables. This could affect the interpretation. This could be included in the methods to include a more elaborated description of the captive group used for this study.
Contacted KSP who have provided the information. This is now included in the Methods section.

261-262 One can question if this is strong evidence as only only one response was measured. Data on general vigilance behaviour is lacking and control used is questionable.

273 As suggested before included observations of phase 2 and 3 or created additional figure

	Additional figures now included. _____ 6. If you would like your identity to be revealed to the authors, please include your name here (optional). Your name and review will not be published with the manuscript. Reviewer #1: (No Response) Reviewer #2: (No Response) [NOTE: If reviewer comments were submitted as an attachment file, they will be accessible only via the submission site. Please log into your account, locate the manuscript record, and check for the action link "View Attachments". If this link does not appear, there are no attachment files to be viewed.]
Additional Information:	
Question	**Response**
Competing Interest For yourself and on behalf of all the authors of this manuscript, please declare below any competing interests as described in the "PLoS Policy on Declaration and Evaluation of Competing Interests." You are responsible for recognizing and disclosing on behalf of all authors any competing interest that could be perceived to bias their work, acknowledging all financial support and any other relevant financial or competing interests. If no competing interests exist, enter: "The authors have declared that no competing interests exist." If you have competing interests to declare, please fill out the text box completing the following statement: "I have read the journal's policy and have the following conflicts" * typeset	The authors have declared that no competing interests exit.
Financial Disclosure Describe the sources of funding that have supported the work. Please include relevant grant numbers and the URL of any funder's website. Please also include this sentence: "The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript." If this statement is not correct, you must describe the role of any sponsors or funders and amend the aforementioned sentence as needed. * typeset	Funding was provided by the Universities of Liverpool and Salford. The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Ethics Statement All research involving human participants must have been approved by the authors' institutional review board or equivalent committee(s) and that board must be named by the authors in the manuscript. For research involving human participants, informed consent must have been obtained (or the reason for lack of consent explained, e.g. the data were analyzed anonymously) and all clinical investigation must have been conducted according to the principles expressed in the Declaration of Helsinki. Authors should submit a statement from their ethics committee or institutional review board indicating the approval of the research. We also encourage authors to submit a sample of a patient consent form and may require submission of completed forms on particular occasions. All animal work must have been conducted according to relevant national and international guidelines. In accordance with the recommendations of the Weatherall report, "The use of non-human primates in research" we specifically require authors to include details of animal welfare and steps taken to ameliorate suffering in all work involving non-human primates. The relevant guidelines followed and the committee that approved the study should be identified in the ethics statement. Please enter your ethics statement below and place the same text at the beginning of the Methods section of your manuscript (with the subheading Ethics Statement). Enter "N/A" if you do not require an ethics statement.	We adhered to ASAB's guidelines for the use of animals in research and ethical clearance to conduct the research was given by the University of Liverpool's (phase 1) and the University of Salford's (phases 2 and 3) research ethics committee. Permission to conduct our research at Knowsley Safari Park was granted by the Knowsley Safari Park Head of the Animal Division. Access to the meerkat facility was under the supervision of the keepers. Animals were not handled as part of our study.

*Cover Letter

Please find our revised manuscript which has undergone the suggested major revision.

Because the major revision–which has also included a re-analysis of all of the data–has been largely carried out by C. O'Hara we have elevated her from the acknowledgments to author. As well as the current work she has been involved in all previous stages of the project (with the exception of data collection – although a site visit when data were being collected was made). Her inclusion only in the acknowledgements, and not authorship, was already a "borderline" decision. We feel her latest contributions firmly justify the change.

We have now replaced our single table with figures for all of our trials. We hope the reviewers will agree that the visual presentation of the data and results is more easily assimilated by readers. The data table, as well as an example video of the trial procedure have been added as Supplementary Information material.

Title: Meat in a carnivore's diet allows a group of captive meerkats to discriminate between the faeces of predators and non-predators.

Short title: Category discrimination in meerkats

Authors:

Sean J. O'Hara (1)*

Heather Williams (2)

Catherine O'Hara (3)

(1) Ecosystems & Environment Research Centre, University of Salford, Greater Manchester, M5 4WT.

s.ohara@salford.ac.uk

(2) School of Biological Sciences, University of Liverpool, Crown Street, Liverpool, L69 7ZB.

(3) Clinical Outcomes Unit, The Christie NHS Foundation Trust, Manchester, M20 4BX.

*corresponding author

The ways in which animals form categories and solve discrimination problems have long been subjects of scientific interest. Here, we measured meerkats' investigation time of stimuli that indicated the presence of a predator, but was not the actual predator. We also asked, if the meerkats do discriminate between different stimuli, on the basis of what feature(s) are they able to make that discrimination? We conducted trials on a large group of predator-naïve captive meerkats at Knowsley Safari Park, Merseyside. In phase 1, meerkats were presented with fresh faeces of potential predators (dog, serval), non-predators (giraffe, elephant) and a control (rubber ball) to record interaction times according to stimulus type. Meerkats interacted for longer with predator faeces. In phase 2 we used one of our predator species (dog) and presented faecal samples to the group that differed by diet (meat versus vegan-fed). Meerkats spent significantly longer inspecting the faeces of meat-fed dogs. Finally, we re-ran phase 2 using aged faeces, predicting that interest would dissipate now that the scents provided no temporally salient information. Interest in both faecal types diminished. Taken together, our results suggest that these predator-naive meerkats might be able to infer the presence of an absent predator on the basis of a secondary cue alone, and that detection is being achieved as a consequence of predators' meat-eating activities.

carnivore, herbivore, predators, meat, odour, *Suricata suricatta*

The ability to perform discriminations in animals is recognised to be more widespread than traditionally supposed [1]. Category formation and discrimination can carry the appearance of being innate and interest now has turned to the extent to which the observed abilities might be acquired through learning. Indeed, the subject continues to be of interest to researchers in a wide variety of fields. One fundamentally important discrimination that animals have to make is the discerning of harmful and non-harmful threats to their life; for example, ingesting toxic versus non-toxic foodstuff or distinguishing between predators and non-dangerous heterospecifics. One way to attempt to disentangle learned behaviour from broadly innate abilities is to test naïve animals, measuring how they perform in response to a defined scenario.

Predator detection mechanisms enable prey species to escape predation more efficiently. These mechanisms permit individuals to act only on real danger cues, thus minimising the interference to other important daily fitness-enhancing activities [2]. Being predated is the ultimate catastrophic event; thus selection for behavioural, physiological or psychological mechanisms that mitigate predation risk are likely to be strongly favoured [3]. Perceiving a predator's presence, before it becomes visible, is further likely to enhance fitness since this 'heterospecific eavesdropping' [4] provides competitive advantages through facilitating predator avoidance; the best means of evading predation.

Olfactory cues left by predators enable odour-sensitive prey to detect their presence. While prey species that co-evolved with predators ought to be sensitive to sympatric predators' cues, it is less clear how animals are able to detect the secondary cues of predators with whom they share no co-evolutionary history or prior exposure. Yet several mammalian prey species have demonstrated predator sensitivity towards odours of unknown-to-them predators [5–14].

A convergent feature of predators is carnivory [15,8]. It has therefore been speculated that sulphurous metabolites, a by-product of meat digestion [16], and other infochemicals [17] might be being detected by prey [18]. Tests demonstrating positive responses to stimuli representing predators are largely restricted to herbivorous prey species (see [19] for a review); those results, however, could also be explained by prey being risk averse to 'different' (carnivore) rather than 'similar' (herbivore). In our experiment, here, we conducted trials on meerkats (*Suricata suricatta*), a social carnivore who itself is preyed upon.

In one of the few studies of a carnivorous prey species, Hollen and Manser reported that meerkats responded to stimuli that indicated the presence of a predator [20]; however, these authors were forced to make a number of concessions in their experimental design that make their conclusion tentative. In worthy pursuit of capturing ecological validity (see [21]) those authors had to make several compromises due to logistical constraints that could have lead to an unmeasured variable(s) accounting for their result. In addition, their results are potentially susceptible to order effects since predator faeces was always made before presentations of herbivore faeces.

Here, we conduct a case study on a single group of meerkats. Our interest was in testing whether the group-level response differed as a function of stimulus type. In the first instance we attempted to corroborate the earlier study's findings while applying a procedure that held constant the variables and attempted to address some of the other issues highlighted. We further introduced a control so we could measure an increase or decrease relative to some baseline; thus, going further than simply establishing a more/less difference. We next posed a second question: If Hollen and Manser [20] are correct and meerkats are able to make a discrimination between carnivore and

herbivore stimuli, how do they make it? Understanding how discriminations are made is important as it allows us to better understand the perceptual world of animal subjects and how they solve problems that are meaningful to them in the real world. Thus, we sought to investigate how any discrimination was made and attempted to exclude the possibility that interest in one or other stimulus type was simply a function of attraction.

89 Methods

90 We presented faecal stimuli to a group of captive meerkats at Knowsley Safari Park (KSP),
91 Merseyside, U.K. The group contained ~30 individuals in each of phases 1 and 2 and contained 17
92 adults (plus four pups) in phase 3. They were housed in a large outdoor enclosure with indoor
93 facilities (fig 1). Exact genealogies for the group are not known but the group has been captive-
94 managed for multiple generations. They are fed at various times through the day. A keeper enters
95 the enclosure and scatter-feeds cat food, mealworms and various other insects, and mixed fruit and
96 vegetables, and sometimes eggs. The group is within ear-shot of other animals in the park. Visual
97 exposure to potential predators is limited to aerial predators (e.g. buzzards) and very occasionally a
98 visitor's dog in the distance being led to the holding kennel facility in the Park.

99

100 Figure 1 about here

101

102 Non-lab studies are always subject to the introduction of two additional types of variable: i) ones
103 that cannot be held constant between natural groups and ii) ones that are present in the background
104 and are unknown to the researchers, or are untested by them. While the second will often be
105 problematic for non-lab studies we made an attempt to nullify the first by testing only one group.
106 This meant that although our procedure sacrificed testing multiple groups it allowed us to have high
107 confidence that our predictor variable was responsible for observed differences in behaviour.
108 Consequently, however, we must encourage against generalising our findings beyond this single test
109 group.

110

111 The group was tested with each class of stimulus six times to gain a robust measure of response.
112 While within-subject replicates represent pseudoreplication under certain circumstances [22], this is

not true here as we are presenting our results as a case study with an *N* of one. In this instance a lack of replicates would only serve to underestimate true variation [23] in our subjects' response. A statistically-limiting feature of our design is that it measured a group, rather than individual, response. Group responses are subject to social facilitation effects and each individual's response might therefore lack biological independence. While this on the one hand compromises replication of treatments, on the other hand it preserves and targets external validity. Our interest here was in understanding whether meerkats responded differently to different classes of stimuli. A group-level response is the typical response for this socially cohesive mammal.

Our trials were conducted over three summers (phase 1: 2009; phase 2: 2010; phase 3: 2011). To test meerkats' ability to perform a discrimination we used a single criterion: duration of interaction with stimuli. A stopwatch was started when the first meerkat reached the tray. Duration ceased when the last meerkat had retreated from the tray and no-one returned to the tray within 1-minute. Only one presentation a day took place. Trials took place when the weather was mild–sunny, and the meerkats were almost exclusively and voluntarily outdoors. We recorded our observations from an unrestricted view vantage point immediately outside of the enclosure.

Data were collected in the morning (typically between 10 and 11 am) shortly after the safari park had opened. The meerkats were outside and active. We found no effect of visitor number on meerkat behaviour. We also found no evidence of habituation to our stimuli over time. In phase 1 we presented the following sample types: carnivore faeces (serval, *Leptailurus serval*, [sympatric predator], *N*=1, 3 presentations; dog, *Canis familiaris*, [not natural predator], *N*=2, 3 presentations), herbivore faeces (giraffe, *Giraffa camelpardaris*, *N*=3, 3 presentations; African elephant, *Loxodonta africana*, *N*=3, 3 presentations). In addition, we used a rubber ball as a control (arbitrary novel object control, 6 presentations). This established a baseline against which we could assess our results; the

time spent investigating the control serving as an index for meerkat response to any novel introduction to their environment. The ball was bright orange in colour and smelled of rubber. We randomised the order of presentation. Faeces was fresh, either provided by KSP keepers or collected by us that morning, double-wrapped in poo-bags, and transported to KSP in a cool bag under ice.

In phase 2 we used two variants of one of our carnivores: domestic dogs. We presented faeces that varied by diet type: meat-fed dogs (*N*=2) and vegan dogs (*N*=2). The former were fed commercially available complete dry dog food supplemented with different fresh meats or tinned dog food. The vegan dogs were fed on a diet of a commercially available organic vegetarian dog food supplemented with rice, pasta and different vegetables. They were vegan from puppies. In sniff tests human volunteers found both variants pungent. Faecal 'amounts' were broadly similar. One sample per day was presented (with 6 presentations of each variant of sample).

Phase 3 followed the same procedures as phase 2 except in that we used aged faeces – after collection, samples were left at room temperature for 48 h before use in trials (with 6 presentations of each category of sample).

All stimuli were presented to the meerkats inside their outdoor enclosure and on a standard lab tray (25 x 35 cm)(see supplementary material). Prior to all trial phases meerkats were first presented an empty, alcohol cleaned, lab tray. It was left in their enclosure for the duration of the morning to habituate them to the presence of a tray (day 1). The tray was set down in the location that subsequent presentations would be placed. On the mornings of days 2 to 7 a faecal sample was placed in the centre of the tray for presentation. The tray was cleaned with alcohol between trials.

Statistical Analyses

We used repeated-measures analysis of variance (ANOVAs) with Duration of Interaction as a within-subjects factor. Since sphericity was not violated (Mauchly's test, $p > 0.05$) we report only sphericity-assumed results. *Post-hoc* pairwise Bonferroni-corrected comparisons are presented. Data were analysed using SPSS 21.

Ethics statement

We adhered to ASAB's guidelines for the use of animals in research and ethical clearance to conduct the research was given by the University of Liverpool's (phase 1) and the University of Salford's (phases 2 and 3) research ethics committee. Permission to conduct our research at Knowsley Safari Park was granted by the Knowsley Safari Park Head of the Animal Division. Access to the meerkat facility was under the supervision of the keepers. Animals were not handled as part of our study.

176 Results

177 There was a significant difference in the way that meerkats responded to the three stimuli presented
178 in phase 1 ($F_{2,10}$ = 14.50, p < 0.001, fig. 2). Meerkats spent significantly more time interacting with
179 carnivore faeces than they did the control (mean difference = 485 s, *SE* = 73.28, p = 0.004). They also
180 spent more time investigating the carnivore faeces than they did the herbivore faeces (carnivore:
181 mean = 636 s, SD = 212; herbivore: mean = 314 s, SD = 149) although this difference did not reach
182 statistical significance (p = 0.116). In contrast, time spent investigating herbivore faeces did not differ
183 from the control (mean difference = 162.83 s, *SE* = 80.19, p = 0.294).

184

185 Figure 2 about here

186

187 We next tested the hypothesis that meerkat discrimination of faeces is due to the meat diet of
188 carnivores (phase 2). Meerkats spent significantly more time interacting with meat-fed dog faeces
189 than vegan-fed dog faeces ($F_{1,5}$ = 26.16, p = 0.004; meat-fed: mean = 732 s, SD = 279; vegan-fed:
190 mean = 306 s, SD = 176, fig. 3).

191

192 Figure 3 about here

193

194 Meerkats may simply have been more attracted to the “meat” faeces, either because they
195 themselves produce meat-derived faeces or the meat faeces was more ‘interesting’ for some other
196 reason. Therefore, we presented our subjects with carnivorous and vegan dog faeces that was old
197 (48 h). Both were met by a lack of interest although aged meat-fed dog faeces received significantly
198 less investigation (mean = 48 s, SD = 27) than the aged vegan-fed dog faeces (mean = 154 s, SD = 51,

199 $F_{1,5}$ = 16.26, p = 0.01, fig. 4). If compared against the control used in phase 1 (control mean = 154 s,
200 SD = 51), neither was significantly different at alpha < 0.05. If compared against the fresh meat
201 faeces used in phase 2, aged-meat faeces received significantly less investigation time ($F_{1,5}$ = 32.55, p
202 = 0.002). Less time was also spent investigating the aged-vegan faeces than the fresh vegan faeces,
203 although this was not statistically significant ($F_{1,5}$ = 5.39, p = 0.068).

204

205 Figure 4 about here

206

Discussion

Our results add further support for the hypothesis that mammals can potentially use faecal derived stimuli to distinguish predators from non-predators. Using difference in time spent inspecting fresh carnivore faeces, relative to other stimuli as our criterion, the results support the proposition that the meerkats in our study performed discriminations. Furthermore, meerkats' response to carnivore faeces – indexed by time spent examining and sniffing the faeces – appears consistent with Hollen and Manser's finding that meerkats can potentially detect a non-present predator's presence from something that only infers its presence [20]. Our phase two trials, using dog faeces that varied by diet type, allowed us to go further and demonstrate that some constituent of digested meat provided the salient information that enabled these meerkats to make a discrimination. This fits with suggestions that there are particular volatile chemicals that are recognised in scent-gathering exploration in olfaction-dominated small mammals (see below). We then went on to show that interest in 'meat' faeces was not just a function of some 'attraction'. The old faeces that we used was essentially the same product as the fresh faeces, yet interest in it diminished when not fresh. Old faeces seemingly provided no temporally relevant information and was therefore quickly ignored.

The recent discovery, in a mechanistic investigation, of a single volatile chemical, 2-phenylethylamine, in the urine excretions of 38 mammalian carnivore species potentially reveals how prey species–even naive ones or others presented with novel predators (like those in this study)–might use a generic cue such as this to detect predator presence [24]. That study's authors point to aversive responses seen in prey when encountering the chemical that is found in high concentrations in carnivores but not herbivores. Meerkats in our study reacted no differently to dog, not a natural predator, as they did to serval faeces, a sympatric predator in the wild. The meerkats did, however, show a clear distinction between the carnivore and the herbivore faeces. Although not

statistically significant, perhaps due to the small number of replicates, meerkats spent on average twice as long investigating carnivore faeces as they did herbivore faeces. Collectively these results point to an ability to detect the presence of some generic feature through olfaction-mediated inspections that is potentially predator-identifying.

Commensurate with these findings is the elegant test performed by Nolte et al. who manipulated the diet of coyotes (*Canis latrans*) and found that their urine, if fed a meat but not a fruit diet, was repellent to test prey subjects [18]. They further went on to chemically remove sulphur constituents from one set of 'meat-fed' urine samples and found aversion to the treated samples diminished, suggesting that predator diet and presence of sulphurous metabolites influenced predator detection. Berton et al. varied a single cat's (*Felis catus*) diet and found some evidence of gross defensive behavioural changes in mice when exposed to meat- but not vegetarian-fed cat faeces [25].

Our meerkat subjects can be considered predator naive yet they were able to behave in a way that discriminated between danger-relevant and irrelevant faeces, suggesting this ability is, at least in part, innate (or very rapidly learned). The group displayed all the behavioural responses to the introduction of alien objects (and especially potential predator faeces) reported in their wild-living counterparts (see e.g. [26]) and behaved in ways consistent with showing an adaptive response.

An alternative explanation to our results is that the meerkats were simply showing an interest in the fresh, meat-derived stimuli because of its similarity to their natural fresh meat diet and were less interested in the aged faeces if that represented "old" meat. While this explanation is plausible, and it cannot be ruled out entirely, we consider it unlikely. These captive meerkats rushed to investigate

almost all introductions in to their enclosure – presumably to investigate its consumption potential; however, here we measured interaction time with stimuli, and the investigation retention time varied considerably depending on stimulus. Furthermore, the meerkats demonstrated no behavioural intent to eat the faeces during stimulus investigations suggesting food-potential exploration is less likely to account for their responses.

The intriguing question is whether on encountering our stimuli prompted meerkats to form mental representations of predators. We cannot currently answer this question. The direction of our results, though, is consistent with the proposition that 'predator' faeces was perceived as representing a greater level of danger. First, twice as much attention was paid to carnivore faeces, relative to herbivore faeces, which did not differ from controls. Second, in phase 2 the meerkats responded to the vegan-fed dog faeces in ways almost identical to that which they displayed toward herbivore faeces in phase 1. Third, in phase 3 meerkat interest dissipated when the faeces was no longer fresh. Thus, when presented with all but fresh carnivore faeces, meerkat interest in the stimuli quickly waned and they returned to pre-trial activities elsewhere.

Our study demonstrates meerkats' ability to perform discriminations consistent with our predictions. If falls short, however, of demonstrating a *cognitive* link to predators in our subjects. Follow-up studies are aimed at demonstrating a 'predator effect' by measuring post-stimulus vigilance and/or alarm calling. Tests of individuals are required to best achieve this. We were unable to undertake this during this study due to the size of the safari park meerkat population and our not being able to manipulate the study population's size or isolate individuals. Further studies of subsets of this population and/or other smaller populations will enable single individual's post-stimulus behaviour to be observed and quantified, and to test whether or not observed behaviour is

congruent with responses to perceived predator presence. Our study here has shown that there is sufficient differential behavioural response to the different stimuli to warrant such a follow-up investigation.

In conclusion, a simple 'similar–dissimilar' rule, which could potentially govern herbivore prey discriminations of predator–non-predator faeces, cannot apply to this social carnivore. As a result of our experimental procedure we were able to confirm that this group of meerkats discriminated between carnivore and non-carnivore faeces; with fresh carnivore faeces eliciting a more protracted, elevated response than other stimulus types. Moreover, the evidence provided on *how* they potentially do it allows insight into how animals perceive the world on their own terms.

Supporting Information

Interaction time with stimulus type for each of the six trials (Table S1). Video example of procedure (Video S1).

295 Acknowledgements

296 Thanks to M. Blanchard, R. Kendal and members of the ELS journal club. We are grateful to Hannah
297 Prynne and Laura Winter for field assistance. We thank the owners of the vegan dogs for facilitating
298 our research and agreeing to participate. Also, Knowsley Safari Park for granting research
299 permission; and especially the keepers for their patience and providing access. And finally, we are
300 grateful to two anonymous reviewers and the editor whose comments helped to improve this ms.

301

Figure 1. Meerkat enclosure at Knowsley Safari Park, Merseyside, UK.

Figure 2. Interaction time by stimulus type (phase 1): Rubber ball control and herbivore and carnivore faces. Mean time indicated by solid lines. Dashed lines indicate 95% confidence intervals.

Figure 3. Interaction time by stimulus type (phase 2): fresh vegan dog faeces and meat-fed dog faeces. Mean time indicated by solid lines. Dashed lines indicate 95% confidence intervals.

Figure 4. Interaction time by stimulus type (phase 3): 48 h old vegan dog faeces and meat-fed dog faeces. Mean time indicated by solid lines. Dashed lines indicate 95% confidence intervals.

313 References

314 1. Pearce JM (2008) Animal learning and cognition: An introduction, 3rd edition. Hove and New York:
315 Psychology Press.

316 2. Lima SL, Bednekoff PA (1999) Temporal variation in danger drives antipredator behavior: The
317 predation risk allocation hypothesis. Am Nat 153: 649-659.

318 3. Lima SL, Dill LM (1990) Behavioral decisions made under the risk of predation: A review and
319 prospectus. Can J Zool 68: 619-640.

320 4. Schoeppner NM, Relyea RA (2009) Interpreting the smells of predation: How alarm cues and
321 kairomones induce different prey defences. Funct Ecol 23: 1114-1121. doi: 10.1111/j.1365-
322 2435.2009.01578.x

323 5. Müller-Schwarze D (1972) The responses of young black-tailed deer to predator odors. J Mammal
324 53: 393-394.

325 6. Sullivan TP, Nordstrom LO, Sullivan DS (1985) Use of predator odors as repellents to reduce
326 feeding damage by herbivores I. Snowshoe hares (*Lepus americanus*). J Chem Ecol 11: 903-919. doi:
327 10.1007/BF01012077

328 7. Sullivan TP, Nordstrom LO, Sullivan DS (1985) Use of predator odors as repellents to reduce
329 feeding damage by herbivores II. Black-tailed deer (*Odocoileus hemionus columbianus*). J Chem Ecol
330 11: 921-935. doi: 10.1007/BF01012078

331 8. Epple G, Mason JR, Nolte D, Campbell DL (1993) Effects of predator odors on feeding in the
332 mountain beaver, *Aplodontia rufa*. J Mammal 74: 715-722.

333 9. Weldon PJ, Graham DP, Mears LP (1993) Carnivore fecal chemicals suppress feeding in alpine
334 goats (*Capra hircus*). J Chem Ecol 19: 2947-2952. doi: 10.1007/BF00980594

335 10. Boag B, Mlotkiewicz JA (1994) Effect of odor derived from lion faeces on behaviour of wild
336 rabbits. J Chem Ecol 20: 631-637. doi: 10.1007/BF02059603

337 11. Engelhart A, Müller-Schwarze D (1995) Responses of beaver (*Castor canadensis* Kuhl) to predator
338 chemicals. J Chem Ecol 21: 1349-1364. doi: 10.1007/BF02027567

339 12. Barreto DR, MacDonald DW (1999) The response of water voles, *Arvicola terrestris*, to the odours
340 of predators. Anim Behav 57: 1107-1112. doi: 10.1006/anbe.1998.1042

341 13. Rosell F, Czech A (2000) Responses of foraging Eurasian beavers *Castor fiber* to predator odours.
342 Wildl Biol 6: 13-21.

343 14. Zhang J -X, Cao C, Gao H, Yang Z -S, Sun L, Zhang Z –B, Wang Z -W (2003) Effects of weasel odor
344 on behaviour and physiology of two hamster species. Physiol Behav. 79: 549-552. doi:
345 10.1016/S0031-9384(03)00123-9

346 15. Abbott DH, Baines DA, Faulkes CG, Jennens DC, Ning PCYK, Tomlinson AJ (1990) A natural deer
347 repellent: Chemistry and behaviour. In: MacDonald DW, Muller-Schwarze D, Netyncnuk SE, editors.
348 Chemical signals in vertebrates 5. Oxford University Press. pp. 599-609.

349 16. Mason JR, Epple G, Nolte DL (1993) Semiochemicals and improvements in rodent control. In:
350 Galef BE, Valsecchi P, Mainardi M, editors. Ontogeny and social transmission in food preferences in
351 mammals: Basic and applied research. London: Horwood Academic Press. pp. 327-346.

352 17. Brennan PA (2009) Pheromones and mammalian behaviour. In: Menini A, editor. The
353 neurobiology of olfaction. Boca Raton, FL: CRC Press. pp. 157-175.

354 18. Nolte DL, Mason JR, Epple G, Aronov E, Campbell DL (1994) Why are predator urines aversive to
355 prey? J Chem Ecol 20: 1505-1516. doi: 10.1007/BF02059876

356 19. Apfelbach R, Blanchard CD., Blanchard RJ, Hayes RA, McGregor IS (2005) The effects of predator
357 odors in mammalian prey species: A review of field and laboratory studies. Neurosci Biobehav Rev
358 29: 1123-1144. doi: 10.1016/j.neubiorev.2005.05.005

359 20. Hollen LI, Manser MB (2007) Persistence of alarm-calling behaviour in the absence of predators:
360 A comparison between wild and captive-born meerkats (*Suricata suricatta*). Ethology 113: 1038-
361 1047. doi: 10.1111/j.1439-0310.2007.01409.x

362 21. Byrne R, Bates LA (2011) Cognition in the wild: exploring animal minds with observational
363 evidence. Biol Lett doi:I 10.1098/rsbl.2011.o352

364 22. Hurlbert SH (1984) Pseudoreplication and the design of ecological field experiments. Ecol
365 Monograph 54: 187-211.

366 23. Bastos AC, Monaghan KA, Pestana JLT, Lillebø AI, Loureiro S (2012) A comment to the Editorial
367 “Replication in aquatic biology: The result is often pseudoreplication”. Aquat Toxicol 126: 467-470.
368 doi: 10.1016/j.aquatox.2012.11.003

369 24. Ferrero DM, Lemon JK, Fluegge D, Pashkovski SL, Korzan WJ, Datta SR, Spehr M, Fendt M,
370 Liberles SD (2011) Detection and avoidance of a carnivore odor by prey. Proc Natl Acad Sci doi:
371 10.1073/pnas.1103317108

372 25. Berton F, Vogel E, Belzung C (1998) Modulation of mice anxiety in response to cat odor as a
373 consequence of predators diet. Physiol Behav 65(2): 247-254.

374 26. Mares R, Young AJ, Levesque DL, Harrison, Clutton-Brock TH (2011) Responses to intruder scents
375 in the cooperatively breeding meerkat: Sex and social status differences and temporal variation.
376 Behav Ecol 22: 594-600. doi: 10.1093/beheco/arr021

Figure 1
Click here to download high resolution image

Figure 2
Click here to download high resolution image

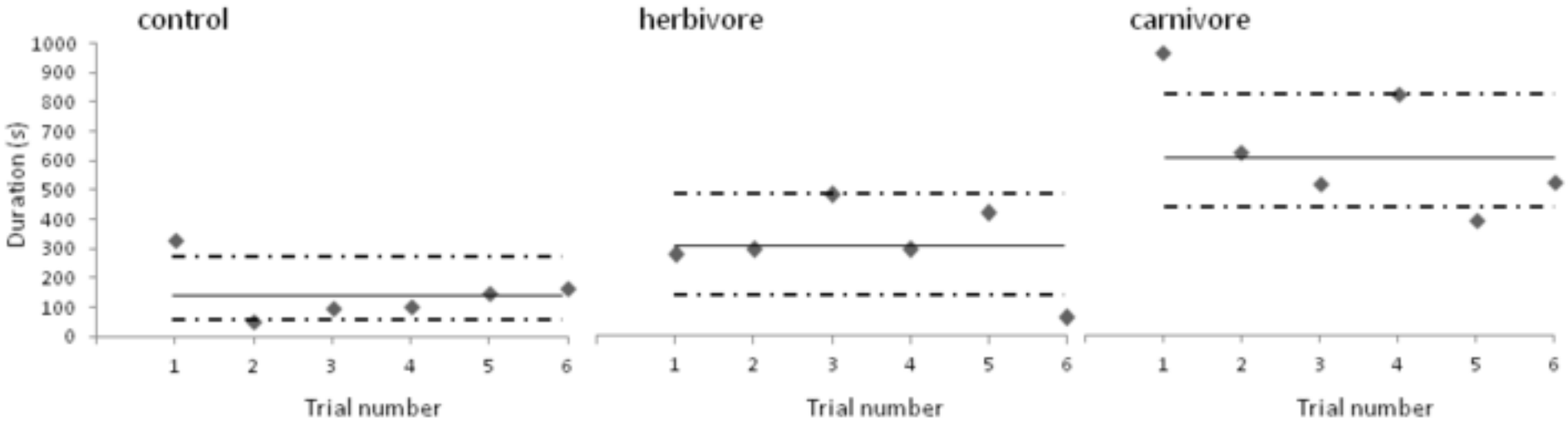


Figure 3
Click here to download high resolution image

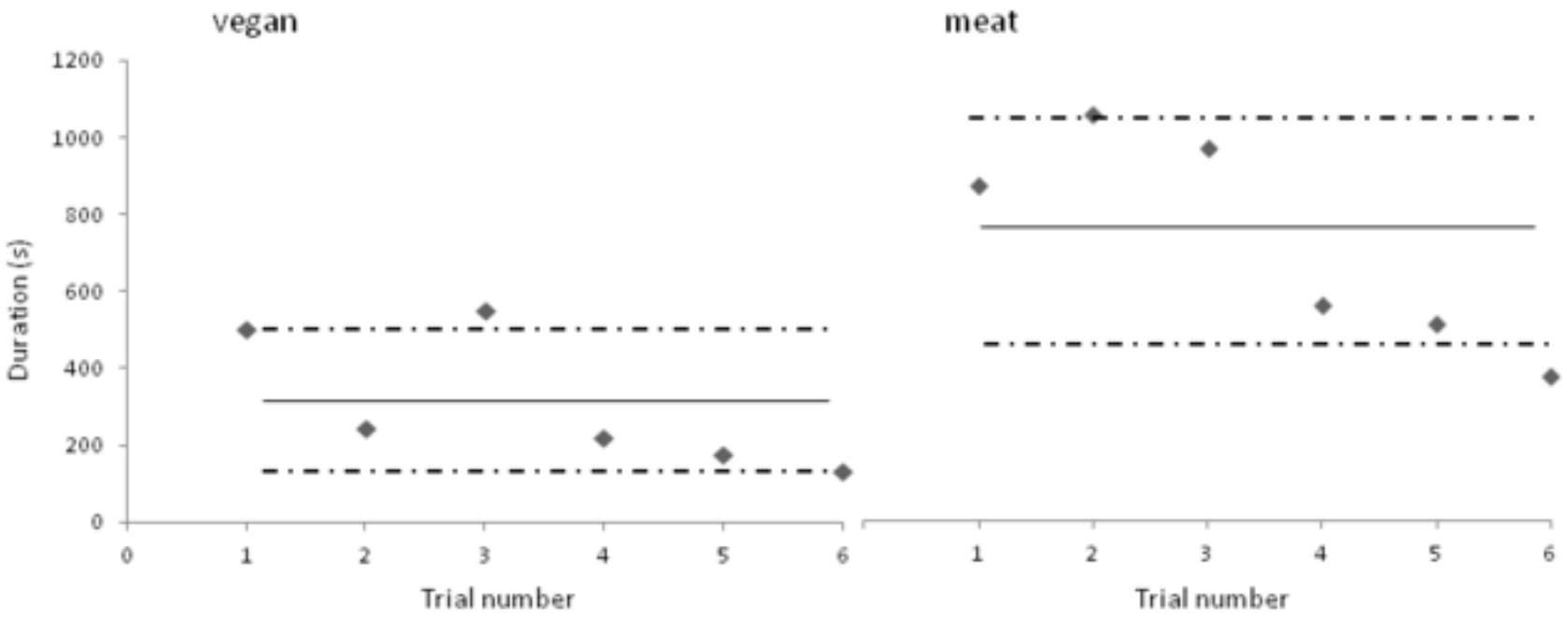
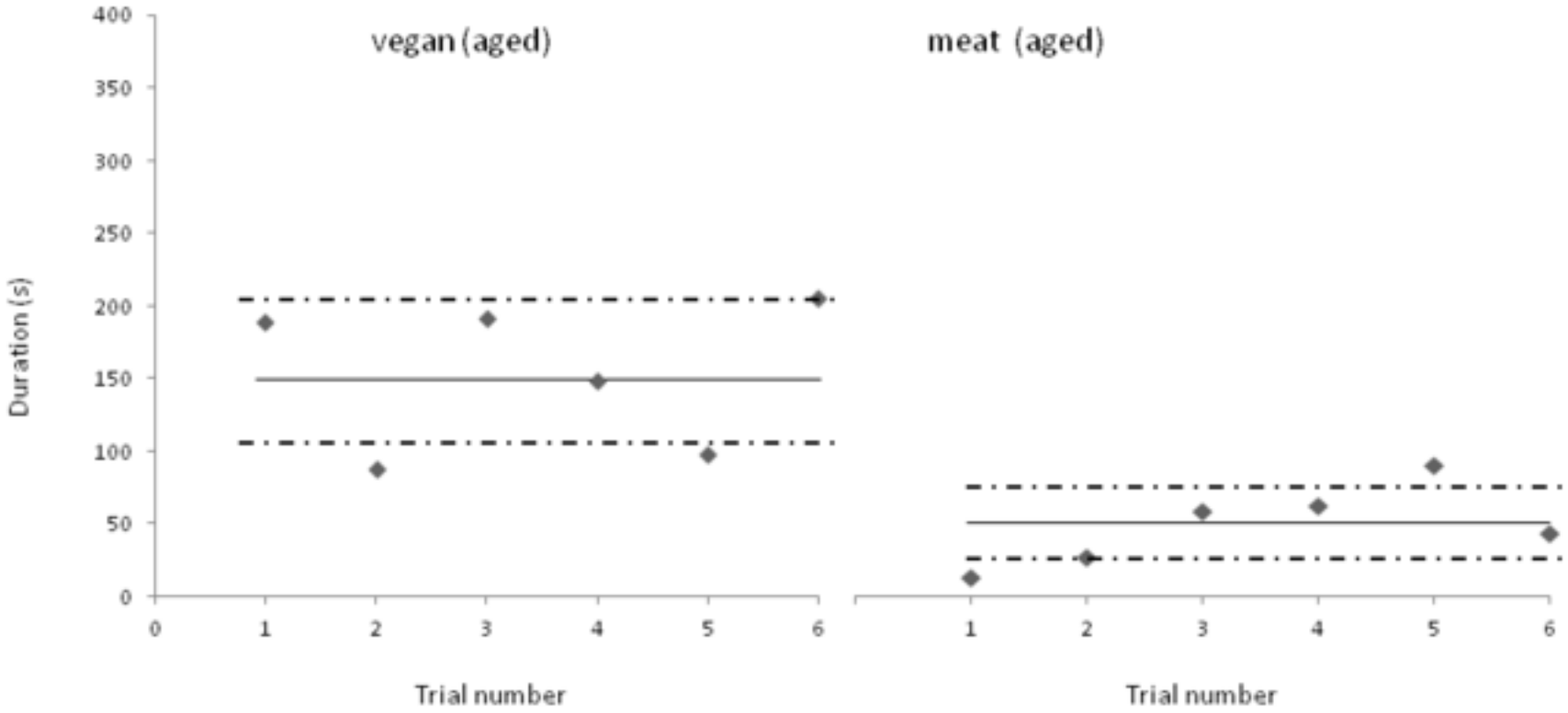


Figure 4
Click here to download high resolution image



Supporting Information Table S1

Click here to download Supporting Information: Table S1.docx

Supporting Information

Click here to download Supporting Information: Video S1 aged vegan trial.mp4

Title: ~~How meerkats detect predators via a stimulus that signals presence, but is not the actual predator~~Meat in a carnivore's diet allows a group of captive meerkats to discriminate between the faeces of predators and non-predators.~~.~~

Short title: Category discrimination in meerkats

Authors:

Sean J. O'Hara (1)*

Heather Williams (2)

Catherine O'Hara (3)

(1) Ecosystems & Environment Research Centre, University of Salford, Greater Manchester, M5 4WT.

s.ohara@salford.ac.uk

(2) School of Biological Sciences, University of Liverpool, Crown Street, Liverpool, L69 7ZB.

(3) Clinical Outcomes Unit, The Christie NHS Foundation Trust, Manchester, M20 4BX.

*corresponding author

The ways in which animals form categories and solve discrimination problems have long been subjects of scientific interest. Here, we ~~attempted to confirm~~ measured ~~whether~~ meerkats' investigation time of stimuli that indicated the presence of a predator~~could detect a predator via a stimulus that signalled its presence~~, but was not the actual predator. We also asked, i~~; and, i~~f the meerkats do discriminate~~so~~ between different stimuli, on the basis of what feature(s) ~~were~~ are they able to make that discrimination? We conducted trials on a large group of predator-naïve captive meerkats at Knowsley Safari Park, Merseyside. In phase 1, meerkats were presented with fresh faeces of potential predators (dog, serval), non-predators (giraffe, elephant) and a control (rubber ball) to record interaction times according to stimulus type. Meerkats interacted ~~significantly~~ for longer with ~~the~~ predator faeces. In phase 2 we used one of our ~~successful~~ predator species (dog) and presented faecal samples to the group that differed by diet (meat versus vegan-fed). Meerkats spent significantly longer inspecting the faeces of meat-fed dogs. Finally, we re-ran phase 2 using aged faeces, predicting that interest would dissipate now that the scents provided no temporally salient information. Interest in both faecal types diminished~~fell below baseline levels~~. Taken together, our results ~~strongly~~ suggest that these predator-naive meerkats ~~are~~ might be able to infer the presence of an absent predator on the basis of a secondary cue alone, and that detection is being achieved as a consequence of predators' meat-eating activities.

carnivore, herbivore, predators, meat, odour, *Suricata suricatta*

The ability to perform discriminations in animals is recognised to be more widespread than traditionally supposed [1]. Category formation and discrimination can carry the appearance of being innate and interest now has turned to ~~and~~ the extent to which the observed abilities might be acquired through learning. Indeed, the subject continues to be of interest to researchers in a wide variety of fields. One fundamentally important discrimination that animals have to make is the discerning of harmful and non-harmful threats to their life; for example, ingesting toxic versus non-toxic foodstuff or distinguishing~~falling victim to a~~ between predators and non-dangerous heterospecifics. One way to attempt to ~~potentially~~ disentangle learned behaviour from broadly innate abilities is to test naïve animals, ~~naïve to the proposed problem to~~ measuring~~e~~ how they perform in response to a defined scenario.

Predator detection mechanisms enable prey species to escape predation ~~frugally~~more efficiently. ~~A trade off exists permitting~~These mechanisms permit individuals to ~~process~~ act only on~~new information discriminatorily to detect~~ real ~~and present~~ danger cues, thus minimising the interference to~~whilst carrying out~~ other important daily fitness-enhancing activities [2]. Being predated is the ultimate catastrophic event; thus selection for behavioural, physiological or psychological mechanisms that mitigate predation risk are likely to be strongly favoured [3]. Perceiving a predator's presence, before it becomes visible, is further likely to enhance fitness since this 'heterospecific eavesdropping' [4] provides competitive advantages through facilitating predator avoidance; the best means of evading predation.

Olfactory ~~signatures~~ cues left by predators enable odour-sensitive prey to detect their presence. While prey species that co-evolved with predators ought to be sensitive to sympatric predators'~~s'~~ cues, it is less clear how animals are able to detect the secondary cues of predators with whom they

share no co-evolutionary history or prior exposure. Yet several mammalian prey species have demonstrated predator sensitivity towards odours of unknown-to-them predators [5–14].

A convergent feature of predators is carnivory [15,8]. It has therefore been speculated that sulphurous metabolites, a by-product of meat digestion [16], and other infochemicals [17] might be being detected by prey [18]. Tests demonstrating positive responses to stimuli representing predators are largely restricted to herbivorous prey species (see [19] for a review); those results, however, could also be explained by prey being risk averse to 'different' (carnivore) rather than 'similar' (herbivore). In our experiment, here, we conducted trials on meerkats (*Suricata suricatta*), a social carnivore who itself is preyed upon.

In one of the few studies of a carnivorous prey species, Hollen and Manser reported that meerkats responded to stimuli that indicated the presence of a predator [20]; however, these authors were forced to make a number of concessions in their experimental design that make their conclusion tentative. In worthy pursuit of capturing ecological validity (see [21]) those authors had to make several compromises due to logistical constraints that could have lead to an unmeasured variable(s) accounting for their result. In addition, their results are potentially susceptible to order effects since predator faeces was always made before presentations of herbivore faeces.

Here, we conduct a case study on a single group of meerkats. Our interest was in testing whether the group-level response differed as a function of stimulus type. In the first instance we attempted to corroborate the earlier study's findings while applying a ~~methodology~~ procedure that held constant the variables and attempted to address some of the other issues highlighted. We further introduced a control so we could measure an increase or decrease relative to some baseline; thus,

going further ~~rather~~ than simply establishing a more/less difference. We next posed a second question: If ~~the assertion~~Hollen and Manser [20] ~~is~~ are correct and meerkats are able to make a discrimination between carnivore and herbivore stimuli, how do they make it? Understanding how discriminations are made is important as it allows us to better understand the perceptual world of animal subjects and how they solve problems that are meaningful to them in the real world. ~~Phase two of our trials was therefore designed~~Thus, we sought to investigate how any discrimination was made and attempted ~~while phase three was implemented~~ to exclude the possibility that interest in one or other stimulus type was simply a function of attraction.

Methods

We presented faecal stimuli to a group of captive meerkats at Knowsley Safari Park (KSP), Merseyside, U.K. The group contained ~30 individuals in each of phases 1 and 2 and contained 17 adults (plus four pups) in phase 3. They were housed in a large outdoor enclosure with indoor facilities (fig 1). Exact genealogies for the group are not known but the group has been captive-managed for multiple generations. They are fed at various times through the day. A keeper enters the enclosure and scatter-feeds cat food, mealworms and various other insects, and mixed fruit and vegetables, and sometimes eggs. The group is within ear-shot of other animals in the park. Visual exposure to potential predators is limited to aerial predators (e.g. buzzards) and verythe occasionally a visitor's dog in the distance being led to the holding kennel facility in the Park.

Figure 1 about here

Non-lab studies are always subject to the introduction of two additional types of variable: i) ones that cannot be held constant between natural groups (e.g. group age, composition, experience, intra-group relationships) and ii) ones that are present in the background and are unknown to the researchers, (e.g. avoidance of an area of the habitat or presence of an undetected behaviour-suppressing noise or odour) or are untested by them (e.g. weather). While the second will often be problematic for non-lab studies we made an attempt to nullify the first by testing only one group. This meant that aWe therefore made a decision that although one procedural decision wasour procedure sacrificed (testing multiple groups) for another (testing one group), ithis allowed us to have high confidence that our predictor variable was responsible for observed differences in

behaviour. Consequently, however, we must encourage against generalising our findings beyond this single test group~~)~~.

The group was tested with each class of stimulus six times to gain a robust measure of response. While within-subject replicates represent pseudoreplication under certain circumstances [22], this is not true here as we are presenting our results as a case study with an *N* of one. In this instance a lack of replicates would only serve to underestimate true variation [23] in our subjects' response. A statistically-limiting feature of our design is that it measured a group, rather than individual, response. Group responses are subject to social facilitation effects and each individual's response might therefore lack biological independence. While this on the one hand compromises replication of treatments, on the other hand it preserves and targets external validity. Our interest here was in understanding whether meerkats responded differently to different classes of stimuli. A group-level response is the typical response for this socially cohesive mammal. ~~Our repeated-measures testing one-group design further meant that the commonly employed statistical techniques were unavailable to us since they assume independence of data points (see below).~~

Our trials were conducted over three summers (phase 1: 2009; phase 2: 2010; phase 3: 2011). To test meerkats' ability to perform a discrimination we used a single criterion: ~~interaction time (duration)~~duration of interaction with stimuli ~~as our criterion~~. ~~We measured meerkat interest in two ways: 1) time to first contact (latency), and 2) duration of interaction. Data on latency, however, are not presented. We found it to be a poor discriminator as the meerkats appeared neophilic and inquisitive, immediately rushing to the stimulus.~~ A stopwatch was started when the first meerkat reached the tray. Duration ceased ~~As a result of pilot study observations duration was operationalised as ceased~~ when the last meerkat had retreated from the tray ~~and none were in~~

~~physical contact with it~~ and no-one returned to the tray within 1-minute. Only one presentation a day took place. Trials took place ~~over three consecutive summers~~ when the weather was mild–sunny, and the meerkats were almost exclusively and voluntarily outdoors. We recorded ~~interaction time on a stopwatch~~our observations from an unrestricted view vantage point immediately outside of the enclosure.

~~Because a morning/afternoon effect was found in phase 1 trials we present data here pertaining only to morning presentations. (Phases 2 and 3 subsequently only tested the meerkats in the morning.)~~Data were collected in the morning (typically between 10 and 11 am) shortly after the safari park had opened. The meerkats were outside and active. We found no effect of visitor number on meerkat behaviour. We also found no evidence of habituation to our stimuli over time. In phase 1 we presented the following sample types: carnivore faeces (serval, *Leptailurus serval*, [sympatric predator], *N*=1, 3 presentations; dog, *Canis familiaris*, [not natural predator], *N*=2, 3 presentations), herbivore faeces (giraffe, *Giraffa camelpardaris*, *N*=3, 3 presentations; African elephant, *Loxodonta africana*, *N*=3, 3 presentations). In addition, ~~in order to establish a baseline against which we could assess our results~~ we used a rubber ball as a control (arbitrary novel object control, 6 presentations). This established a baseline against which we could assess our results; the time spent investigating the control serving as an index for meerkat response to any novel introduction to their environment. The ball was bright orange in colour and smelled of rubber. We ~~randomised and counter-balanced~~ randomised the order of presentation ~~order~~. Faeces was fresh, either provided by KSP keepers or collected by us that morning, double-wrapped in poo-bags, and transported to KSP in a cool bag under ice.

In phase 2 we used two variants of one of our ~~'successful~~ carnivores~~'~~: domestic dogs. We presented faeces that varied by diet type: meat-fed dogs (*N*=2) and vegan dogs (*N*=2). The former were fed commercially available complete dry dog food supplemented with different fresh meats or tinned dog food. The vegan dogs were fed on a diet of a commercially available organic vegetarian dog food supplemented with rice, pasta and different vegetables. They were vegan from puppies. In sniff tests human volunteers found both variants pungent. Faecal 'amounts' were broadly similar. One sample per day was presented ~~and, again, we used a counter-balanced design~~ (with 6 presentations of each variant of sample).

Phase 3 followed the same procedures as phase 2 except in that we used aged faeces – after collection, samples were left at room temperature for 48 h before use in ~~presentation~~ trials (with 6 presentations of each category of sample).

All stimuli were presented to the meerkats inside their outdoor enclosure and on a standard lab tray (25 x 35 cm)(see supplementary material). Prior to all trial phases meerkats were first presented an empty, alcohol cleaned, lab tray. It was left in their enclosure for the duration of the~~a~~ morning to habituate them to the presence of a tray (day 1). The tray was set down in the location that~~where~~ subsequent presentations would be placed. On the mornings of days 2 to 7 a faecal sample was placed in the centre of the tray for presentation. The tray was cleaned with alcohol between trials.

Statistical Analyses

We used repeated-measures analysis of variance (ANOVAs) with Duration of Interaction as a within-subjects factor. Since sphericity was not violated (Mauchly's test, $p > 0.05$) we report only sphericity-

Formatted: Font: Italic

assumed results. *Post-hoc* pairwise Bonferroni-corrected comparisons are presented. Data were analysed using SPSS 21. ~~Single-subject analyses are seldom presented in animal behaviour research. Quantitative reporting of such results might be considered challenging and the problem of autocorrelation and pseudoreplication in analyses is a genuine concern for researchers when using traditionally-employed statistical procedures. However, it is not uncommon in human medically-related research to report findings from single-subject case studies, in which subjects serve as their own control. Data there are handled in one of two ways: they are either examined critically using visual inspection (graphical data) or they are subjected to some form of quantitative assessment that allows data from different experimental phases to be contrasted using some statistical criterion [22]. Here we used a modified form of one such single-subject analysis, borrowed from medical research, that adopts a quantitative stance, to report our results. The procedure is the 2 standard deviations method [23]. The method requires that a baseline mean is calculated and a value for two standard deviations from the mean is computed (see [22]). Significant differences occur where experimental means fall beyond two standard deviations of the control mean i.e. equivalent to $p < 0.05$.~~

Formatted: Font: Italic

Ethics statement

We adhered to ASAB's guidelines for the use of animals in research and ethical clearance to conduct the research was given by the University of Liverpool's (phase 1) and the University of Salford's (phases 2 and 3) research ethics committee. Permission to conduct our research at Knowsley Safari Park was granted by the Knowsley Safari Park Head of the Animal Division. Access to the meerkat facility was under the supervision of the keepers. Animals were not handled as part of our study.

Results

There was a significant difference in the way that meerkats responded to the three stimuli presented in phase 1 ($F_{2,10}$ = 14.50, p < 0.001, fig. 2). Meerkats spent significantly more time interacting with carnivore faeces than they did the control (mean difference = 485 s, SE = 73.28, p = 0.004). They also spent more time investigating the carnivore faeces than they did the herbivore faeces (carnivore: mean = 636 s, SD = 212; herbivore: mean = 314 s, SD = 149) although this difference did not reach statistical significance (p = 0.116). In contrast, time spent investigating herbivore faeces did not differ from the control (mean difference = 162.83 s, SE = 80.19, p = 0.294). ~~Meerkats' spent significantly more time interacting with carnivore faeces than herbivore faeces and a control (carnivore: mean = 636 s, SD = 212; herbivore: mean = 314 s, SD = 149; control: mean= 151 s, SD = 97). Herbivore faeces interaction time did not differ from the control (ns., fig. 2).~~

Formatted: Font: Italic
Formatted: Subscript
Formatted: Font: Italic
Formatted: Font: Italic
Formatted: Font: Italic
Formatted: Font: Italic
Formatted: Font: Italic
Formatted: Font: Italic

Figure 2 about here

We next tested the hypothesis that ~~meat in one carnivore sample, but not the other, leads~~ meerkat~~s~~ ~~to making the~~ discrimination of faeces is due to the meat diet of carnivores (phase 2). Meerkats spent significantly more time interacting with meat-fed dog faeces than vegan-fed dog faeces ($F_{1,5}$ = 26.16, p = 0.004; meat-fed: mean = 732 s, SD = 279; vegan-fed: mean = 306 s, SD = 176, ~~table 1~~fig. 3).

Formatted: Font: Italic
Formatted: Subscript
Formatted: Font: Italic

Figure 3 about here

~~Because the m~~Meerkats may simply have been more attracted to the “meat” faeces, either because they themselves produce meat-derived faeces or the meat faeces was more ‘interesting’ for some other reason. Therefore, we presented our subjects with carnivorous and vegan dog faeces that was old (48 h). Both were met by a lack of interest although aged ~~. M~~meat-fed dog faeces received ~~little~~ significantly less investigation (mean = 48 s, SD = 27) than~~and~~ the aged vegan-fed dog faeces (mean = 154 s, SD = 51, $F_{1,5}$ = 16.26, p = 0.01, fig. 4). If compared against the control used in phase 1 (control mean = 154 s, SD = 51), neither was significantly different at alpha < 0.05. If compared against the fresh meat faeces used in phase 2, aged-meat faeces received significantly less investigation time ($F_{1,5}$ = 32.55, p = 0.002). Less time was also spent investigating the aged-vegan faeces than the fresh vegan faeces, although this was not statistically significant ($F_{1,5}$ = 5.39, p = 0.068). ~~interest diminished to baseline levels (mean = 154 s, SD = 51, ns.).~~

Figure 4 about here

Formatted: Font: Italic
Formatted: Subscript
Formatted: Font: Italic
Formatted: Font: Italic
Formatted: Subscript
Formatted: Font: Italic
Formatted: Font: Italic

248

249 ~~Table 1 about here~~

Discussion

Our results add further support for the hypothesis that mammals can potentially use faecal derived stimuli to distinguish predators from non-predators. Using ~~consistent~~ difference in ~~average~~ time spent inspecting fresh carnivore faeces, relative to other stimuli as our criterion, the results support the proposition that the~~our~~ meerkats in our study performed discriminations. ~~an adaptive response to predation risk.~~Furthermore, meerkats' response to carnivore faeces – indexed by time spent examining and sniffing the faeces ~~duration of interest in extracting olfactory information from the stimulus~~ – appears consistent with Hollen and Manser's finding that ~~they are~~ meerkats can potentially ~~able to~~ detect a non-present predator's presence from something that only infers its presence [20]. Our phase two trials, using dog faeces that varied by diet type, allowed us to go further and demonstrate that ~~the existence of~~ some constituent of digested meat provided the salient information that~~in the diet informs meerkats,~~ enabled these meerkats to make a ~~ing them to perform this~~ discrimination. This fits with suggestions that there are particular volatile chemicals that are recognised in scent-gathering exploration in olfaction-dominated small mammals (see below). We then~~further~~ ~~went on to~~went on to show that interest in 'meat' faeces was not just a function of some 'attraction'. The old faeces that we used was essentially the same product as the fresh faeces, yet interest in it diminished when not fresh. ~~This hints at the possibility that meerkats may be extracting information that informs them about the presence of a predator; O~~old faeces seemingly provided no ~~such~~ temporally relevant information and was therefore quickly ignored.

~~Olfactory-mediated examination appears to be prioritised in meerkat close quarter investigation. Detection of non-present predators by this means has the potential to be widespread since, by definition, meat in the diet would be common to all predators [15,8]. It is worth mentioning that in our pilot study we presented meerkats with fake faeces purchased in a joke shop. This plastic product provided the meerkats with a realistic visual representation of faeces but one that lacked~~

~~the appropriate olfactory component(s); meerkat interest in it was short-lived. This fits with suggestions that there are particular volatile chemicals that are recognised in scent-gathering exploration in olfaction-dominated small mammals.~~

The recent discovery, in a mechanistic investigation, of a single volatile chemical, 2-phenylethylamine, in the urine excretions of 38 mammalian carnivore species potentially reveals how prey species ~~s,~~ even naive ones or others presented with novel predators (like those in this study) ~~,~~ might use a generic cue such as this to detect predator presence [~~24~~24]. That study's authors point to aversive responses seen in prey when encountering the chemical that is found in ~~coupled with its~~ high concentrations in carnivores but not herbivores. Meerkats in our study reacted no differently to dog, not a natural predator, as they did to serval faeces, a sympatric predator in the wild~~, strongly suggesting the presence of some generic predator-identifying feature~~. The m~~M~~eerkats did, however, show a clear distinction between the carnivore and the herbivore faeces. Although not statistically significant, perhaps due to the small number of replicates, meerkats spent on average twice as long investigating carnivore faeces as they did herbivore faeces. Collectively these results point to an ability to detect the presence of some generic feature ~~apparently categorised predator/non-predator during~~through olfaction-mediated inspections that is potentially ~~and detected differences relating to the diet type of its agent.~~ predator-identifying.

Commensurate with these findings is the elegant test performed by Nolte et al. who manipulated the diet of coyotes (*Canis latrans*) and found that their urine, if fed a meat but not a fruit diet, was repellent to test prey subjects [18]. They further went on to chemically remove sulphur constituents from one set of 'meat-fed' urine samples and found aversion ~~diminished to~~in the treated samples diminished, suggesting ~~then~~ that predator diet and presence of sulphurous metabolites influenced

predatory detectionability. Berton et al. varied a single cat's (*Felis catus*) diet and found some evidence of gross defensive behavioural changes in mice when exposed to meat- but not vegetarian-fed cat faeces [2525].

Our meerkat subjects can be considered predator naive yet they were able to successfully behave in a way that discriminated e between danger-relevant and irrelevant carnivore and herbivore faeces, suggesting this abilityability is, at least in part, innate (or very rapidly learned). The group displayed all the behavioural responses to the introduction of alien objects (and especially potential predator faeces) reported in their wild-living counterparts (see e.g. [2626]) and , behaved in ways consistent with showing an adaptive response.

An alternative explanation to our results is that the meerkats were simply showing an interest in the fresh, meat-derived stimuli because of its similarity to their natural fresh meat diet and were less interested in the aged faeces if that represented "old" meat. While this explanation is plausible, and it cannot be ruled out entirely, we consider it unlikely. These captive meerkats rushed to investigate almost all introductions in to their enclosure – presumably to investigate its consumption potential; however, here we measured interaction time with stimuli, and the investigation retention time varied considerably depending on stimulus. Furthermore, the meerkats demonstrated no behavioural intent to eat the faeces during stimulus investigations suggesting food-potential exploration is less likely to account for their responses.

The intriguing question is whether on encountering our stimuli prompted meerkats to form mental representations of predators. We cannot currently answer this question. The direction of our results, though, is consistent with the proposition that 'predator' faeces was perceived as representing a

greater level of danger. First, twice as much ~~much greater~~ attention was paid to carnivore faeces, relative to herbivore faeces, which did not differ from controls. Second, in phase 2 the meerkats ~~now~~ responded to the vegan-fed dog faeces in ways almost identical to that which they displayed toward~~s~~ herbivore faeces in phase 1 ~~(fig. 3)~~. Third, in phase 3 meerkat interest dissipated when the faeces was no longer fresh. Thus, when presented with all but fresh carnivore faeces, meerkat interest in the stimuli quickly waned and they returned to pre-trial activities elsewhere.

~~Furthermore, the fact that~~These captive meerkats ~~rushed to investigate almost all introductions in to their enclosure – presumably to investigate its consumption potential; however, here we measured interaction time with stimuli, and that time varied considerably depending on stimulus. meerkats demonstrated no behavioural intent to eat the faeces during stimulus investigations suggests food-potential exploration cannot account for their responses.~~

~~While O~~our study demonstrates meerkats' ability to perform discriminations consistent with our predictions. If falls short, however, of demonstrating a *cognitive* link to predators in our subjects. ~~, we suggest f~~Follow-up~~uture~~ studies are aimed at ~~might be able to~~ demonstrating ~~e~~ a 'predator effect' by ~~even more conclusively. For example, if~~ measuring post-stimulus vigilance and/or alarm calling. ~~-~~ Tests of individuals are required to best achieve this. We were unable to undertake this during this study due to the size of the safari park meerkat population and our not being able to manipulate the study population's size or isolate individuals. Further studies of subsets of this population and/or other smaller populations will enable single individual's post-stimulus behaviour to be observed and quantified, and to test whether or not observed behaviour is congruent with responses to perceived predator presence. Our study here has shown that there is sufficient differential behavioural response to the different stimuli to warrant such a follow-up investigation.

Formatted: Font: Italic

~~can be accurately measured and can be shown to be congruent with responses to actual predator presence this would solidify claims that the observed behaviour is an adaptive response to predation risk.~~

In conclusion, a simple 'similar–dissimilar' rule, which could potentially govern herbivore prey discriminations of predator–non-predator faeces, cannot apply to this social carnivore. ~~and an 'attraction effect'~~ As a result of our experimental procedure we were able to confirm that this~~our~~ group of meerkats discriminated between carnivore and non-carnivore faeces; with fresh carnivore faeces eliciting a more protracted, elevated response than other stimulus types. Moreover, the evidence~~we~~ provided on ~~strong evidence of~~ *how* they potentially do it ~~which~~ allows insight into how animals perceive the world on their own terms.

Supporting Information

Interaction time with stimulus type for each of the six trials (Table S1). Video example of procedure (Video S1).

Acknowledgements

Thanks to M. Blanchard, R. Kendal~~, C. O'Hara~~ and members of the ELS journal club. We are grateful to Hannah Prynne and Laura Winter for field assistance. We thank the owners of the vegan dogs for facilitating our research and agreeing to participate. Also, Knowsley Safari Park for granting research permission; and especially the keepers for their patience and providing access. And finally, we are grateful to two anonymous reviewers whose comments helped to improve this ms.

Figure 1. Meerkat enclosure at Knowsley Safari Park, Merseyside, UK.

Figure 2. Interaction time by stimulus type (phase 1): Rubber ball control and herbivore and carnivore faces. Mean time indicated by solid lines. Dashed lines indicate 95% confidence intervals. ~~Dashed line represents two standard deviations of the mean for the control stimulus (baseline).~~

Figure 3. Interaction time by stimulus type (phase 2): fresh vegan dog faeces and meat-fed dog faeces. Mean time indicated by solid lines. Dashed lines indicate 95% confidence intervals.

Figure 4. Interaction time by stimulus type (phase 3): 48 h old vegan dog faeces and meat-fed dog faeces. Mean time indicated by solid lines. Dashed lines indicate 95% confidence intervals.

Formatted: Font: Bold

Formatted: Font: Not Bold

Formatted: Font: Not Bold

385 References

386 1. Pearce JM (2008) Animal learning and cognition: An introduction, 3rd edition. Hove and New York:
387 Psychology Press.

388 2. Lima SL, Bednekoff PA (1999) Temporal variation in danger drives antipredator behavior: The
389 predation risk allocation hypothesis. Am Nat 153: 649-659.

390 3. Lima SL, Dill LM (1990) Behavioral decisions made under the risk of predation: A review and
391 prospectus. Can J Zool 68: 619-640.

392 4. Schoeppner NM, Relyea RA (2009) Interpreting the smells of predation: How alarm cues and
393 kairomones induce different prey defences. Funct Ecol 23: 1114-1121. doi: 10.1111/j.1365-
394 2435.2009.01578.x

395 5. Müller-Schwarze D (1972) The responses of young black-tailed deer to predator odors. J Mammal
396 53: 393-394.

397 6. Sullivan TP, Nordstrom LO, Sullivan DS (1985) Use of predator odors as repellents to reduce
398 feeding damage by herbivores I. Snowshoe hares (*Lepus americanus*). J Chem Ecol 11: 903-919. doi:
399 10.1007/BF01012077

400 7. Sullivan TP, Nordstrom LO, Sullivan DS (1985) Use of predator odors as repellents to reduce
401 feeding damage by herbivores II. Black-tailed deer (*Odocoileus hemionus columbianus*). J Chem Ecol
402 11: 921-935. doi: 10.1007/BF01012078

403 8. Epple G, Mason JR, Nolte D, Campbell DL (1993) Effects of predator odors on feeding in the
404 mountain beaver, *Aplodontia rufa*. J Mammal 74: 715-722.

405 9. Weldon PJ, Graham DP, Mears LP (1993) Carnivore fecal chemicals suppress feeding in alpine
406 goats (*Capra hircus*). J Chem Ecol 19: 2947-2952. doi: 10.1007/BF00980594

10. Boag B, Mlotkiewicz JA (1994) Effect of odor derived from lion faeces on behaviour of wild rabbits. J Chem Ecol 20: 631-637. doi: 10.1007/BF02059603

11. Engelhart A, Müller-Schwarze D (1995) Responses of beaver (*Castor canadensis* Kuhl) to predator chemicals. J Chem Ecol 21: 1349-1364. doi: 10.1007/BF02027567

12. Barreto DR, MacDonald DW (1999) The response of water voles, *Arvicola terrestris*, to the odours of predators. Anim Behav 57: 1107-1112. doi: 10.1006/anbe.1998.1042

13. Rosell F, Czech A (2000) Responses of foraging Eurasian beavers *Castor fiber* to predator odours. Wildl Biol 6: 13-21.

14. Zhang J -X, Cao C, Gao H, Yang Z -S, Sun L, Zhang Z –B, Wang Z -W (2003) Effects of weasel odor on behaviour and physiology of two hamster species. Physiol Behav. 79: 549-552. doi: 10.1016/S0031-9384(03)00123-9

15. Abbott DH, Baines DA, Faulkes CG, Jennens DC, Ning PCYK, Tomlinson AJ (1990) A natural deer repellent: Chemistry and behaviour. In: MacDonald DW, Muller-Schwarze D, Netyncnuk SE, editors. Chemical signals in vertebrates 5. Oxford University Press. pp. 599-609.

16. Mason JR, Epple G, Nolte DL (1993) Semiochemicals and improvements in rodent control. In: Galef BE, Valsecchi P, Mainardi M, editors. Ontogeny and social transmission in food preferences in mammals: Basic and applied research. London: Horwood Academic Press. pp. 327-346.

17. Brennan PA (2009) Pheromones and mammalian behaviour. In: Menini A, editor. The neurobiology of olfaction. Boca Raton, FL: CRC Press. pp. 157-175.

18. Nolte DL, Mason JR, Epple G, Aronov E, Campbell DL (1994) Why are predator urines aversive to prey? J Chem Ecol 20: 1505-1516. doi: 10.1007/BF02059876

19. Apfelbach R, Blanchard CD., Blanchard R-J, Hayes RA, McGregor IS (2005) The effects of predator odors in mammalian prey species: A review of field and laboratory studies. Neurosci Biobehav Rev 29: 1123-1144. doi: 10.1016/j.neubiorev.2005.05.005

20. Hollen LI, Manser MB (2007) Persistence of alarm-calling behaviour in the absence of predators: A comparison between wild and captive-born meerkats (*Suricata suricatta*). Ethology 113: 1038-1047. doi: 10.1111/j.1439-0310.2007.01409.x

21. Byrne R, Bates LA (2011) Cognition in the wild: exploring animal minds with observational evidence. Biol Lett doi:I 10.1098/rsbl.2011.o352

22. Hurlbert SH (1984) Pseudoreplication and the design of ecological field experiments. Ecol Monograph 54: 187-211.

23. Bastos AC, Monaghan KA, Pestana JLT, Lillebø AI, Loureiro S (2012) A comment to the Editorial "Replication in aquatic biology: The result is often pseudoreplication". Aquat Toxicol 126: 467-470. doi: 10.1016/j.aquatox.2012.11.003

~~22. Nourbakhsh MR, Ottenbacher KJ (1994) The statistical examination of single-subject data: A comparative examination. Phys Ther 74: 768-776.~~

~~23. Gottman JM, Leiblum SR (1974) How to do psychotherapy and how to evaluate it. New York: Holt, Reinhart and Winston.~~

~~24~~24. Ferrero DM, Lemon JK, Fluegge D, Pashkovski SL, Korzan WJ, Datta SR, Spehr M, Fendt M, Liberles SD (2011) Detection and avoidance of a carnivore odor by prey. Proc Natl Acad Sci doi: 10.1073/pnas.1103317108

~~25~~25. Berton F, Vogel E, Belzung C (1998) Modulation of mice anxiety in response to cat odor as a consequence of predators diet. Physiol Behav 65(2): 247-254.

~~26~~26. Mares R, Young AJ, Levesque DL, Harrison, Clutton-Brock TH (2011) Responses to intruder scents in the cooperatively breeding meerkat: Sex and social status differences and temporal variation. Behav Ecol 22: 594-600. doi: 10.1093/beheco/arr021

PONE-D-13-17133
How meerkats detect predators via a stimulus that signals presence, but is not the actual predator
PLOS ONE

Dear Dr O'Hara,

Thank you for submitting your manuscript to PLOS ONE. After careful consideration, we feel that it has merit, but is not suitable for publication as it currently stands. Therefore, my decision is "Major Revision."

We invite you to submit a revised version of the manuscript that addresses the points below:

Both reviewers make many important and helpful comments - I would like all these points addressed, but in particular:

Both reviewers are unconvinced by the statistical analysis. I share their concern that in a comparison of 2 variables it is unclear which mean and SD you should consider - if you ask if the mean of herbivore investigation is more than 2SD less than the mean of the carnivore the answer is no - yet the other way around the answer is yes. Insufficient explanation and justification for these results is given - reviewers encourage you to explore alternatives.

We agree with reviewer one that if we write the paper specifically about *this* group of meerkats a repeated-measures approach would be justified and appropriate. At your suggestion we have made major revisions: the data have been re-analysed and the paper re-written.

The nature of the control trials - neither reviewer was convinced that a rubber ball was an appropriate control - the plastic 'fake' poo mentioned in pilot work would seem much more appropriate. Given most of your analyses hinge on the control results it is vital that this an appropriate control and I am not convinced of this currently.

We believe the reviewers adopted that view because it was not clear that the ball provided an olfactory cue. We have made changes to our wording to now make it clear that it did. We would disagree that the fake poo would have served as a better control as it is visually related to the test stimuli. As such, it would distinguish less well between a meerkat response towards our experimental stimuli and a control than *an entirely novel item* such as a rubber ball. Please see further justification of our control choice below in response to referees' specific comments.

Given the only DV is time spent investigating all claims of 'inferring an absent predator' should be removed. If appropriate alarm calling or anti predator behaviour was elicited by these stimuli this may be a justified speculation, but without this, this is not justified by the data presented.

Careful re-write undertaken.

I think the simple explanation that meerkats may be more interested in stimuli containing

fresh meat, given its similarity to their food items, has not been sufficiently addressed. Meerkats are not scavengers, so old meat may not be considered a potential food item. Faeces containing fresh meat, may be interesting due to its similarity to their natural fresh meat diet - Just because no meerkat was seen eating the faeces, does not rule this out in a convincing manner. I don't see how you can address this experimentally, but ackowledgement of this alternative explanation for these results should be clearly made in the discussion.

Inclusion of additional wording to address this has been placed in the discussion. We agree that ruling this out convincingly is difficult. And, although we realise that phase 3 trials do not do rule out this explanation entirely, the reason we implemented phase 3 was to go so way to addressing that problem.

We encourage you to submit your revision within forty-five days of the date of this decision.

When your files are ready, please submit your revision by logging on to http://pone.edmgr.com/ and following the Submissions Needing Revision link. Do not submit a revised manuscript as a new submission. Before uploading, you should proofread your manuscript very closely for mistakes and grammatical errors. Should your manuscript be accepted for publication, you may not have another chance to make corrections as we do not offer pre-publication proofs.

If you would like to make changes to your financial disclosure, please include your updated statement in your cover letter.

Please also include a rebuttal letter that responds to each point brought up by the academic editor and reviewer(s). This letter should be uploaded as a Response to Reviewers file.

In addition, please provide a marked-up copy of the changes made from the previous article file as a Manuscript with Tracked Changes file. This can be done using 'track changes' in programs such as MS Word and/or highlighting any changes in the new document.

If you choose not to submit a revision, please notify us.

Yours sincerely,

Katie Slocombe, Ph.D
Academic Editor
PLOS ONE

Journal requirements:

When submitting your revision, we need you to address these additional requirements.

1) Thank you for including the following details of field study permits and/or approvals in the Acknowledgments section of your manuscript: [Knowsley Safari Park for granting research permission; and especially the keepers for their patience and providing access]. To comply with PLOS ONE submissions requirements for field studies, please include this information in the Methods section of the manuscript and in the "Ethics Statement" field of the submission form (via "Edit Submission"). Your submission will then be able to progress

towards peer review. If you have any questions or concerns about this request, please let us know. Additional information about PLOS ONE guidelines for reporting observational and field studies can be found at http://www.plosone.org/static/guidelines#observational

[Note: HTML markup is below. Please do not edit.]

Reviewers' comments:

Reviewer's Responses to Questions

Comments to the Author

1. Is the manuscript technically sound, and do the data support the conclusions?

The manuscript must describe a technically sound piece of scientific research with data that supports the conclusions. Experiments must have been conducted rigorously, with appropriate controls, replication, and sample sizes. The conclusions must be drawn appropriately based on the data presented.

Reviewer #1: No

Reviewer #2: Partly

Please explain (optional).

Reviewer #1: I think this is an interesting piece of work, and I agree (from eyeballing the raw data) that there does seem to be a greater reaction to carnivores. Nevertheless, there is insufficient information available to judge whether the data presented really support this conclusion.

As the experiment is based on a single study population, presentation order could be a significant factor. Although the authors state that presentation order was "randomised and counter-balanced", they should still check for decline in interest across successive trials. This is particularly important as trials seem to have conducted on successive days (in blocks of 6). How much time elapsed between these blocks? Why were dogs considered a "successful" predator? Was the Serval unsuccessful? Meerkats did have previous exposure to dogs, but not to Servals - how might this effect the results? Why was a ball, and not the fake poo, used as the control? As your hypotheses are based on differences in diet and smell, a fake (and presumably scent-free) poo would better control. In phase 1, there seems to be little difference between responses to herbivores and carnivores.

With further clarification and more information, this research has potential.
Figures for *all* trials have now been included so as to provide clear visual information concerning successive trials. They show a waning response wasn't apparent. (We were happy to proceed with the study trials as we did, incidentally, test for habituation to stimuli in our earlier pilot study and found no evidence for temporal patterns.)

"Successful" has been removed.

Our hypotheses were based on diet, smell and vision. We chose a rubber ball as an arbitrary object control as we wanted to be sure that the meerkat response was a response to our experimental stimuli and not to *any* novel stimuli that entered their enclosure. The ball was novel and distinctive, bright orange in colour and smelled of rubber. Thus, we feel it had all of the necessary components to make an appropriate control here. It appeared to elicit the desired response as it received immediate and thorough investigation by the group. Meat-containing stimuli, however, proved to elicit more concerted attention.

Reviewer #2: Although the study is a nice attempt to study the way meerkats investigate (and potentially interpretation), however setup has some problems and manuscripts could be clearer and better written. The used control (with plays a major role in analysis) is questionable for phase 1 and unclear or absent for phase 2 and 3.

see comments to authors for details

---

2. Has the statistical analysis been performed appropriately and rigorously?

Reviewer #1: No

Reviewer #2: No

Please explain (optional).

Reviewer #1: As you are testing the hypothesis that "meat in one carnivore sample, but not the other, leads meerkats to making the discrimination", I think in phase 2 you should be comparing meat-fed and vegan-fed dogs, not comparing them to the baseline (it's not clear whether you have done this, but Table 1 seems to imply this).

In phase 1, although I appreciate there is greater difference between the control and carnivore than control and herbivores, I also think you should be comparing the carnivore and herbivore presentations - there's little evidence the meerkats react differently to the two feces types.

I'm not convinced by the "2 standard deviations method". Previous research in animal behaviour has relied on single subjects, and they have not used this method. To determine if THIS GROUP can distinguish between the presentations, then standard non-parametric tests (e.g. kruskal wallis) would be fine, as long as the presentations are independent (and I'm not convinced they are if they are on subsequent days). A longitudinal analysis approach could control for this by quantifying the extent to which the trial from the previous day influenced the result.

The results section has had a complete re-write. The statistical analysis has been re-done using a repeated-measures approach and the discussion has been re-written referring only to

this group of meerkats when discussing our specific results. We have included figures for all the tests in all three phases to provide better clarity to the reader.

Reviewer #2: The choosen statistical analysis seems to be ok, although it is unclear how "post-hoc" difference can be calculated with this method. It clear how one can test if the observed response for a variable is different from the mean of the observed (e.g. larger of smaller than mean +/- 2SD), but it is unclear how this works to test for difference between herbivore and carnivore. Also it is unclear how the SD of the tested variable influences the test. Some clarifications in the statistical analysis section are needed.

Taking the analysis of the second trial as an example
vegan mean = 306 sd= 176 --> value with needs to be passed to reach sig. 306+2*176=648
meat mean = 732 sd = 279, just looking at the mean one would conclude that there is a significant difference, however if one includes the sd of this then 732-2*279=174 with is no where close to significance. More details are needed to be able to conclude if used analysis is appropriated.

Statistical approach addressed above.

A more general point is the question if the used control is the appropriated control. The inspection of a ball may be driven by different factors as the inspection of a feaces. A ball bay be inspected because it is novel or unexpected, feaces potentially is rather inspected to establish the presents of predators. More justification for the use as the ball as control is needed.

Please see above.

---

3. Does the manuscript adhere to standards in this field for data availability?

Authors must follow field-specific standards for data deposition in publicly available resources and should include accession numbers in the manuscript when relevant. The manuscript should explain what steps have been taken to make data available, particularly in cases where the data cannot be publicly deposited.

Reviewer #1: Yes

Reviewer #2: No

Please explain (optional).

Reviewer #1: (No Response)

Reviewer #2: At present it is unclear if data is available and it seems no video data was recorded with makes validation tricky.

---

4. Is the manuscript presented in an intelligible fashion and written in standard English?

PLOS ONE does not copyedit accepted manuscripts, so the language in submitted articles must be clear, correct, and unambiguous. Any typographical or grammatical errors should be corrected at revision, so please note any specific errors below.

Reviewer #1: No

Reviewer #2: No

Please explain (optional).

Reviewer #1: Greater care on English use should be taken - the manuscript has many sentences which would be clearer if tidied up. As an example:
"We next tested the hypothesis that meat in one carnivore sample, but not the other, leads meerkats to making the discrimination" (line 178), would read better as: "We tested the hypothesis that meerkat discrimination of feces is due to the meat diet of carnivores"
There are many sentences like this which could be improved.

Reviewer #2: Manuscript contains several long and poorly structured sentences and needs revision.
Detailed comments are given below in comments to authors

---

5. Additional Comments to the Author (optional)

Please offer any additional comments here, including concerns about dual publication or research or publication ethics.

Reviewer #1: (No Response)

Reviewer #2: Title is confusing and should potentially include reference to secondary predator cues.

New title

We've provided some responses to reviewer 2's line-specific comments below; however, the paper has since undergone a significant re-write which we hope addresses the points raised.

Numbers below refer to line numbers in manuscript.

15 When animals detect secondary predator cues they don't detect the predator, they rather potentially establish the possible presence of a predator

Sentence corrected.

24 what are baseline levels? How where these established?

32-35 very long sentence. please rephrase

36-37 only examples of harmful are given rather present a situation of harmful versus non harmful that animals face.

Wording changed to include non-harmful.

37-38 confusing sentence. Needs to be rephased.

40. try to avoid the use of uncommon words such as frugally, it makes the text hard to understand and unclear.

Changed.

40-42 sentence is incomplete and needs to be restructured.

49 Olfactory signatures rather refer to these as Olfactory cues and in same sentence make reference to the commonly used terminology of secondary predator cues

Changed to Olfactory cues.

63-69 authors are critical towards the work done by Holken and Manser, but fail to provide details of the potential concessions and constrains.. ...more details are needed to justify the critique and to clarify what the present study does to avoid these.

We are extremely reluctant to expand on this. It is definitely *not* our intention to have the paper read as any type of assault on their work; expansion could lead to that impression (We scaled back earlier edits of the ms for that reason). We make reference to specific points in the introduction in two paragraphs and further allude to more in the Methods. We directly refer to and cite their paper and feel that any reader interested in the exact details of their study and how they differ from ours could easily access that information if they wished to do so.

74 the word assertion seems to be out of place here as it refers to "Something declared or stated positively, often with no support or attempt at proof"... ...as conclusions here are based on experimental testing a different word seems needed.

76-78 again long sentence that needs to be restructured.

78-80 reference is made to phases of experiment before general experiment is introduced. An additional sentence is needed to introduce the general experiment and that it consisted of multiple phases.

95-99 needs restucturing e.g. ... i) ones that cannot be held constant between natural groups (e.g. group age, composition, experience, intra-group relationships); ii) ones that are present

in the background (add example); iii) are unknown to the researchers (e.g. avoidance of an area of the habitat or presence of an undetected behaviour, suppressing noise or odour); and/or iv) are untested by them (e.g. weather).

100-103 The testing of only one group will only control for a part of the problems mentioned in the sentences between 95-99. Things like weather or unknown background factors could still vary between trials and days within the same test group.

Wording revised to take away that (non-intended) insinuation.

103-105 This problem could have partly have been avoided by testing individual animals within the group rather than using group responses.

We agree. But not an option for us as these were not our animals. Wording addressing issue expanded and added to.

107-109 why was inspection time the only response measured? One could imagine that factors such as vigilance or guarding behaviour would also be affected depending on exposure to different cues.

We agree. Had these trials been conducted on a small number of study individuals that would have been done. But, as we were working with a large group we did not have the resources available for this study to undertake that type of observational approach in a sufficiently robust way. We have adjusted wording accordingly to not make claims about inferring presence of a predator to not claims beyond our data. Further explanation has been added in to the discussion.

110-112 How where stimuli presented? Where the presented in sight of meerkats or rather hidden. If cues were presented in sight of meerkats, meerkats could have associated cues with researchers.

Presentations were made in full view of the meerkats. All introductions to them are e.g. feeding by the keepers. Additional wording to clarify added. The point, however, is a valid one; but we were interested in differences in response between stimuli – and all stimuli were introduced in exactly the manner and to the same location.

115-117 Was the response also video recorded? Or are where only times recorded?

Timings were made using a digital stopwatch.

119 morning/afternoon effect what was this effect? and could it affect the interpretation of the results

We don't think so. We simply wished to hold constant the time of day (cf. Hollen & Manser). Our pilot study suggested meerkats were more active in the morning than in the afternoon; hence it was chosen.

122-126 why the in-balance in number of trials per stimuli?

All trials categories are n=6 presentations. The additional information given is to provide full information on how many individuals contributed to the classes of presentations. For example, three different elephants each contributed once to the three elephant samples that were presented; two vegan dogs provided the six samples that were used etc.

126-127 randomised and counterbalanced presentation order seem to be incompatible. More details should be provided

Wording revised.

143-147 (see comment line 110-112).

General comments statistics and results. see remarks in statistical section (2)

178-181 what was the control here as this is needed for the used statistical analysis

186-187 it is again unclear what control is for the phase 3. is it the control of phase 1? If this is the case than one can really ask the question of the control using a ball (with in itself is a questionable control) in 2010 can be used as a control for experiments in 2011 and 2012. If control with ball was repeated in other years data needs to be presented. Maybe these results would be clearer if figure 2 was repeated or included phase 2 and 3.

193 our meerkats??

Text revised throughout.

194-196 again a unclear long sentence try the rephrase

198 "informs meerkats" of what?

205 "Olfactory-mediated examination appears to be prioritised in meerkat close quarter investigation." On what is this statement based? and what is meant with close quarter investigation?

206 non-present predators rather non-visible predators as predator may be present as it deposit feaces

208-212 The lack of interest in the faeces that lacked the appropriate olfactory component re-emphasises the question if the used control is the correct one. I assume the ball used also lacked these olfactory cues and therefore potential interest by meerkats. These observations suggest that a olfactory cues that is not related to either herbivores or carnivores should have been used as control.

We agree. Indeed we used a stimulus with an olfactory cue that was neither herbivore nor carnivore: rubber. The rubber ball was chosen for its potential to provoke a response on three levels: 1) novelty, 2) visibility, 3) rubbery smell.

212 add reference

243-245 not dissimilar to that which they displayed towards herbivore faeces in phase 1. Was this tested?

Test result now included.

251-255 Why where these observations not made during the current study?

See above.

257-258 Although I generally agree with this statement in would be more confincing if data on the diet of the meerkats would be provided. Whilst wild meerkats are indeed small carnivores, captive groups are often fed with fruit and vegetables. This could affect the interpretation. This could be included in the methods to include a more elaborated description of the captive group used for this study.

Contacted KSP who have provided the information. This is now included in the Methods section.

261-262 One can question if this is strong evidence as only only one response was measured. Data on general vigilance behaviour is lacking and control used is questionable.

273 As suggested before included observations of phase 2 and 3 or created additional figure

Additional figures now included.

---

6. If you would like your identity to be revealed to the authors, please include your name here (optional).

Your name and review will not be published with the manuscript.

Reviewer #1: (No Response)

Reviewer #2: (No Response)

[NOTE: If reviewer comments were submitted as an attachment file, they will be accessible only via the submission site. Please log into your account, locate the manuscript record, and check for the action link "View Attachments". If this link does not appear, there are no attachment files to be viewed.]