# The role of behavioral flexibility in a rapid expanding bird

**Esther Sebastián González** 🆔 *and* **Julia Astegiano** 🆔 *based on peer reviews by 3 anonymous reviewers*

Submitted: 27 May 2024, Recommended: 26 May 2025

Behavioral flexibility (i.e., the ability of some animal species to adapt their actions to new conditions) is a feature that may be critical for the local persistence and, therefore, the geographic distribution of many animal species in our fast-changing world. However, most studies have measured it using different aspects of foraging, social or habitat use behavior as proxies, lacking an empirical demonstration of the relationship between behavioral flexibility and these variables (e.g., Sol et al. 2002). In this study, Corina Logan and co-authors (Logan et al. 2025) undertake a set of well-described experiments that start using two methods (i.e., reversal learning of a color preference and a multiaccess box study weather behavioral flexibility) to measure the behavioral flexibility of great-tailed grackles, a species that is rapidly expanding its geographic range. The grackles are then released to the wild and their foraging, social or habitat use behaviors are measured and compared to those of wild unmanipulated individuals.

This experimental setup allowed the authors to test several hypotheses and predictions on the relationship between behavioral flexibility and foraging, social or habitat use behaviors. The results of the study are of great interest for those using different proxies of behavioral flexibility, as number of foraging habitats and techniques, because the relationships are not as expected, finding positive, negative and no relationships depending on the flexibility measure and the sex of the bird. Interestingly, they find that the less flexible individuals are those that use more human foods. Sociality and habitat use showed mostly no association with behavioral flexibility. They also find a new variable that can be used to measure behavioral flexibility using focal observations, without the need of controlled experiments (i.e., switching among different foraging techniques).

1

This study is part of a preregistered project (Astegiano and Sebastían González, 2019; Logan et al., 2019), and thus all the steps taken during the research had already been published and any deviation from the pre-registration has carefully been explained, being a great example of transparency and reproducibility. The study also highlights the importance of manipulative experiments to test this type of behavioral hypotheses, as some variables used as proxies does not seem to work under all circumstances. **References**

Astegiano, J, Sebastián González, E (2019) Understanding geographic range expansions in human-dominated landscapes: does behavioral flexibility modulate flexibility in foraging and social behavior?. Peer Community in Ecology, 100026. https://doi.org/10.24072/pci.ecology.100026

Logan CJ, McCune K, Bergeron L, Folsom M, Lukas D (2019). Is behavioral flexibility related to foraging and social behavior in a rapidly expanding species? Recommended by Peer Community In Ecology. http://corinalogan.com/Preregistrations/g_flexforaging.html

Logan CJ, Lukas D, Geng X, Hardy K, LeGrande C, Marfori Z, MacPherson M, Rowney C, Smith C, McCune KB (2025) Behavioral flexibility is related to foraging, but not social or habitat use behaviors, in a species that is rapidly expanding its range. EcoEvoRxiv, ver.3 peer-reviewed and recommended by PCI Ecology https://doi.org/10.32942/X2T036

Sol, D., Timmermans, S., & Lefebvre, L. (2002). Behavioural flexibility and invasion success in birds. Animal Behaviour, 63(3), 495–502. https://doi.org/10.1006/anbe.2001.1953

# Reviews

## Evaluation round #2

DOI or URL of the preprint: https://doi.org/10.32942/X2T036
Version of the preprint: 2

### Authors' reply, 25 April 2025

**COMMENT 1:** Decision for round #2 : Revision needed
Dear Corina,
The two reviewers that previously read your manuscript have now done it again and they are overall very satidfied with the new version. One of the reviewers is happy with this version and doe snot have any further comments, but the second one has a few extra comments that I agree can improve the discussion of your paper, so I would like to give you the oportunity to incorporate at least some of them. I will be happy to write my recommendation after this round of comments, so you are almost there!
Looking forward for this last version.
Regards,
Esther
by Esther Sebastián González and Julia Astegiano, 10 Apr 2025 07:44
Manuscript: https://doi.org/10.32942/X2T036
version: 2

**RESPONSE 1:** Thank you so much for going through the latest version and giving this feedback! The comments are really helpful and we have revised accordingly, as outlined below and in the track changes version. We are excited to share this research with the world!
Review by anonymous reviewer 1, 01 Apr 2025 23:42

**COMMENT 2:** I have reviewed the manuscript again and found no further suggestions or points to question in the current version. Furthermore, I am satisfied with the authors' answers to my questions in the previous revision of this manuscript. Based on the above, I believe the current version of the manuscript is acceptable without further changes.

   **RESPONSE 2:** Thank you very much for having another look at our submission! We are really happy that you are satisfied with the changes!

   Review by anonymous reviewer 2, 29 Mar 2025 19:14

   **COMMENT 3:** I have read the revised version of the manuscript. As suggested in the PCI recommendations for the second revision, I primarily reviewed the authors' responses to all reviewers' comments.

I believe all the reviewers' comments, including mine, were adequately addressed. However, some additional details were missing. Clarifying these would enhance the real impact of the MS. The most important concerns to details on your discussion. In the present paper, you tend to discuss the overall finding of your MS and why your results agree or disagree with your predictions. There is also a very detailed search for alternative explanations when a result was unexpected. However, I think that the discussion can be reorganized to emphasize the implications of your findings with respect to current knowledge of flexibility and the misuse of proxies during comparative studies. In the present version, the paragraph that discuss it correspond to the second paragraph (the one begging with: "The finding that the manipulated birds…"). As this thematic is presented in the first part of the discussion, and the next paragraph resume whether your finding support or not your predictions and the possible causes when they do not, that important conclusion is lost. I think most of your actual second paragraph should be moved and would be the last paragraph of the true discussion (i.e. the one just before your last "conclusion" paragraph) in the new version. Finally, I suggest adding somewhere in the discussion, a comment about what is known about Grackles before the large habitat modification by humans. See my specific comments. As there are not line numbering in the MS, I will refer to the page, subtitle, and line within the subtitle.

**RESPONSE 3:** We really appreciate your taking the time to provide us with additional feedback, which we find really useful! You have great insights and we revised per your comments as explained here and below. We are so sorry for forgetting to add line numbers! We know how much it helps make the review process easier! It totally slipped our minds and we apologize. Regarding your comment about moving the second paragraph (starting with "The finding that the manipulated birds…") to just before the conclusion, you are so right about this. It was an unregistered extra analysis we did and we agree that it does break up the flow of the main predictions we had. We moved the paragraph as you suggested.

   **COMMENT 4:** Specific comments
   1)Page 8, "5)" in the list, line 2-3 within the "5)": "To equalize". I think perhaps it would say: "To standardize". Anyway, I think you must clarify what do you mean with "equalize" (or standardize).

**RESPONSE 4:** Thank you for pointing this out. We have now clarified this as follows: "To standardize observation time across individuals such that all individuals would have the same amount of total observation time"

   **COMMENT 5:** Another doubt. Idem page and also "5)", but lines 9-12 (it's in bold in the text). If the analyses used were GLMs, the usual method to correct for different observation times when the response variable is a count, is to include time as an offset. With an offset, you don't want to see the effect of different observation times on your response variable. However, you know it has an effect and you want to correct for it. I do not know if this or something similar is appropriate with Bayesian analyses.
   **RESPONSE 5:** By incorporating time the way we did in the Bayesian model, it allowed the model to accurately

assess the number of food types and foraging techniques even though birds were observed for different amounts of time. We revised the explanation to clarify as follows: "we changed this to using the number of different foods eaten and the number of foraging techniques used by an individual as the response variable and included the total observation time per individual as an explanatory variable (which, in this case is more like a random variable in a GLMM, but it is treated differently in Bayesian analyses). Including time in this way allows us to derive the slope of the expected number of foraging techniques a bird would have if it had been observed for a given amount of time. This allows the model to assess whether, after we account for the differences in the amount of time that different individuals have been observed, manipulated individuals deviate more or less from the expected values than control individuals"

We also added a clarification to the Methods >Analyses > P2: "The term $b\_\i\$time$ allows us to derive the slope of the expected number of food types or foraging techniques a bird would have if it had been observed for a given amount of time. Whereas, $a\_\i\$[$treatment$] explains the remaining variation in whether treatment has an effect on how different the actual versus expected values are for the number of foraging techniques."

**COMMENT 6:** 2)Page 11, "Reversal learning", line 2. The (Fig 1a). I think that you refer to the one within the protocol of reversal learning whose Link you list in the bottom lines. I suspect that when you send the MS to a Journal, that "Fig 1a" would be part of Supplementary Materials. Without such information, readers tend to look within the main text of the MS. The same doubt applies to your "Fig 1b" (page 12, second line).

**RESPONSE 6:** Thank you for catching these errors! We added Supplementary Material 5, which consists of a figure showing the reversal learning tubes and the two multiaccess boxes (Figure SM5.1) so readers don't have to read through the protocols.

**COMMENT 7:** Page 14, "Open materials". The two Links listed are not more available. You have moved them to other Links displayed later places within the text.

**RESPONSE 7:** Thank you for notifying us that the links are now broken! We replaced the links with the current links. It looks like the links are the same, but they are now working for us and we made sure the sharing settings at these Google docs are set to Commenter so that everyone with the link should be able to see the files.

**COMMENT 8:**
Discussion

Please see my general comments. The last paragraph of the new discussion (i. e. the one before your conclusions) would emphasize the implications of your findings concerning current knowledge of flexibility and the misuse of proxies during comparative studies. Aside from the bias introduced by attempting to conduct comparative analyses, what implications does the finding that flexibility is not directly related to foraging technics, habitat use, or sociability?

**RESPONSE 8:** Thank you for this detailed feedback! We took the relevant part of the last paragraph in the discussion and moved it up to make a new paragraph just before it where we now expand on your feedback:

"To understand whether flexibility is directly involved in facilitating adaptations to new environments, manipulative experiments are needed. Manipulating one variable of interest is crucial to determining whether it has an effect on one or more other variables because it reduces the noise in correlations enough to resolve relationships. We recently demonstrated this in @mccune2025exploration, which investigated relationships between flexibility and exploration, boldness, persistence, and motor diversity. Flexibility was found to only relate to exploration and only when comparing the control versus flexibility manipulated grackles: the manipulated grackles were more exploratory. We demonstrate this principle again here with the finding that flexibility and foraging behavior are related only when comparing control versus flexibility manipulated grackles: the manipulated grackles have more foraging behaviors. The accumulating evidence from our manipulative investigations implicates flexibility as a facilitator of exploratory behavior, which likely leads to discovering

more foraging behaviors. Both of these features could contribute to the rapid geographic range expansion this species is undergoing. This evidence also indicates that, to arrive at robust conclusions about how behaviors relate to each other, the variables of interest must not only be measured directly at the individual level, rather than via proxies [@logan2018beyond], but also investigated in an experimental context where the key behavior can be manipulated. This reveals that using proxies of behaviors (i.e., not directly testing behavior) at the individual and cross-species levels to investigate correlations between flexibility and foraging, social, and habitat use behaviors result in a high degree of uncertainty and an insufficient ability to draw conclusions. Similar conclusions have been reached in research on flexibility in clinical psychology, where unclear definitions and issues of measurement have hindered the understanding of ecological and construct validity [@uddin2021cognitive]."

And we added to the conclusion:

"Our findings highlight the importance of directly testing the causal effects of increasing flexibility and the subsequent changes in other behaviors that are potentially involved in the range expansion and how individuals adapt to changing environments. Additionally, in humans, researchers have argued that, to fully understand cognitive and behavioral flexibility, interventions are required to reveal the relevance and transferability of the increased flexibility to daily life [@conesa2021effects]. Until recently, it was unknown how to manipulate flexibility effectively and, even if there was such an intervention, it was unlikely to be possible to implement it in the wild outside of a lab context. Our approaches solved these problems and have opened up the field to investigating the causal role of flexibility in the wild [@logan2023flexmanip; @logan2022manyindividuals]."

**COMMENT 9:** Finally, I think that it would be very interesting to give information within the discussion, about the original habitats and food habits of the Grackles in their native areas. Best if there is information before important habitat modification due to European colonization or the implementation of more intensive land uses. There are at least three reviews that you can consult: Jaramillo & Burke (1999), Fraga (2011), and Winkler, et al. (2020). Or you have access to some of the original papers listed there. It would be very interesting if you discuss briefly yours finding on flexibility in Grackles and how do you think such flexibility would be maintained in their original habitats before the new human modify habitats and food sources were abundant to Grackles.

**RESPONSE 9:** This is a really interesting question and one that Logan has been working on (very slowly) in a separate project for a few years. The short answer is that there is no information about what this species ate before urban areas popped up because urban areas were established thousands of years ago in Central America where this species originated. This species has been associated with human-modified habitats throughout all of the literature we could find on historical observations (e.g., Christensen (2000) and Haemig (2011) discuss 15th century observations, while Skutch (1954) discusses observations from the 1950s in Central America). Of the three references you pointed out, we were only able to gain access to Jaramillo & Burke (1999) - thank you for the suggestion. The foraging details they (and other natural history resources we are aware of) gave for great-tailed grackles were from contemporary observations and did not specify which geographical areas the observations came from. There is currently text in paragraph 3 in the discussion about the grackle's habitat changes that we studied in Summers et al. (2023), however, if we were to try to discuss what they were eating in particular regions in historical time periods, it would be pure speculation. Therefore, we decided not to add this piece here, but we did add the Christensen (2000) and Haemig (2011) references as follows: "Because this species is highly associated with human modified landscapes going back as far as we have records for [e.g. @christensen2000fifteenth; @haemig2011introduction], we predicted they would likely rely on human foods as part of the reason for this association"

## Decision by **Esther Sebastián González** and **Julia Astegiano** , posted 10 April 2025, validated 10 April 2025

Dear Corina,
The two reviewers that previously read your manuscript have now done it again and they are overall very

satidfied with the new version. One of the reviewers is happy with this version and doe snot have any further comments, but the second one has a few extra comments that I agree can improve the discussion of your paper, so I would like to give you the oportunity to incorporate at least some of them. I will be happy to write my recommendation after this round of comments, so you are almost there!

Looking forward for this last version.

Regards,

Esther

## Reviewed by anonymous reviewer 1, 01 April 2025

I have reviewed the manuscript again and found no further suggestions or points to question in the current version. Furthermore, I am satisfied with the authors' answers to my questions in the previous revision of this manuscript. Based on the above, I believe the current version of the manuscript is acceptable without further changes.

Title and abstract

Does the title clearly reflect the content of the article? [x ] Yes, [ ] No (please explain), [ ] I don't know

Does the abstract present the main findings of the study? [x Yes, [ ] No (please explain), [ ] I don't know

Introduction

Are the research questions/hypotheses/predictions clearly presented? [ x] Yes, [ ] No (please explain), [ ] I don't know

Does the introduction build on relevant research in the field? [ x] Yes, [ ] No (please explain), [ ] I don't know

Materials and methods

Are the methods and analyses sufficiently detailed to allow replication by other researchers? [x] Yes, [ ] No (please explain), [ ] I don't know

Are the methods and statistical analyses appropriate and well described? [x ] Yes, [ ] No (please explain), [ ] I don't know

Results

In the case of negative results, is there a statistical power analysis (or an adequate Bayesian analysis or equivalence testing)? [ ] Yes, [ ] No (please explain), [ x] I don't know

Are the results described and interpreted correctly? [x ] Yes, [ ] No (please explain), [ ] I don't know

Discussion

Have the authors appropriately emphasized the strengths and limitations of their study/theory/methods/argument? [x ] Yes, [ ] No (please explain), [ ] I don't know

Are the conclusions adequately supported by the results (without overstating the implications of the findings)? [ x] Yes, [ ] No (please explain), [ ] I don't know

## Reviewed by anonymous reviewer 2, 29 March 2025

**Download the review**

# Evaluation round #1

DOI or URL of the preprint: https://doi.org/10.32942/X2T036

Version of the preprint: 1

## Authors' reply, 19 February 2025

AUTHOR'S REPLY Decision for round #1 : Revision needed Dear authors,

First of all, we am very sorry for the delay in the response. We have had many problems in finding reviewers and some of those reviewers needed extra time to complete the process.

As you can see, the three reviewers found your study very interesting but they also include some comments that can help to improve the quality of the manuscrit. Thus, we would like to invite you to incorporte those changes in the text and submit a new improved version of the manuscript.

We are looking forward to it. Best,

Esther Sebastián González and Julia Astegiano

by Esther Sebastián González and Julia Astegiano, 21 Jan 2025 14:54 Manuscript: `https://doi.org/10.3 2942/X2T036` version: 1

RESPONSE: thank you very much for taking on this submission and for trying so hard to find reviewers! We are also editors and we know how difficult that can be, so we really appreciate your diligence. Also, no problem with the delay! Some of us only just got back from field work and only now had a chance to work on the revision. We have now revised the article based on the feedback. Thank you again! Please note that we have added a co-author, K Hardy, because a mistaken email address meant that she did not get the original email informing the co-authors that it was their chance to revise the post-study article before we submitted it to PCI Ecology in May. She has finished contributing to the article by providing helpful edits, which are shown in the track changes version. Changes are shown in the tracked changes version at the PCI Ecology website and at the Rmd file at GitHub: `https://github.com/corinalogan/grackles/blob/84efe125ee75e32310deba335 872e8f222c3f990/Files/Preregistrations/g_flexforaging.Rmd`.

Review by anonymous reviewer 1, 25 Aug 2024 20:09

•Title and abstract

o Does the title clearly reflect the content of the article? [x] Yes, [ ] No (please explain), [ ] I don't know

o Does the abstract present the main findings of the study? [x] Yes, [ ] No (please explain), [ ] I don't know

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• Discussion

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o Are the conclusions adequately supported by the results (without overstating the implications of the findings)? [ x] Yes, [ ] No (please explain), [ ] I don't know

COMMENT 1: Comments for authors. The authors presented an interesting paper in which they analysed the

relationship at the individual level between the performance of grackles in two tests of behavioural flexibility in captivity and their flexibility in foraging, habitat use, and social behaviour measured in the field. They point out that the lack of direct evidence between flexibility and ecological or social traits, such as dietary breadth, measured at the individual level, is an important limitation in predicting the actual role of behavioural flexibility in animals' adaptation to new or changing environments. This paper is therefore very welcome in this respect. The manuscript is well written and clear to the reader, even considering the number of analyses performed to test each hypothesis/prediction. The statistical analysis seems correct and appropriate for the questions and type of data the authors are dealing with, although I have only a superficial knowledge of Bayesian analysis, so my assessment on these matters may not be the most appropriate. It seems that this paper has already passed previous rounds of peer review, so I don't have much to add, but a few comments/questions, listed below. 1. Regarding predictions: If I'm not mistaken, I think that what is not taken into account here is that, a) the authors do not know the abundance of prey or natural food in the areas used by the birds, so one cannot say that an individual is selecting human food if you do not know if it really has the natural alternative available; b) demonstrating more diverse feeding techniques is probably associated with human food, precisely because of the way it is presented in nature (the different packages), and that individuals probably do not use them to consume natural prey. This is not necessarily evidence that they do not have such a repertoire, but that they do not need it to feed on the natural resources of the species; c) individuals could generalize when using different techniques to access food from the different human food packages, so they would not need to innovate (or change) in techniques just because of the change in the package format.

RESPONSE 1: Thank you very much for your review and for your great feedback! We are so glad the manuscript is readable even though there are so many analyses.

a) Our hypothesis is about how flexibility relates to their foraging behavior, and not about the abundance of the various food types in the environment, which would be a separate, and very interesting question to ask in the future. That said, we can say that all individuals could access natural and human foods throughout the day as they preferred. In both populations, all individuals are part of the same social groups (see the social network analyses), frequently interacting and using the same spaces. Accordingly they all should have the same access to the same resources (e.g., earth, trees, bushes) and could include multiple human food sources (e.g., cafe, people moving through while eating and dropping food on the ground). In California, grackles had very large daily ranges (on the order of several miles) that overlapped with each other. On a daily basis, they roosted in a natural area, and moved through it and urban environments when foraging during the day. Even when they were in a shopping mall parking lot, there were trees and dirt in little islands where they could access non-human foods. Additionally, both populations ate almost the exact same food types. We added the details to the Results: "The Arizona population took 20 food types, including lizard, bird poop, candy, vomit, condiment, and carcass, which the California population did not have. The California population took 15 food types, including mulch, which the Arizona population did not have"

b) In prediction 2, we found that the more flexible individuals used more foraging techniques. In prediction 3, we found that the less flexible individuals used a higher proportion of human foods and had more human food sources within their home range, suggesting that they specialize on human foods. So we didn't find an increase in foraging techniques that related to human foods, but we also didn't have a specific preregistered analysis to investigate that directly. Regardless of the direction of the results, the foraging techniques that were used (gape, lift or nudge, stalk catch, flip, food share, break into pieces, dunk in water, theft, dig, pick up, and sweep), could be applied to human and non-human foods. For example, a grackle might gape or flip to look under a rock or a sugar packet, or pick up a worm from the ground or a french fry from the cement. There is only one technique that would only be seen for natural food items, such as stalk catch where the individual is pursuing a flying insect. However, the rest of the techniques could apply to both natural and human foods. Therefore, it

isn't possible for there to be a relationship between more diverse techniques being used on human foods. Additionally, we added to the Results: "The Arizona population used 9 foraging techniques, including break into pieces, dunk in water, tolerated theft, and theft, which the California population did not have. The California population used 8 foraging techniques, including pick up, and sweep, which the Arizona population did not have"

c) Our response in b above also applies here in that the same techniques could be used for natural and human foods, so any differences found would be due to individual differences in how they deploy the techniques. Also, it would be difficult to quantify what a foraging technique innovation is for a grackle because they have so many different techniques, and there is no comprehensive list of what their known techniques are. Our research question does not assume foraging is innovative, but rather focuses on the number of different foraging techniques, regardless of whether they are new or not. This is one of the reasons we really like the new flexibility measure of technique (and food type) switches because switching between techniques/types doesn't involve needing to know whether a technique/type is novel or not.

COMMENT 2: 2. Hypothesis 3: Is it possible that after the period of captivity, released individuals may exhibit behavioural alterations until they are properly integrated back into their social-ecological environment?

RESPONSE 2: We don't have focal follow data from before the individuals were caught to compare with after they were released back to the wild, however, from our focal follow observations, the grackles released from the aviaries appeared to immediately reintegrate back into their home range and social group. Additionally, we conducted focal follows for months after their release back to the wild, so the data used in the analyses would primarily be from after any reintegration period, if there was any. Regardless, even if there was such an integration period, it would not matter for our analyses because all birds we compare were in captivity. So even if the birds behave differently right after the release, we would still expect that flexibility could lead to variation among them.

COMMENT 3: 3. Trapping. Since the authors used several types of traps to capture their birds, knowing that some type of trapping methodology could filter bolder individuals, did they consider the possibility that this could have happened with their set of individuals tested?

RESPONSE 3:  Experimental data shows mixed support for whether trapping methods lead to a selection bias for more bold or exploratory individuals (e.g., Michelangeli et al., 2016; Carter et al., 2012; Brehm et al., 2018). However, we used multiple methods to trap grackles, including one method (mist nets) which they could not see and so there is unlikely to be selection bias for individuals with certain personality traits. For the visible trapping methods, we conducted extensive habituation that minimizes bias towards more bold or exploratory individuals. We added to the Methods:

   "Some of these trapping methods decreased the likelihood of a selection bias for exploratory and bold individuals because grackles cannot see the traps (i.e., mist nets).  For the visible trapping methods we conducted extensive trap habituation to increase the chance of catching the individuals that were initially more cautious approaching these traps. "

Michelangeli M, Wong B, Chapple D. 2016. It's a trap: sampling bias due to animal personality is not always inevitable. Behavioral Ecology, 27(1): 62-67.

Carter A, Heinsohn R, Goldizen A, Biro P. 2012. Boldness, trappability and sampling bias in wild lizards. Animal Behaviour, 83(4): 1051-1058.

Brehm A, Mortelliti A. 2018. Mind the trap: large-scale field experiment shows that trappability is not a proxy for personality. Animal Behaviour, 142: 101-112.

COMMENT 4: 4. At any point in the manuscript, did the authors set out to compare the flexibility of the captured birds as a function of their environment? I wonder if grackles in populations that are in expansion (at the edge of their geographic range) are more flexibles (in all the measurements) than those in populations with more years habiting the same location.

RESPONSE 4: We also had this very same question and we published that article in 2023: `https://peerco mmunityjournal.org/articles/10.24072/pcjournal.320/`. We found that the grackles in the edge population were more persistent and had a higher variance in flexibility than grackles in the more central population, which was older. There were no differences in average flexibility, exploration, or innovativeness. Review by anonymous reviewer 2, 21 Jan 2025 03:22 COMMENT 5: I have not addressed the specific categories listed above, as my general comments below apply to all (except power analysis, to which my answer is "yes"). This paper addresses the important goal of disentangling flexible behavior and underlying cognitive processes within the broad ecological context of range expansion. Novel aspects are the combined use of experimental and observational protocols, the attempt to measure flexibility across a range of behavioral contexts, and the explicit separation of seemingly flexible behaviors and underlying cognitive processes. A particular strength is that the main hypotheses tested were pre-registered, which constrained and directed the analyses, averting the high potential in this kind of research for post-hoc pattern-seeking. However, to their credit, the authors were sufficiently agile to also test modified hypotheses as suggested by the study results. I have a few recommendations for improvement.

My primary recommendation is that the paper is simplified. As is, it reads more like a thesis than a scientific paper, and I suspect that few readers will persevere sufficiently to get something out of the study. Perhaps the most obvious option for shortening and simplifying is to present only a brief summary of the preregistered hypotheses and associated updates in the body text and move the long and detailed descriptions to supplementary material.

RESPONSE 5: Thank you so much for taking the time to go through our submission and give your insightful feedback! We really appreciate your positive comments about this having been a preregistration - thank you for supporting this open research practice! On the point about the article structure and readability, the other two reviewers pointed out the opposite: "well written and clear to the reader, even considering the number of analyses performed" and "The MS is well written". Therefore, we will keep the format and structure as is, but we added the following to help give more sign posts:

First sentence of the Methods: "We first describe the changes we made from our original plans, then we describe the sampling methods to measure behavior, and then outline the analyses we used for each prediction."

First paragraph of the Results: "We share the results from each prediction below"

COMMENT 6: A second concern is that underlying the methodical, seemingly objective, scientific aspects, is a morass of definitional and philosophical complexity around such issues as cognition and behavioral flexibility, and the associated interpretations and assumptions upon which the hypothesis tests and their interpretation are based. This applies even to the fundamental assumption that the experimental measures of "flexibility" do in fact reflect cognitive flexibility as defined in the paper and in the 2017 Mikhalevich paper, rather than some more basic mechanism such as a rule of thumb of the sort "if expected outcome b doesn't follow behaviour B, then switch to behaviour A". In fairness, this partly reflects the status quo in complex fields at the interface of psychology and ecology, such as cognitive ecology and cognitive ethology, and it is difficult to recommend that relevant terms and assumptions are more fully discussed, especially in a paper that is already dense

and long. I do, however, recommend that the language is changed to more closely reflect the relationships between observed outcomes and what was actually manipulated/measured (e.g., reversal learning/multiaccess switching) rather than the inferred meaning of these manipulations/measures. This could be complemented in the discussion by more critically examining how these assumptions impact on the interpretation of the results.

RESPONSE 6: We have now accumulated an extensive body of work on grackle behavioral flexibility, and, one article in particular, Lukas et al. 2024, goes into more detail about how reversal and serial reversal learning relate psychologically to flexibility using our definition of flexibility. Specifically, by applying and expanding "newly developed Bayesian reinforcement learning models to gain additional insights into how individuals might dynamically modulate their behavioral flexibility if they experience serial reversals". It took a whole article to get into the juicy details, so we added a sentence to the discussion to point people in that direction if they want more information. We kept the term flexibility as it was because we define what we mean by this term in the introduction and again throughout the discussion. We added to the first sentence of the discussion that flexibility as measured by a reversal learning task, and we also added to the discussion:

"Indeed, grackle flexibility is itself flexible - it changes across multiple color reversals to match the reliability and stability of the environment they experience [@lukas2024flexmanip]"

Review by anonymous reviewer 3, 19 Nov 2024 20:11

1. Be supportive: Avoid rude, emotive, and accusatory language and tone.

2. Be precise: In references to manuscript text, analyses and figures, include the line number and figure panels in question.

3. Fully reference all of your non-trivial scientific claims (as you would in a manuscript).

4. Review the claims: Perceived novelty or impact of the work must not factor into reviewer comments, unless these form part of the specific claims of the manuscript (e.g., a claim of novelty).

5. Be constructive: Whenever possible, suggest a solution for the problem or concern you raise. Note, this will always only be one of different possible solutions – the authors may choose a different one. In addition, we encourage you to answer the following questions to help recommenders make well-informed and efficient decisions.

If you answer "No" to a question, please explain why and list your suggestions for

improvement by the authors in your free-text review or below each question.

Title and abstract

o Does the title clearly reflect the content of the article? [X] Yes, [ ] No (please explain), [ ] I don't know

o Does the abstract present the main findings of the study? [X] Yes, [ ] No (please explain), [ ] I don't know

Introduction

o Are the research questions/hypotheses/predictions clearly presented? [X] Yes, [ ] No (please explain), [ ] I don't know

o Does the introduction build on relevant research in the field? [X] Yes, [ ] No (please explain), [ ] I don't know

Materials and methods

o Are the methods and analyses sufficiently detailed to allow replication by other researchers? [X] Yes, [ ] No (please explain), [ ] I don't know

o Are the methods and statistical analyses appropriate and well described? [X] Yes, [] No (please explain), [ ] I don't know

Results

o In the case of negative results, is there a statistical power analysis (or an adequate Bayesian analysis or equivalence testing)? [X] Yes, [ ] No (please explain), [ ] I don't know

o Are the results described and interpreted correctly? [X] Yes, [ ] No (please explain),[ ] I don't know

Discussion

o Have the authors appropriately emphasized the strengths and limitations of their study/theory/methods/argument? [X] Yes, [ ] No (please explain), [ ] I don't know

o Are the conclusions adequately supported by the results (without overstating the implications of the findings)? [X] Yes, [ ] No (please explain), [ ] I don't know

COMMENT 7: The MS discusses the term "behavioral flexibility" and how to measure it. The authors discuss that currently since it is very difficult to measure, proxy equivalents are used without having previously verified that these approximations are correct. Common proxies used for behavioral flexibility are sociability, use of urban habitats, and eating new human foods. The main idea is that based on a correct estimation of behavioral flexibility, this concept can be used in studies that will include multiple species. The MS is well written. The introduction presents the background with the defects that the authors want to correct. With this objective, the authors have presented a project that studies the great-tailed grackle (Quiscalus mexicanus; hereafter grackle). This species is a good model since it is social and has expanded its distribution by taking advantage of modifies habitats and by eating new foods generated by human activity. The project contemplates capturing wild individuals and later in captivity, measuring and training some individuals to promote their behavioral flexibility. Subsequently, following individuals released into the wild, the authors corroborate whether the commonly assumed predictions are met when using a proxy instead of behavioral flexibility "per se". Originally, the project involved testing individuals from three populations: one within the species' historical range and the remaining two from urban and recently expanding areas. Finally, and due to justified difficulties, the authors only worked with individuals from the two recently expanded areas. The authors captured individuals in the wild and tested them in the laboratory with two devices that measured their ability to cope with new technics for access to food under conditions of deprivation. Half of these individuals were then randomly assigned to training experiments in which they were forced to reverse learning those new capabilities. Furthermore, the time required to proceed with reversal learning was used as a measure of its flexibility. Finally, when released into the wild, all individuals (tested only and tested plus trained) were followed to measure many characteristics commonly assumed to be indicators of behavioral flexibility. The authors tested many predictions based on commonly used indicators. Overall, they corroborated the predicted association between flexibility and breadth of foraging techniques. However, contrary to expectations, the least flexible individuals used the highest proportion of human foods within their ranges, suggesting that they specialize in those types of foods. Finally, the authors found that there was no association between flexibility and social or habitat use behaviors. Overall, the hypothesis, predictions, and the MS is really interesting. The results are correctly presented, analyzed, and discussed in detail. In addition, when the results did not hold some predictions, the authors added new analyses. I have read all the models presented and think that they are correct. However, I did not test the models by running them. To the best of my knowledge, I consider MS to be very valuable and would be of great interest to scientists working in many branches of ethology and comparative biology or behavioral evolution. My comments are mostly of form or related to the presentation of data and results. However, based on some results, I have a personal comment that I think the authors can discuss related to the use of human foods and the quality of the individuals who depend on them. There is a growing literature on the effect of human diet on animal health, longevity, lifetime reproductive success, and population viability. For example, in the house sparrow (Passer domesticus) the decline of its populations has been attributed to its expansion into agricultural and urban habitats. Possible causes of such decline included poisoning from the use of agrochemicals, exposure to air pollutants, low reproductive success in modified habitats, and dependence on human foods that have very low nutritional quality (Vincent 2005, Shaw et al. 2008, Seress et al. 2012, Bichet et al. 2013, Morrison et al. 2014, Berigan et al. 2020). Furthermore, house sparrows from a rural population forced to follow urban diet resulted in a decrease in their body condition and nutritional physiology, and the induction of oxidative stress (Bernat-Ponce et al. 2023). Therefore, I wonder if the high dependence of the least flexible individuals on human food found in this MS may be a consequence of those individuals being the "low quality" ones. As low-quality individuals are not able to obtain all their nutritional requirements from high-quality natural sources because they cannot compete with individuals of higher quality or rank, they are forced to rely on less preferred foods. Expanding that concept to the population level, these low-quality

individuals could not survive in the natural environment. Modification of human habitat and food sources only allows those "low quality" individuals to survive and reproduce. In summary, I think it can be argued whether in those species currently expanding their range into human-disturbed or even urban habitats, the individuals that took advantage of those new opportunities could truly be characterized as "winners" and those species and populations as "successful."

RESPONSE 7: Thank you very much for taking the time to review our submission and for your great feedback! In terms of whether the less flexible individuals (who use more human foods than the more flexible individuals), dominance rank was included in the P1, P3, and P4, models, so the effects we found are in spite of any dominance rank differences that might exist. In addition, grackles are sexually dimorphic, which might also influence their competitiveness. However, our models investigating potential differences in foraging accounted for sex. In terms of measuring quality in other ways, we do have data on, for example, body size, body condition (using scaled mass index), hematocrit, white blood cell count, and more, and this would be an interesting avenue for future research. In terms of the whether the grackles at the population/species level could be considered successful in human-disturbed or urban environments, we are only able to find this species in human modified environments, so it appears that there is not enough population variation in this species to answer your question about whether the low quality individuals are the ones who move into human modified environments. We added to the discussion:

"In contrast, a cross-species meta-analysis by @maclean2017species found that habitat breadth, but not diet breadth, was associated with geographic range shifts. Even if our habitat categories were at too small of a resolution to be able to detect differences, grackles exist almost exclusively in human-modified habitats. Therefore there would likely not be enough variation at the individual or population level to evaluate hypotheses about variation in habitats as it relates to other variables"

COMMENT 8: More specific comments below. Line 144. P1 alternative 2. I have a doubt. Always the more caloric foods be considered as being more valuable? I am not a specialist on food quality, but I suspect that there may be some king of foods that are more easily digested. Or that they may have specific micronutrients for example. Of course, number of calories may be appropriate proxy for quality. But the authors may discuss their approximation and alternatives in the discussion of the MS.

RESPONSE 8: Good idea to discuss more about the choice of this measure, thank you for bringing this up. In Prediction 1 alternative 2, we said we would "examine what food types the more flexible grackles eat and whether these food types are potentially more valuable (measured as having more calories)". So we were interested in the value of the food, as measured by calories, not necessarily the quality of the food, as in nutrient content. We added to the discussion: "While calories are a common measure of the energy value of food [@merrill1955energy], other measures of value might be interesting to investigate in the future, for example nutrient content"

COMMENT 9: Is there information on the reproductive success of individuals and populations of grackles from natural or native vs modified and urban areas? Perhaps more modified habitats imply low breeding success and are used by low quality individuals.

RESPONSE 9: This is an interesting question and one that we were interested in as well when we set out to study this species. We scoured eBird.org for populations of great-tailed grackles that live in non-human modified areas, but we could not find any. We are also not aware of literature that compared these variables historically when there might have been grackles who lived in non-human modified areas.

COMMENT 10: Is food from human sources healthy for grackles? See the paragraph where I cite the known

examples for the house sparrow.

RESPONSE 10: We do not know about the health impacts of the different foods the grackles eat and we are unaware of research that has investigated this question. It would be an interesting avenue of future research.

COMMENT 11: Line 233. "grackles" no "rackles"

RESPONSE 11: Good catch! We fixed the typo.

COMMENT 12: Line 339. You add microhabitat as a random factor because you want to account for differences in microhabitat but you were not interested in measuring the effect? Please clarify.

RESPONSE 12: Thank you for pointing this out. We agree that it isn't clear. We re-checked our preregistration, which says "We will examine the proportion of focal follows associated with each microhabitat per individual and relate this to their flexibility scores on their most recent reversal learning color tube experiment." The Bayesian analysis we wrote, does exactly that, so we don't actually need to write that a change was made. We deleted the text from the Changes section (formerly bullet point 13).

COMMENT 13: Line 398. I suspect your Fig 1. previously had a format more similar to a figure. Now I think is more of a Table with schemes in the upper line displaying the apparatus you had used during flexibility trials. Therefore, I think it would be more clear if you cite the Fig 1 as "Table 2" And refers to the apparatus: see, Table 2 upper line "B". This would be more clear if you add a letter (A, B, C, D) to corresponding cells of the upper line in the Table.

RESPONSE 13: Good point that this is actually a table. We made the change and we removed the pictures from the top row.

COMMENT 14: Line 413. Idem my previous comment. Replace Fig 1c for: Table 2, upper line "C".

RESPONSE 14: We are sorry for the confusion! Figure 1c was a typo - carried over from a previous version. We didn't intend for the newly labeled Table 2 (from Response 13) to have clarifying pictures of the apparatuses, so we removed this typo.

COMMENT 15: Line 554. I am not familiar with Bayesian analyses. The "89% compatibility interval" is commonly used in Bayesian analysis? Please clarify.

RESPONSE 15: The 89% compatibility interval was made popular by McElreath (2018) as a way of pointing out that the 95% or 89% intervals are arbitrary choices. It is now commonly used.
   McElreath, R. (2018). Statistical rethinking: A Bayesian course with examples in R and Stan. Chapman and Hall/CRC.
   **Download tracked changes file**

### Decision by **Esther Sebastián González** and **Julia Astegiano** , posted 21 January 2025, validated 21 January 2025

   Dear authors,
   First of all, we am very sorry for the delay in the response. We have had many problems in finding reviewers and some of those reviewers needed extra time to complete the process.

As you can see, the three reviewers found your study very interesting but they also include some comments that can help to improve the quality of the manuscrit. Thus, we would like to invite you to incorporte those changes in the text and submit a new improved version of the manuscript.

We are looking forward to it. Best,

Esther Sebastián González and Julia Astegiano

### Reviewed by anonymous reviewer 1, 25 August 2024

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### Reviewed by anonymous reviewer 3, 21 January 2025

I have not addressed the specific categories listed above, as my general comments below apply to all (except power analysis, to which my answer is "yes").

**Download the review**

### Reviewed by anonymous reviewer 2, 19 November 2024

**Download the review**