Dear Dr.'s Astegiano, Sebastián-González, and an anonymous reviewer,

We apologize for the delay in our resubmission - we were ill for a few weeks and then there was a very intense period when we were finishing modifications to the grackle aviary experiments, which delayed our ability to attend to this revision.

We greatly appreciate the time you have taken to give us such useful feedback! We are very thankful for your willingness to participate in the peer review of preregistrations, and we are happy to have the opportunity to revise and resubmit.

We revised our preregistration and associated files at https://github.com/corinalogan/grackles/blob/master/EasyToReadFiles/g_flexforagingForReviewers.md, and we responded to your comments (which we numbered for clarity) below (our responses are preceded by "> Response X").

We think the revised version is much improved due to your generous feedback!

All our best,

Corina, Luisa, Kelsey, and Dieter

Note: Carolyn Rowney has removed herself as a co-author because she doesn't feel she has contributed enough to warrant authorship on this manuscript.

Is behavioral flexibility related to foraging and social behavior in a rapidly expanding species?

Corina Logan, Luisa Bergeron, Carolyn Rowney, Kelsey McCune, Dieter Lukas

https://github.com/corinalogan/grackles/blob/master/EasyToReadFiles/g_flexforagingForReviewers.md version v1.5
Submitted by Corina Logan 2018-10-23 00:47

Abstract
This is one of the first studies planned for our long-term research on the role of behavioral flexibility in rapid geographic range expansions. Project background: Behavioral flexibility, the ability to change behavior when circumstances change based on learning from previous experience (Mikhalevich, Powell, and Logan (2017)), is thought to play an important role in a species' ability to successfully adapt to new environments and expand its geographic range (e.g., (Lefebvre et al. 1997), (Griffin and Guez 2014), (Chow, Lea, and Leaver 2016), (Sol and Lefebvre 2000), (Sol, Timmermans, and Lefebvre 2002), (Sol et al. 2005)). However, behavioral flexibility is rarely directly tested at the individual level, thus limiting our ability to determine how it relates to other traits, which limits the power of predictions about a species' ability to adapt behavior to new environments. We use great-tailed grackles (a bird species) as a model to investigate this question because they have rapidly expanded their range into North America over the past 140 years ((Wehtje 2003), (Peer 2011)) (Fig. 1). Results will allow us to determine whether, as predicted by hypotheses and cross-species correlational data, in this expanding species, individual-level variation in flexibility is linked with diet breadth, foraging proficiency, social interactions, habitat use, and movement into new geographic areas. This investigation: In this piece of the long-term project, we will assess whether performance in experiments that assess behavioral flexibility relates to variation in ecological and social behavior in the natural environment. In particular, we aim to determine whether the more behaviorally flexible (measured by reversal learning and solution switching on a multi-access box in a separate preregistration) grackles have more flexible foraging

behavior (eat a larger number of different foods, use a wider variety of foraging techniques), are more flexible in their habitat use (are found in more diverse habitat types, disperse farther from their natal area), and are more flexible in their social relationships (have stronger social bonds particularly with less related individuals). We will be able to compare the grackle's ability to adapt behavior according to social context with data from other species, as well as determine whether it is linked with measures of flexibility in asocial contexts.

Keywords: Behavioral flexibility, innovation, foraging, habitat use, social behavior, relatedness, comparative cognition, avian cognition

Round #1

Decision

by Julia Astegiano, 2019-03-16 13:59
Manuscript: [10.17605/OSF.IO/GCA5V](10.17605/OSF.IO/GCA5V)

Review of your pre-registration

Dear Dr. Corina Logan and co-authors,
Thank you for submitting your preregistration "Is behavioral flexibility related to foraging and social behavior in a rapidly expanding species? " to PCI Ecology. My sincere apologies for the delay in giving you a first answer.

Dr. Esther Sebastián-González, an anonymous reviewer and myself have now reviewed your preregistration. You will find our general and specific comments below. I hope that they will help to improve your project. We all agree that this is an interesting, original and timely project. However, Dr. Sebastián González have some concerns on your hypotheses and the way you will carry out fieldwork related to the characterization of foraging and social behavior. Reviewer 2 suggests that (1) you should include general background information supporting your hypotheses, (2) reformulate your hypotheses to include the alternative predictions, and (3) rethink about how you will measure and characterize some of your variables. I agree with all their comments and suggestions, and I have some additional ones in the same line that are explained below.

I would like to invite you to submit a revised version of this pre-registration considering all comments and suggestions.

Sincerely
Julia

General comments
Comment 1: About the populations you will sample My main concern is related with the design of your study. You decided to conduct your study in three populations and consider them as blocks in your statistical analyses since you recognize that different relationships among variables can be expected in each population, which I agree. However, I am not sure that the populations you chose will be the most informative for your general question concerning the role of behavior flexibility on rapid range expansion. I mean you will use one population from the center, one from the middle and one from the edge of the geographic range of the species. If you are trying to understand the role of behavior flexibility in the expansion of a species range, why focusing on a population from the center of the species distribution and not different populations from the edge (i.e. trying to represent different edge conditions)? It is widely recognized that the different edges of the geographic range of species will impose different limitations for range expansion. Moreover, I expect geographic range expansion be more linked with populations in the edge of the

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geographic distribution of a given species. Then I think it will be more informative to perform your study in different populations from the edge of the geographic range of grackles, having central populations as controls.

>Response 1: Thank you very much for this feedback. We have struggled with this issue in the past and we ended up selecting only three populations because that is the maximum number we can sample in the 5 years we have funding for - both because we only have enough funding to run one field site at a time, but also the project is running at maximum capacity in terms of needing to figure out how to make the project run in a new place and with a completely different population of grackles. The problem is that it takes a really long time to set up and get data from each field site. For example, it took 2.5 years just to get the permits for the Santa Barbara population, and in Arizona the grackles are proving very difficult to catch (relative to the Santa Barbara grackles), which has greatly slowed down our progress. Given these constraints, we decided to have one population in the center of their original range as a sort of baseline (in terms of this being where they originally evolved) from which to compare the edge population. We expect there to be the largest differences between individuals in the center population and individuals in the edge population, and we wanted to ensure we captured this variation, which would give us the best chance at actually detecting differences in flexibility if there are any. We would expect the center population to have less and/or less individual variation in flexibility than edge populations (see more details in H1 in our cross-population comparison preregistration: https://github.com/corinalogan/grackles/blob/master/EasyToReadFiles/g_expansion.md). Therefore, if we were to only sample edge populations, we might not be able to detect site differences in flexibility because individuals in these edge populations might be similar to each other. To know whether flexibility is important for a range expansion, our prediction is that flexibility is higher where it is more needed on the expanding edge. Flexibility is a continuous variable, particularly the way we measure it, therefore we need a baseline (the population in the middle of their original range) to understand whether flexibility is relatively higher on the edge.

In terms of considering the differences in habitat between the populations, we have searched all observations at eBird.org and we were unable to find a non-urban great-tailed grackle population. Therefore, their environments will differ in so much as city types differ. The city that hosts the center population will be in Central America and at the moment we are considering a small town in the middle of the tropical rainforest. The tropical rainforest is very different from the two younger populations we will sample: the desert for the current urban population (Tempe, Arizona), which will likely be similar to the environment of the next urban population we are setting up (likely in northern California). From our observations of grackles at the Santa Barbara and Tempe field sites, individuals at both locations seem to use their very similar urban environments in the same ways: they feed at outdoor cafes during open hours when people are eating meals, they steal food set out for other species (e.g., at the Zoo and at feral cat feeding stations), they raid garbage cans, and they forage on insects and other animals they encounter. So we expect the two sites nearer the edge to be quite similar to each other, while the center population will likely differ due to being a different type of city (e.g., less cement, fewer buildings, more water).

Considering all of this, we are open to changing the locations of the edge population and the center population, but we are unclear about how we could make better choices given our constraints of being limited to a maximum of 2 more field sites during the remaining 3.5 years.

As a partial solution to this problem, we have discussed running a flexibility/individual differences test on groups of unmarked grackles at additional sites, which could really help with the issue that you bring up. The extremely time consuming part of the project is catching grackles and also setting up aviaries and testing them there, so eliminating these components could make additional sites feasible and perhaps we could catch a few birds at these other sites so we would have some indication of their blood and DNA measures at the group level. The test we are thinking of running at these additional sites is the multiaccess puzzle box. It would occur in the wild and not in visual isolation of other individuals because that wouldn't be feasible. The individuals would not be marked, but perhaps we could make it so the box marks an individual with paint as it interacts with the 4 solving options, thus we would know who has already solved versus who hasn't. We will write a preregistration for this later in the project because we expect we will

want to try gathering this additional data on the edge when we are at the primary edge population field site and when we are at the primary population in the original part of their range.

Comment 2: I also suggest that you provide more information on the ecology of the species and the expansion of its range, which certainly may help understand your decisions about the populations that will be sampled.

>Response 2: We agree that it is good to include more background about this species so thank you for bringing it up! We made the following changes:

Added to H1: "We expect this species to be behaviorally flexible because they are fast at reversal learning (Logan 2016), they often encounter human-made "puzzle boxes" in the wild as they attempt to open packaging to access food when digging through garbage cans and eating at outdoor cafes, and they may track resources across time and space. Foraging behavior is considered central to the rapid geographic range expansion of this species and it is thought that they have been so successful by following human urban and agricultural corridors ([@wehtje2003range], [@peer2011invasion]). Therefore, as humans continue to modify landscapes, this increases the amount of suitable grackle habitat."

Added to H2: "To give an example of the types of social relationships this species engages in, in terms of male social relationships, @johnson2000male found during the breeding season in a population in Texas that one or more territorial males defend a territory with several nests from females, that non-territory holding resident males will queue to gain access to a territory, and that transient males move from colony to colony. There could be varying needs for males to manage their relationships with each other and flexibility could potentially play a role in such management."

Added to H3: "Although we were only able to find this species in association with human-modified landscapes based on eBird sightings (i.e., there appear to be no forest-based populations), individuals could use these landscapes in a variety of ways. For example they could specialize on particular foods or at particular types of locations (e.g., foraging exclusively at cafes or in grassy areas), they could generalize across all foods and location types, or they might fall somewhere in between these extremes"

Comment 3: In regard with your study design, have you thought about the possibility of comparing the association between behavioral variation and foraging and social behavior across species with contrasting geographic ranges or that expanded their range at different speeds? Have you considered performing such comparative analyses in the future?

>Response 3: Yes we have considered this and we have a field site ready to go to compare boat-tailed grackles (BTGR) with great-tailed grackles (GTGR). The BTGR species split from GTGR about 500,000 years ago and they are the species most closely related to GTGR. What makes this comparison interesting is that these species are very similar (according to the literature), but BTGR are not rapidly expanding their geographic range. Therefore, investigating flexibility in BTGR could help elucidate what it is about GTGR that makes them so successful. If BTGR have less/less variability in flexibility, than it provides more support to the argument that flexibility is involved in the GTGR range expansion. If, on the other hand, BTGR is similar in their flexibility, then this would call into question the role of flexibility in a range expansion and lead to additional alternative explanations to investigate in the future. The issue we run into here is that we don't have the time or resources in this 5 year project to run the BTGR field site. We are hoping to get funding for this in the subsequent 5-year period.

Comment 4: Data analyses Social network analyses are comparatively less explained than other analyses. Please, include more information on which variables you will measure and how you will do it, how you will construct random networks and how you will compare your observed social network with randomized ones.

>Response 4: Thank you for pointing this out! We have elaborated on our methods for conducting social network analysis in Analysis Plan > P4 by adding the following text:

To quantify social relationships, we will conduct at least four 10-minute focal follows on each subject. We will find subjects in the wild because we will attach radio transmitter tags to all grackles that are released from the aviaries upon completion of their test battery. To ensure we fully sample social and foraging behavior, we will prioritize conducting focal follows on these tagged grackles for which we have a much larger amount of individualized data, including multiple measures of flexibility. We will also sample many other banded grackles that were never tested in the aviaries, and thus do not have measures of flexibility. By conducting focal follows on grackles that were not in captivity, we can verify that the time in captivity had no effect on grackle social behavior after release because aviary-tested birds should be indistinguishable from non-aviary-tested birds in these analyses (this is accounted for by the new variable in the analyses: Condition).

To measure affiliative bonds, during each focal follow we will record when another grackle comes within one body length of the focal bird (and does not engage in aggressive interactions). In case we do not observe enough of these close associations, we will also record when another grackle comes within 3m of the focal subject (and does not engage in aggressive interactions). Finally, we will conduct a scan sample at the end of the follow to determine group size as the number of other grackles within 10m of the focal individual. Unbanded grackles that are seen in proximity of the focal individual will be recorded and included in the count of group size and individual degree (the number of unique associates), but because we cannot distinguish unbanded individuals from each other, we will exclude unbanded bird data from calculations of an individual's summed bond strengths (see details in the next paragraph).

We will conduct subsequent follows on the same individual only when 3 or more weeks have passed since the previous focal follow to prevent temporal autocorrelation in behavior (@whitehead2008analyzing). From the data sheet of dyadic associations during focal follows, we will create a matrix of association strengths between all banded grackles by calculating the Half-Weight association index. This index determines association strength based on the proportion of observations in which two individuals are seen together versus separately, and accounts for bias arising from subjects that are more likely to be observed separately rather than together in the same group (@cairns1987comparison). From the matrix of association values, we will use the R package igraph (@csardi2006igraph) to create a social network, and calculate each individual's strength (sum of all association values) and degree (maximum number of unique associates) values (@croft2008exploring).

Social network data are not independent (@croft2011hypothesis), therefore, to determine whether individuals are associating non-randomly based on flexibility (i.e., association strength between two grackles is larger than would be expected by random chance), we will compare our model results to those obtained from random networks. To make a random network, we will use the R package sna (@butts2016sna) and the function "rperm" to randomly rearrange the association strengths (edges) of grackles in our network. We will conduct this edge randomization (called permutation) 10,000 times to create our sample of random networks. We will then re-calculate our dependent variables from the random networks and re-run the same models (as in @croft2011hypothsis and @whitehead2005testing). We will conclude that social bonds are significantly related to flexibility if the coefficients describing the relationship in our observed data are in the top 2.5% and bottom 2.5% of the coefficients resulting from models run on the random networks.

Specific comments
Comment 5: Abstract "However, behavioral flexibility is rarely directly tested at the individual level, thus limiting our ability to determine how it relates to other traits, which limits the power of predictions about a species' ability to adapt behavior to new environments". This sentence is not clear to me. Which other traits? Other traits that are already know to relate to rapid geographic range expansion? What do you mean by rapid geographic range expansion? Please, try to be as specific as you are in the following sentence: "Results will allow us to determine whether (…)

individual-level variation in flexibility is linked with diet breadth, foraging proficiency, social interactions, habitat use, and movement into new geographic areas".

>Response 5: Thank you for your help in making this clearer! To address your comments we made the following changes (in bold):

Abstract:
"However, behavioral flexibility is rarely directly tested at the individual level, thus limiting our ability to determine how it relates to other traits **(e.g., behavior, invasion success, diet generalism, foraging techniques, foraging innovations, mortality, brain size)**"

"they have rapidly expanded their range into North America over the past 140 years (**i.e., they increased their nesting range by over 5500% between 1880 and 2000**"

Comment 6: "We use great-tailed grackles (a bird species) as a model to investigate this question because they have rapidly expanded their range into North America over the past 140 years ((Wehtje 2003), (Peer 2011)) (Fig. 1)". Here I suggest you provide more information about the natural history explaining the expansion of the geographic range of this species, in order to link it with the mechanisms you are proposing to evaluate in this project. Does this rapid expansion include new biomes? Or is the species just moving on the same kind of environments (i.e. expanding more by modifying dispersal ability and not new habitat use?). Are grackles using different vegetation cover types?

>Response 6: Good point. Not much is actually published on the details of how grackles are using habitats as they expand, but we added what we know so far from the literature. To address your comments we made the following changes (in bold):

Abstract: "We use great-tailed grackles (a bird species) as a model to investigate this question because they have rapidly expanded their range into North America over the past 140 years (i.e., they increased their nesting range by over 5500% between 1880 and 2000 [@wehtje2003range], [@peer2011invasion]) (Fig. 1). **Foraging behavior is considered central to the rapid geographic range expansion of this species and it is thought that they have been so successful by following human urban and agricultural corridors ([@wehtje2003range], [@peer2011invasion]). Therefore, as humans continue to modify landscapes, this increases the amount of suitable grackle habitat. We expect this species to be behaviorally flexible because they are fast at reversal learning (@logan2016behavioral), they often encounter human-made "puzzle boxes" in the wild as they attempt to open packaging to access food when digging through garbage cans and eating at outdoor cafes, and they may track resources across time and space.**"

Comment 7: "This investigation: In this piece of the long-term project, we will assess whether performance in experiments that assess behavioral flexibility relates to variation in ecological and social behavior in the natural environment". Are you referring to individual performance and individual variation? Or is it individual performance and across-individual (population) variation in ecological and social behavior? By being more specific in the formulation of these questions you may allow readers to evaluate their match with the methodology you are proposing. From this abstract is difficult to see if you will evaluate variation in flexibility, ecological and social behavior just at the individual level.

>Response 7: Thank you again for helping us get clear about this. To address your comment we made the following changes (in bold):

Abstract: "In this piece of the long-term project, we will assess whether **individual** performance in experiments that assess behavioral flexibility relates to **individual** variation in ecological and social behavior in the natural environment."

Comment 8: Hypotheses "H1: Individuals that are more behaviorally flexible (measured by reversal learning and switching between options on a multi-access box) will differ in their foraging behavior in the wild (measured with focal follows)". I suggest you rewrite your hypothesis as: Behavioral flexibility is related to/modulates/influences foraging behavior. "Prediction 1: (…) validating the cross-species correlational finding that technique breadth (Overington et al. (2009)) and diet breadth (Ducatez, Clavel, and Lefebvre (2015)) indicate flexibility". I suggest you change "indicate" by "are associated to".

>Response 8: Great point because we will not know whether one of these variables causes the other and, if so, which direction the causal relationship would be in. We made the following changes (in bold):

H1: "Behavioral flexibility (measured by reversal learning and switching between options on a multi-access box) **is related to** foraging behavior (measured with focal follows) in wild individuals."

P1: "validating the cross-species correlational finding that technique breadth (@overington2009technical) and diet breadth (@ducatez2015ecological) **is associated with** flexibility"

Comment 9: "P1 alternative 1: If there is no correlation, this suggests that flexibility is an independent trait from the number of foods eaten and foraging techniques used." Why you use a statistical term in your prediction? I suggest you use "relationship" or association. On the other hand, since flexibility is the factor of analysis in fact this will suggest that the number of foods eaten and foraging techniques used are independent from behavioral flexibility.

>Response 9: Sorry for the confusion here - I think we were not clear that by "independent trait" we did not mean "independent variable in a model", but rather that flexibility is a trait that appears to be disconnected from foraging (foods eaten and techniques used). We reworded to (change in bold):

P1alt1: "suggests that flexibility is a trait **that is not related to** the number of foods eaten and foraging techniques used "

Comment 10: "P1 alternative 1: (…) Flexibility is not necessarily associated with diet and foraging technique breadth because flexibility could be constrained in a foraging context due to social competition (e.g., subordinates are outcompeted while foraging and thus try new foods and techniques) or ecological limitations (e.g., constrained by what is available)". In the same line of my previous comment and following the logic you presented previously, you should state that foraging techniques and items might be constrained by other factors such as… For me, this will constitute an alternative hypothesis and not an alternative prediction.

>Response 10: Since our focus is on the relationship between flexibility and foraging behavior, in the event that there is no correlation between these variables, then it certainly could be that other factors are constraining foraging behavior. We see now that we worded this prediction as if the explanation we gave for why flexibility wouldn't be correlated with the foraging variables makes it look as if this was the only explanation for the lack of an association. Since we are not investigating other factors that could affect foraging behavior and we are only looking at its relationship with flexibility, we consider this part of a prediction in the current hypothesis. We reworded to (changes in bold):

P1alt1: "Flexibility **may** not necessarily be associated with diet and foraging technique breadth because flexibility could be constrained in a foraging context due to social competition (e.g., subordinates are outcompeted while foraging and thus try new foods and techniques) or ecological limitations (e.g., constrained by what is available). **Additional research would be required to determine the factors that might constrain foraging behavior.**"

Comment 11: "P3: The more flexible individuals eat more human food, potentially due to A) having stayed in their parent's home range (i.e., they eat human food because it happens to be more prevalent in their home range than in other home ranges; local specialization) or B) because these individuals move around to seek out such opportunities (potentially seeking out habitat edges within their population). Foods eaten will be recorded during focal follows". What does it mean "more human food"? More quantity? More items? Eating human food will be considered an expansion of their diet (i.e. adaptation to new environments) or not? How innovative techniques will function in this case? Do you have predictions on these techniques? Thinking on the populations you chose (see general comments): Do the populations that you propose to sample differ on their proximity to urban areas? How may this proximity affect the relationships you expect to find? "H3: Individuals that are more behaviorally flexible (measured by reversal learning and switching between options on a multi-access box) will use a wider range of habitats (measured with GPS point for each focal follow)." Can you be more specific about what you will consider different habitats and therefore how you will measure the range of habitats? In P5 and P6 you use diversity of habitats so I think it will help to have a more specific definition of habitat here. Will you use different vegetation cover types? Or compare wild, semi-natural and urban habitats?

>Response 11: Thanks to your requests for clarifications in Comments 2, 5, and 6, we think the resulting revision should place P3 and H3 in a better context in general. We now include foraging techniques in P3 where we address the predictions for this piece. To address your comments, we made the following changes (in bold):

P3: "**The proportion of their diet that is human foods and the proportion of their foraging techniques involving human foods is higher** for the more flexible individuals, **who will consistently occur in locations closer to known outdoor human food locations like picnic areas and outdoor cafe seating (measured as the repeatability of the individual's distance from cafes across multiple separate focal follows)**. **For the diet**, this is potentially due to A) having stayed in their parent's home range (i.e., they eat human food because it happens to be more prevalent in their home range than in other home ranges; local specialization) or B) because these individuals move around to seek out such opportunities (potentially seeking out habitat edges within their population). **For the foraging techniques, this is potentially due to human foods and their packaging changing at a faster rate than natural foods and prey items and their accessibility.** Foods eaten and foraging techniques used will be recorded during focal follows. **Because this species is highly associated with human-modified landscapes, it is likely that consuming human foods is part of the reason for this association, and that flexible individuals are better at solving these human-made "puzzle boxes" to access food.**"

P3 alternative: "There is no correlation between an individual's flexibility and **the proportion o**f human foods"

H3: Individuals that are more behaviorally flexible (measured by reversal learning and switching between options on a multi-access box) **will differ in their use of micro**habitats **within human-modified landscapes** (**substrate qualification during** each focal follow), **but the macrohabitat (square kilometer) of each population will not differ in human population density (**measured with a GPS point for each focal follow **and using census information to obtain human population density [US: https://www.census.gov/quickfacts/fact/note/US/LND110210, Central American countries: http://worldpopulationreview.com/continents/central-america-population/])**.

P6: Flexible individuals will be found more regularly in a wider diversity of **micro-**habitats **(human-modified substrates including cement, dumpsters or cafes; or natural substrates including grass, shrubs, and trees [additional substrates will be added as they are encountered]) during focal follows.**

P6 alternative: Flexibility is not associated with presence in diverse **micro-**habitats because the more flexible individuals might specialize in specific foraging strategies.

P7: There will be no difference in human population density among the sites for our 3 grackle populations because all great-tailed grackle populations are highly associated with human-modified landscapes.

Comment 12: METHODS P6: flexible = wider range of habitats 1.Evenness in the proportion of time spent in each habitat type (grass, gravel and other natural substrate, cement, cafe, dumpster). Comment: Why you use one measure of habitat diversity? I suggest you use different qualitative and quantitative measures to fully understand habitat use or provide a strong argument to just use evenness (which I found will provide you a highly constrained definition of habitat use).

>Response 12: We revised the preregistration to justify our use of the Shannon diversity and evenness index for quantifying habitat use by adding:
Analysis Plan > P6: "This species is primarily found within urbanized environments, however there are many different substrates within urban habitats that could provide a variety of food items. Since we are interested in the flexibility of grackle foraging behaviors within the urban habitat, we have focused our habitat diversity measures on the different substrates on which we are mostly likely to see individual variability in foraging behaviors and food types, if present. For example, cement, cafe, and dumpster substrates are all likely to contain human-provided food (either because people leave food out for wild animals or wild animals are able to scrounge human foods), whereas grass, gravel, or other natural substrates such as trees likely contain non-human provided prey items including insects and small vertebrates. Using the Shannon diversity index to understand the evenness of substrate use within urban habitats has been recommended by others in the field of urban ecology (@alberti2001quantifying & @tews2004animal)."

Comment 13: About the use of G tests It is not clear for me why you present those G tests if (1) you cannot include the exact variance structure of your future models, which will modify the sample size you will need; (2) you are planning to use the Bayesian approach.

>Response 13: By "G tests" do you mean the G*Power analyses? If so, this also came up in the reviews of a different preregistration (https://github.com/corinalogan/grackles/blob/master/EasyToReadFiles/g_flexmanip.md, reviews at https://ecology.peercommunityin.org/public/rec?id=17&reviews=True) and parts of our response applies here as well:

"We realize that the power analyses used were the wrong tool for the job. However, we didn't know of a better option and we wanted to have some representation of our ability to detect effects, which is why we used them. We had tried a few R packages that could have been more effective, but we were not able to get any of them to work with our mock data."

As a result of this reviewer's comment, we tried running simulations to get a better idea of how our models will behave (using the R package SQuID). Here is our response detailing how that didn't end up working either:
"After working with SQuID to simulate our model in P2, we were unable to find a way to represent the complexity of our model (e.g., compare population means between control vs. manipulated conditions) while manipulating effect sizes. Additionally, we have no prior information about what values to provide in the input for the simulation without looking at the data we are currently collecting (e.g., the multi-access box has never been presented to grackles and

we were unsure of how many options they would be able to solve). We are interested in the effect of the manipulation vs. everything else we are controlling for and, because of the complexity of the model, the effect is going to depend on the factors we control for as well as the boundaries of the dependent and independent variables. We currently don't have any estimates for any variables because these tests have never been done in grackles and we have not encountered previous research that has manipulated flexibility in this way."

We are finally getting a chance to go through Richard McElreath's Statistical Rethinking book and online course and we are beginning to understand what the second part of your comment likely implies - that the Bayesian approach actually takes care of this piece for us. We need to finish the course before we will be able to competently update the preregistration. We have now deleted the G*Power analyses throughout the Analysis Plan and revised:

Analysis Plan > Ability to detect actual effects: "To begin to understand what kinds of effect sizes we will be able to detect given our sample size limitations and our interest in decreasing noise by attempting to measure it, which increases the number of explanatory variables, we will revise the Analysis Plan after reading @statrethinkingbook. We currently don't have any estimates for any of our measured variables because these tests have never been done in grackles and we have not encountered previous research that has manipulated flexibility in this way. We will use Bayesian analyses to estimate our likely confidence in the results given simulated data. We will revise this preregistration to include these new analyses before conducting the planned analyses on our actual data. Based on the simulations, we might adapt the number of focal follows per individual or decide to collect much more data just with the aviary-tested birds to increase the amount of information per individual."

If you know of specific ways in which the Bayesian approach can eliminate the need for a G*Power analysis, we would be very happy to hear your ideas.

Reviews

Reviewed by Esther Sebastián González, 2019-01-10 10:43
Comment 14: Dr. Logan et al have pre-registered an interesting project to study the relationship between the flexibility in the behavior of grackles and several individual characteristics such as their diet, foraging strategies and social relationships. The main novelty of this project is that it focuses on individual differences, while most previous studies have worked at population level. The hypotheses are in general well described and tailed to the methods that will be used. The authors have also provided with a very detailed description of the statistical analyses that they will perform, including the R code. I have some concerns with the methodological design and the hypotheses, as well as suggestions of improvement in the description of the variables and of the stats.

>Response 14: Thank you very much for your feedback! We look forward to responding to your comments below.

Comment 15: My main concern is with the fieldwork. Besides being very informative in other aspects, the pre-registration does not provide with much information about how the fieldwork will be carried out, and it is vey important. For example, how will you gather the information on the diet, bonding, foraging strategies, or the probability of being an immigrant for the individuals that are in the field? Will you follow them? For the bonds analysis you indicate that "To quantify social relationships, we will conduct at least four 10-minute focal follows", but nothing is said for the other variables. How will you do the survey for the remaining variables? Will you try to resample in different days? How likely is that you resample one individual? I am worried that if you cannot resample the individuals enough times, that won´t give you the information on their entire diet or foraging techniques used. It is also not very clear to me how will you do the behavioral test in the field, will you keep individuals until they solve a loci and then do the reverse learning test? It looks to me that some of the hypotheses can be only tested with aviary individuals while others with field individuals, or with both of them. It will help to understand how you will perform the

study if you include the information on what group of individuals you will use to test each prediction. It may be that this is well explained in another pre-submission, but it would be nice to have it here a bit more detailed. Finally, are you planning to compare the behavior of the individuals that have been in the aviary for some time with those that won´t? It looks like you will measure both. I think it would be very interesting at least to compare the diet, bonding and foraging techniques among them to be sure that being in the aviary is not affecting them. Or maybe this can be an additional predictor variable to be included in your analyses.

>**Response 15:** We understand your concern, and we have now provided more methodological details to answer your questions. Please see our Response 4 (above) where we now give more detail on how we will measure social bonds, we compare the social behavior of aviary-tested and non-aviary-tested birds to make sure the aviary time did not affect their behavior in the wild, we describe the minimum number of days between focal follows on the same individual (resampling), we discuss that we record what a focal grackle eats and how it searches for food and we link to the ethogram, and we describe that we place radio tags on all of the aviary birds that are released so we can find them and increase the likelihood of conducting focal follows.

We measure the remaining variables via the focal follows using an ethogram (https://docs.google.com/spreadsheets/d/1N8wsA3geaRGlMjRxYTRpdG2i5oCXNGq9zBlTnj02Gho/edit?usp=sharing), which we provided a link to in the Open Materials section, but now we make it more explicit by including this link in H1 and H2 (see the changes summarized in the paragraph below the next paragraph).

We clarified in each hypothesis whether birds had been tested in the aviaries and that their behavior measured for this preregistration is conducted after they are released back to the wild. You are correct in that the flexibility tests were carried out in aviaries, and then the data for this preregistration is collected on these individuals after they are released back to the wild. We have made the following clarifications (changes in bold):

H1: "Behavioral flexibility (measured by reversal learning and switching between options on a multi-access box **in aviaries**) is related to foraging behavior (measured with focal follows using this [ethogram](https://docs.google.com/spreadsheets/d/1N8wsA3geaRGlMjRxYTRpdG2i5oCXNGq9zBlTnj02Gho/edit?usp=sharing)) in wild individuals **(after their release from the aviaries)**"

H2: "Behavioral flexibility (measured by reversal learning and switching between options on a multi-access box **in aviaries**) is related to social behavior (measured with focal follows using this [ethogram](https://docs.google.com/spreadsheets/d/1N8wsA3geaRGlMjRxYTRpdG2i5oCXNGq9zBlTnj02Gho/edit?usp=sharing)) in wild individuals **(after their release from the aviaries)**"

H3: "Behavioral flexibility (measured by reversal learning and switching between options on a multi-access box **in aviaries**) is related to variation in the use of microhabitats within human-modified landscapes (measured with a GPS point for each focal follow **after their release from the aviaries**;"

We added Condition (aviary-tested or non-aviary tested) to Analysis Plan > Independent Variables > P1-P4 and P6 (and to the R code in the subsequent analyses) to make sure that the wild foraging behavior of the aviary-tested birds is the same as those who were not tested in the aviaries.

We describe how we measure relatedness and probability of being an immigrant in:
Methods > Dependent variables:
P4: "5) Relatedness for the strongest bond **(following the protocol in @thrasher2018double to estimate pairwise relatedness between all individuals based on the extent of sharing of genetic variants as determined by ddRADseq)**"

Comment 16: I also have some comments for some of your hypotheses: P3: You can easily test hypothesis P3 A) if you do a fast estimation of the amount of food available in the surroundings of your observation and compare how frequent human food is and how frequent it is consumed by the species. Additionally, I can also think about a P3 C) the more flexible individuals eat more human food because they have the foraging techniques required to consume those new food items.

>Response 16: Thank you for the suggestions! We have been discussing how we could implement these and we aren't sure how we would quantify the amount of human food available. This is because we really only find the grackles in the middle of areas that are surrounded by human foods. For example, if we were to conduct a focal follow in a nearby park, there would be several trash cans scattered throughout the park and there might be a few people eating at the picnic tables. Given that we are not able to quantify how much non-human food is available at any given location (i.e., we can't see the worms under the grass or how many insects there are or how many fruits are in the area), then we aren't quite sure how to give this a quantitative measure. However, we will be able to quantify how frequently human foods are consumed because we can see what they eat and this is measured during the focal follows. We will also measure the substrate they were last observed on for each focal follow so this will give us an indication of whether that focal occurred in a grassy area (where they were more likely eating non-human foods) or on cement (where they would be more likely to be eating human foods).

Regarding your suggestion for a P3 C, please see our Response 11 for how we incorporated foraging techniques and our predictions about them into this prediction.

Comment 17: In P3 alternative A, I´d complete the sentence specifying that "their daily range sizes encompass many different food resources, including human foods, but they are not specialized on them". Also, I think that if P3 alternative B happens, then the correlation between an individual's flexibility and the amount of human food in their diet would be negative.

>Response 17: Great point! We revised the sentence to say "A) their daily range sizes encompass many different food resources, including human foods (though they are likely not specialized on human foods),". Regarding P3 alternative, point B, the relationship could still be neutral if both the flexible individuals and the less flexible individuals have a high proportion of human foods in their diet. The less flexible individuals might achieve this by specializing on human foods, while the more flexible individuals might rely on this or another mechanism. However, you are right in that there could also be a negative correlation outcome, therefore we added P3 alternative 2 to address this: "There is a negative correlation between an individual's flexibility and the proportion of human foods in their diet, potentially because some of the less flexible individuals might specialize on human foods, thus increasing their consumption above that of the more flexible individuals."

Comment 18: I had to read P4 alternative 1 a couple of times to fully understand it due to the negative on it. I think it would be easier to read if you change it to something like "Individual flexibility is not related to having stronger bonds…"

>Response 18: Thank you for this. We changed it to "Individual flexibility is not related to having stronger bonds"

Comment 19: I also think it would help the reader to see a short description on how you will calculate the following variables, as you do with the others: 1. Relatedness for the strongest bond 2. Probability of being an immigrant 3. Dominance rank

>Response 19: We now clarified points 1 and 2 as described in our Response 15. We also now describe how we will calculate dominance in Analysis Plan > P4: "We will also measure aggressive behavioral interactions, as indicated in our ethogram. The outcome of these dyadic interactions will be used to create our index of dominance (wins - losses / wins + losses). "

Comment 20: In the "analysis" sections there is a lot of repetition about how you will perform the GLMMs and the Power analysis. This is a matter of taste, but the sections of the description that are the same can potentially refer to the first time written and avoid repetition. Alternatively, you can write a section about the models and the power analysis and refer to it while describing the analyses for each hypothesis and prediction.

>Response 20: Thank you for your help with the overall flow! We cut out the repetition of these descriptions by referring to their first appearance in subsequent sections. To aid in improving the flow, we also moved the Planned Sample section up to just below the Methods title. Additionally, we learned a better way to do data checking from reviewer comments on a different preregistration and we updated these sections in this preregistration as well (Analysis Plan > Data Checking, as well as the associated R code for each prediction). In going back through the code, we also realized the P3 GLMM needed to use a binomial distribution because the response variable is a proportion so we changed it accordingly.

Comment 21: In the section "P4: flexible = stronger bonds" I have a couple of things to say: First, I am not sure about how you will create the social network. If you plan to use field data, you will not be able to identify all the individuals, as many of them will not be banded. If you use the data from the aviary, you will be sampling a very reduced "community", unless you have a huge aviary to do the study. Also, you describe the power analysis and then say that tit is the same than in P1-P2, so maybe you can exclude the description from here. In the last paragraph of the section P4: flexible = stronger bonds there is a missing reference also, written as (???).

>Response 21: We now describe our methods for creating social networks in much more depth in Response 4 above, and in the Analysis Plan > P4. Briefly, we will collect field data on natural social associations during focal follows. You are correct that we won't be able to identify all individuals, however our hope is that we will be able to band most individuals in a given area. Unbanded grackles will not be included in our calculations of bond strength and its relation to behavioral flexibility. However, we will measure the number of grackles within 10m of the focal individual at the end of the focal follow (group size), to get an estimate of Degree (maximum number of other individuals that the focal subject associated with) where we do not need to have each individual banded.

Regarding cutting out repetition in the power analysis text throughout, we have now done this (see details in Response 20). Sorry about the missing reference - we fixed this (Methods > Dependent Variables > P4).

Reviewed by anonymous reviewer, 2019-03-08 05:05
Comment 22: Here, I have reviewed the preregistered manuscript title 'Is behavioural flexibility related to foraging and social behaviour in a rapidly expanding species' by Logan and colleagues. Overall, I think this is a timely and interesting study that can contribute to the understanding about the evolution of behavioural flexibility. I do find the manipulation of flexibility experience particularly interesting! While I am happy with the overall presentation of the

manuscript (as a pre-registered standard), I do have some comments/suggestions which hope the authors would address them in their final submission.

>**Response 22:** Thank you so much for your feedback! Please see our responses to your comments below.

Comment 23: Missing general background information. Although I know this is a pre-registered manuscript, I would appreciate if authors would provide a brief background information about how hypotheses/ predictions are formed; it is a bit awkward when authors just provided information about more behaviourally flexible birds would differ in their foraging behaviours right at the beginning of the document... A similar comment for the methods; a brief information about what is reversal learning or what the multi-access box is like (at least how many solutions are there??) would be helpful to understand how authors would like to examine flexibility (as opposed to clicking a link that actually related to another registration). For example, what indicate flexibility in literature; 'faster' to reverse preference in reversal learning is measured as the total number of choices/the number or errors/the number of correct choices that a grackle made before reaching a learning criterion?

>**Response 23:** Thank you for helping us figure out what readers need to more easily navigate this preregistration! We have now provided more background information - please see our Responses 2, 5, and 6 for details about our revisions. To address the remaining changes you brought up regarding describing more about the multiaccess box and reversal measures, we made the following changes (in bold):

H1: "Behavioral flexibility (measured by reversal learning **(where they must learn to prefer one of two options that contain food and then reverse this preference)** and switching between options on a multi-access box **(where they must learn to switch to a new option, out of four available options, when an option becomes non-functional)** in aviaries) is related to foraging behavior"

How we measure reversal learning is described in Methods > Independent variables > P1-P4 and P6, but your questions prompted us to provide more detail (changes in bold):

Methods > Independent variables > P1-P4 and P6 > Flexibility 1: "Number of trials to reverse a preference in the last reversal (**in the reversal learning experiment**) an individual experienced (**individuals in the flexibility control group only experience 1 reversal so this data will come from their first and only reversal; individuals in the flexibility manipulation group experience serial reversals until they pass a certain criterion, therefore we will only use data from their most recent reversal**)."

Comment 24: Hypotheses should be presented in a general sense (e.g. 'the level of flexibility in individual would be correlated with their foraging behaviour in the wild') so as to cover different directions of predictions.

>**Response 24:** Good point! We changed H1 to:
"Behavioral flexibility (measured by reversal learning and switching between options on a multi-access box) is related to foraging behavior (measured with focal follows) in wild individuals"

H2 to:
"Behavioral flexibility (measured by reversal learning and switching between options on a multi-access box) is related to social behavior (measured with focal follows) in wild individuals"

H3 to:
"Behavioral flexibility (measured by reversal learning and switching between options on a multi-access box) is related to variation in the use of habitats (measured with a GPS point for each focal follow)."

Comment 25: Additional point to consider: 1) In hypothesis one, if there is no correlation between the flexibility of reversal learning and the number of food consumed or the number of types of habitats used, do authors really think the results are reflecting independent traits? Or both are under the umbrella term of 'flexibility' but reflecting a different 'form' of flexibility? likewise for P1 alternative 2 and alternative 3: would the negative correlation actually help to reflect these flexible behaviours are different 'forms' of flexibility? 2) Hypothesis 1 P2- Have authors thought of how quick would the birds learn not to eat certain food as a measure of flexibility too? That said this may not be ethically manipulated through experiments… 3) Hypothesis 2. Social bonding may not only be assessed in quality but also quantity – the number of 'buddies' they interact with rather than being 'best friends' with everyone. 4) P4 alternative 2 – 'because they frequently change their behaviour and are difficult to form bonds with' - This needs to be measured properly because the change of behaviour is also a form of flexibility though not in social context. 5) P6 alternative – it would also well be individuals prefer certain type(s) of habitats after initial exploration. If this is the case, authors may have to limit or quantify the use of different types of habitats at the initial stage.

>Response 25:

1) There are many, many definitions of behavioral flexibility and we agree that it is important to define what we mean by it. Our definition of behavioral flexibility is discussed in detail in Mikhalevich et al. 2017, but perhaps that wasn't quite clear, so we added:

H1, H2, H3: "see Mikhalevich et al. 2017 for a detailed definition"

We can see how the wording in P1alternative1 was confusing because it indicated that we were treating flexibility as a broad trait that applied across all contexts. What we actually meant, and have now included in the revision, is that we were referring to flexibility as we measured it. If there is no correlation between our flexibility measures and the number of foods eaten, then we can't exclude the possibility that flexibility in a wild foraging context (which we are not measuring) could be independent of the flexibility variables we measured in the aviaries. Nevertheless, this would indicate that in a wild foraging context there is no selection on flexibility the way we are measuring it, which would be necessary according to the hypothesis that rapidly expanding species require flexibility to move into new environment. We are hoping that our manipulation of flexibility (in a separate preregistration) will allow us to determine how independent flexibility is relative to a variety of other variables. In a way, it is difficult for us to give definitive answers about what flexibility is and how it should relate to other traits because this is what our research program is setting out to test. The way we define flexibility, as individuals needing to functionally change their behavior when circumstances change, excludes it from being the same as how we measure foraging behavior (the number of foods eaten and the number of different foraging techniques used). It is possible that they could use their flexibility when foraging by changing their foraging behavior as their environment changes, but we are not measuring this because it would take a much more long-term field investigation to collect this kind of data. We can see how the wording of P1alternative1 was confusing in that it looked like the explanation we were suggesting was the only one possible. We changed the wording as described in Response 10, which we think also addresses your comment here. We further changed P1alternative1 to reflect this discussion (change in bold):
"If there is no correlation, this suggests that flexibility **as we measured it represents** a trait that is not related to the number of foods eaten and foraging techniques used."

2) This is a really interesting avenue and we are able to test this experimentally in one of the inhibition tests using a go no-go experiment (see the inhibition preregistration here: https://github.com/corinalogan/grackles/blob/master/EasyToReadFiles/g_inhibition.md). It involves a rewarded stimulus or a non-rewarded stimulus appearing on a touchscreen. The correct response for the rewarded stimulus is to peck the stimulus and then the food is automatically available for 5s. The correct response for the non-rewarded stimulus is to not peck the stimulus, which results in a short intertrial interval and then the next trial appears on the

screen. In terms of testing this in the field, we have not yet devised a good experimental protocol, but we are open to suggestions!

3) This is a very good point: social bond quantity might be just as important as social bond quality! We have added this to our Analysis Plan > P4. We will count the number of other grackles within 10m of the focal individual at the end of each focal follow. These measures of group size will inform the individual-level measure "Degree" and we will model the relationship between behavioral flexibility and degree. See more details of the changes we made in Response 4 above.

4) Our aim with the various predictions and their alternatives is to provide potential explanations for why that result might arise. However, we are not able to test all of the alternatives to determine whether our potential explanation was supported or not. This would be something we would propose to test in a future study depending on what we find in this study (and then we would know which of our potential explanations to explore). In this case, we are not proposing to measure how frequently they change their behavior in the wild, but rather we are proposing that it is a possible explanation if we find no correlation between flexibility and bond strength. We revised P4alternative2 text to reflect this discussion:
"Flexible individuals **may have fewer affiliates** or be less likely to **regularly affiliate with the same individuals**, potentially because they frequently change their behavior and are difficult **to associate with**. **We are not able to test this alternative in this study, but could propose experimental designs for future research if this alternative is supported by the data.**"

5) Good point that individuals might prefer particular microhabitats. We are now quantifying the microhabitat where each focal follow is conducted. Please see our Response 11 above for more details on how we revised the preregistration to address this.

Comment 26: Independent variables: 1) Flexibility 2. 'no choice' trials are a good way to control the confounding factor 'exploration'. But the independent variable should focus on what the ratio really represents – how do correct and incorrect choices really relate to flexibility? 2) Authors are right to predict that the number of trials to reach a learning criterion in the reversal phase may not necessarily relate to the latency of solve new loci on the multi-access box. But have authors also considered using the number of experience that requires a bird to reach asymptotic performance in the multi-access box as an indicator of flexibility? This is because both are measuring flexibility after a change occurs.

>Response 26:
1) Good catch! This sparked a lively discussion involving lots of whiteboard drawings. In the end, we concluded that actually, the Flexibility 1 measure already controls for exploration because "no choice" trials are NOT included. When we tried variations of the Flexibility 2 measure using mock data, what ended up happening is that we were actually looking at individual differences in exploration by including the "no choice" trials. If we were to look at individual differences in participation, it would remain unclear what these "no choice" trials actually mean for the bird: are they not participating because they are not hungry, or do they understand what the correct option is and they are just frustrated and refusing to participate? In a separate preregistration, we actually measure exploratory behavior in these individuals and examine its relationship with flexibility. Given that we are unsure what Flexibility 2 would be measuring and that we are already specifically measuring exploratory behavior in these individuals, we decided to delete the Flexibility 2 measure from this and the other preregistrations. Thank you for this feedback!

2) Great point. We received a similar comment from a reviewer of the behavioral flexibility preregistration, and we now revised this preregistration in the same way:

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Methods > Independent variables > P1-P4 and P6 > Flexibility 3: we replaced the latency to attempt/solve with the number of trials to attempt to solve a new locus and the number of trials to solve (pass criterion) a new locus.