Behavioral flexibility is similar in two closely related species where only one is rapidly expanding its geographic range

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Abstract

Human modified environments are rapidly increasing, which puts other species in the precarious position of either adapting to thea new challengesarea or, if they are not able to adapt, shifting their range to a more suitable environment. It is generally thought that behavioral flexibility, the ability to change behavior when circumstances change, plays an important role in the ability of a species to rapidly expand their geographic range. To determine whether species differences in range expansion propensity are linked to differences in behavioral flexibility, we compared two closely related species, great-tailed grackles (*Quiscalus mexicanus*; GTGR) and boat-tailed grackles (*Quiscalus major*; BTGR). The former is rapidly expanding their geographic range by settling in new areas, whereas the latter is not. We previously found that GTGR are behaviorally flexible, however not much is known about BTGR behavior, which provides an ideal way to test the hypothesis that behavioral flexibility plays a key role in the GTGR rapid range expansion using the comparative method. We compared behavioral flexibility of GTGR from two populations across their range (an older population in the middle of the northern expansion front: Tempe, Arizona, and a more recent population on the northern edge of the expansion front: Woodland, California) with BTGR from Venus, Florida, to investigate whether the rapidly expanding GTGR are more flexible. We found that both species, and both GTGR populations, have similar levels of flexibility (measured as food type switching rates during focal follows). Our results elucidate that, while GTGR are highly flexible, flexibility in foraging behavior may not be the primary factor involved in their successful range expansion. If this were the case, we would expect to see a rapid range expansion in BTGR as well. This comparative perspective adds further support to our previous intraspecific findings that persistence and the variance in flexibility (rather than population average flexibility)variance play a larger role in the edge GTGR population than in the GTGR population away from the edge. Our research indicates that the hypothesis that higher average levels of flexibility ias the primary facilitator of rapid geographic range expansions into new areas needs to be revisited.

Preregistered Stage 1 protocol: http://corinalogan.com/Preregistrations/gxpopbehaviorhabitat.html (date of in-principle acceptance: 06/10/2020)

Keywords: behavioral flexibility, boat-tailed grackle, Quiscalus major, great-tailed grackle, Quiscalus mexicanus, focal follow, food type, range expansion, comparative approach

Programmatic registered report

This Stage 2 (focused on research question 4) is one of four Stages 2s resulting from one programmatic Stage 1 registered report. A programmatic registered report means that multiple Stage 2 articles result from the one Stage 1. Two of the other three Stage 2s have already been published: @logan2023xpopppcj (research question 1) and @summers2023xpop (research question 3). The third, on research question 2, is currently in review at PCI Ecology: @lukas2025xpopdispersal.

Introduction

Human modified environments are rapidly increasing [@liu2020high; @wu2011quantifying; @goldewijk2001estimating], which puts other species in the precarious position of either requiring the ability to adapt to the~~a~~ new challenges~~area~~ or, if they are not able to adapt, moving to a different area if they are able to [@sol2014urbanisation; @sol2017urbanisation]. Behavioral flexibility (hereafter, 'flexibility'), the ability to change behavior when circumstances change via processing information that becomes available to other cognitive processes [see @mikhalevich_is_2017 for theoretical background], is hypothesized to play an important role in the ability of a species to adjust to new areas and rapidly expand its geographic range [@chow2016practice; @griffin2014innovation; @lefebvre1997feeding; @sol2002behavioural; @wright2010behavioral]. The prediction is that flexibility, along with behaviors such as exploration, and innovation, facilitate the expansion of individuals into completely new areas, and that the role of these behaviors diminishes after an initial adjustment stage [@wright2010behavioral]. This prediction is supported by experimental studies that have shown that latent abilities are primarily expressed when needed [e.g., @taylor2007spontaneous; @bird2009insightful; @manrique2011spontaneous; @auersperg2012spontaneous; @laumer2018spontaneous]. Therefore, the founding individuals who initially dispersed out of their original range should not have unique behavioral characteristics that are passed on to their offspring. Instead, the continuation of a range expansion should rely on flexibility, and potentially behaviors such as exploration, innovation, and persistence. These behaviors should therefore be expressed more on the edge of the expansion range where there have not been many generations to accumulate relevant knowledge about the environment, and expressed more in species that are rapidly expanding their range relative to species that are not.

Whether and how flexibility relates to a rapid range expansion is still an open question [@wright2010behavioral]. To determine whether behaviors are involved, they must be directly measured in individuals in populations across the range of the species, and, ideally, also include cross-species comparisons using the same methods [see a discussion~~,~~ in @logan2018beyond~~,~~ and direct evidence in~~,~~ @logan2024flexforaging~~,~~ on the danger of flexibility proxies]. There is only a small amount of direct evidence that is beginning to answer this question and it suggests that populations on the range edge express certain behaviors more than populations away from the edge. @cohen2020innovation showed that common mynas (*Acridotheres tristis*) in populations on the edge were more innovative and less neophobic with food than individuals in populations away from the edge, while there were no differences in object neophobia. @logan2023xpop found that edge great-tailed grackles (*Quiscalus mexicanus*; hereafter GTGR) were more persistent and had a higher flexibility variance than individuals in a more central population, while there were no differences in average flexibility, innovation, or exploration. Evidence in invasive species in regions where they were introduced also shows that individuals on the edge or in newer populations were faster to eat new foods [house sparrows, *Passer domesticus*; @martin2005taste; @liebl2014living] and were more risk averse [bank voles *Myodes glareolus*; @eccard2022timid] than individuals away from the edge or in older populations. In contrast, invasive spiders (*Cyrtophora citricola*) at newer sites in their non-native range were less exploratory and less bold than spiders at older sites [@chuang2021personality]. Such differences can also vary seasonally: @liebl2012exploratory found that invasive house sparrows closer to the edge of their non-native range were more exploratory than those away from the edge, but only during the breeding season. This suggests that behavior is differentially involved in expanding a range, however flexibility may not play the primary role and the relative expression of the behaviors might be contrary to predictions. Most evidence on this topic comes from invasive species in their non-native range. GTGR are not technically considered invasive because their rapid range expansion is not due to human introductions [see @logan2023xpop for discussion]. However, it is useful to compare GTGR

with invasive species because the range expansion dynamics after arrival appear similar [@chapple2012can].

While some intra-species comparisons of behaviors in edge and non-edge populations exist, we were not able to find inter-species investigations that directly measure flexibility or similar behaviors in closely related species that differ in their range expansion rates. This comparative method is a useful approach because it can serve as a type of natural experiment when testing closely related species that are known to differ in one of the two traits of interest [@davies2012introduction]. Here, we investigate flexibility in two closely related species, ~~great-tailed grackles (hereafter~~ GTGR~~)~~ and boat-tailed grackles (*Quiscalus major*, hereafter BTGR), that differ in their range expansion rates: GTGR are rapidly expanding, while BTGR are not [@dacosta2008historic; @wehtje2003range]. GTGR are highly flexible [@logan2016behavioral; @logan2023flexmanip], but no flexibility data exist for BTGR. Both species are associated with human-modified environments, and are social and polygynous.~~,~~ Both species ~~and~~ eat the same kinds of foods: a variety of human foods in addition to foraging on insects and on the ground for other natural food items [@johnson2001great; @post1992dominance; @post2000boat]. ~~We aimed to compare flexibility, measured as food type switches during focal follows, in two populations of GTGR (an older population in the middle of the northern expansion front, Tempe, Arizona, and a very recent population on the northern edge of the expansion front: Woodland, California) with one population of BTGR in the center of their range (Venus, Florida). We investigated whether the GTGR edge population has higher flexibility averages and variances relative to BTGR and to the older GTGR population.~~

We previously found that GTGR individuals that were faster at reversal learning, a common method to quantify flexibility, had more food type switches during their focal follows [@logan2023xpop] and were faster at switching between options on a puzzlebox [@logan2023flexmanip]. Using food type switches as a measure of flexibility is theoretically similar to using switches between options on puzzleboxes as a measure of flexibility in that individuals need to consider their options and decide when to switch and what to switch to [e.g., @logan2023flexmanip]. Food type switching also more directly reflects whether individuals rely on flexibility when dealing with the challenges in their environment [@wiggins2006diet]. We aimed to compare flexibility, measured as food type switches during focal follows, in two populations of GTGR (an older population in the middle of the northern expansion front, Tempe, Arizona, and a very recent population on the northern edge of the expansion front: Woodland, California) with one population of BTGR in the center of their range (Venus, Florida). The comparison of food type switching across these three populations first allows us to confirm that flexibility is generally high across the range of GTGR, which is what we previously found using reversal learning, and second to determine whether BTGR have lower flexibility than GTGR in general or GTGR at the edge. ~~We investigated whether the GTGR edge population has higher flexibility averages and variances relative to BTGR and to the older GTGR population.~~

Determining whether GTGR are more flexible will allow us to accumulate more evidence to determine whether there is support for the hypothesis that flexibility might play a key role in the GTGR rapid geographic range expansion. Alternatively, if BTGR and GTGR perform similarly, this would suggest that other abilities, ~~either~~ ecological, behavioral, or physiological, may play a larger role in restricting the BTGR range expansion.

PREREGISTERED RESEARCH QUESTION

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Are there differences in behavioral traits (flexibility, innovation, exploration, and persistence) between boat-tailed and great-tailed grackles?

Prediction: If behavior modifications are needed to adapt to new locations, then GTGR~~great-tailed grackles~~, which are rapidly expanding their geographic range [@wehtje2003range], will have higher averages and/or larger variances than BTGR~~boat-tailed grackles~~, which are not rapidly expanding their range [@wehtje2003range], in at least some behavioral traits (behavioral flexibility: speed at reversing a previously learned color preference, innovativeness: number of options solved on a puzzle box, exploration: latency to approach/touch a novel object, and persistence: proportion of trials participated in). Higher averages in behavioral traits indicate that each individual can exhibit more of that trait. If resources are regularly distributed in time and space, perhaps BTGR~~boat-tailed grackles~~ require less flexibility when visiting these resources and attend less to their temporal availability or the individual's food preferences. Perhaps the problems BTGR solve do not require much exploration or persistence. Lower variances in behavioral traits indicate that there is less diversity of individuals in the population, which means that there is a lower chance that some individuals in the population would innovate new foraging techniques and be more flexible, exploratory, and persistent.

Prediction alternative 1: Human-modified environments are suitable habitat for GTGR and BTGR and the amount of human-modified environments has been and is increasing. If the original behaviors exhibited by these species happen to be suited to the uniformity of human-modified landscapes, then averages and/or variances of these traits will be similar in the GTGR~~great-tailed~~ and BTGR ~~boat-tailed grackles~~ sampled. This supports the hypothesis that, because these species are closely associated with human-modified environments [@wehtje2003range], which may be similar across the geographic range, individuals in new areas may not need to learn very much about their new environment: they can eat familiar foods and access these foods in similar ways across their range (e.g., fast food restaurant chains likely make the same food and package it in the same packaging in Central and North America, outdoor cafes and garbage cans also look the same across their range).

Prediction alternative 2: If BTGR have higher averages and/or larger variances in the behavioral traits measured, this could indicate that perhaps these traits are not the primary facilitators of the GTGR~~great-tailed grackle~~'s rapid geographic range expansion. Alternatively, perhaps these species differ in a life history variable that restricts the BTGR from expanding, or there is some geographic feature that prevents the BTGR from rapidly expanding its range.

METHODS

Updates and changes to the preregistration

This study began as a preregistration, which received in principle acceptance at PCI Ecology in 2020: https://github.com/corinalogan/grackles/blob/master/Files/Preregistrations/gxpopbehaviorhabitatPassedPreStudyPeerReview16Dec2021.pdf. The preregistration contains the pre-planned analyses. Here, we report the rationale for the ways in which we conducted the study differently from the plan, and then describe the methods we used to obtain the results.

Changes made in the middle of data collection

1) After the first BTGR field season in 2022, we **stopped the aviary experiments and switched to measuring reversal learning in the wild**. We received permission to make the change in Feb 2023 from the PCI Ecology Managing Board and from the recommender, Esther Sebastián González, who incorporated it into our in principle acceptance. The reason for stopping the aviary experiments is that we discovered that BTGR are less robust to captivity compared to ~~very different from~~ GTGR. BTGR seem to be having health issues as indicated by multiple deaths in the wild (including two that we witnessed), which unfortunately also carried over to the aviaries where two of the six aviary birds died. This meant that we were not able to obtain measures for three of the four variables we planned on investigating: innovation, exploration, and persistence. These three variables would have required aviary testing to conduct a comparison between the two species because this is the setting in which the GTGR were tested. We planned to measure reversal learning in the wild using an automated feeder experiment that we were planning on running in GTGR [as in @logan2022manyindividuals]. After data collection started in November 2023, and after extensive work in trying to get the automated feeders to function, we discovered in January 2024 that the feeders were not usable. Therefore, we have no data on reversal learning in BTGR.
2) We previously measured flexibility in the wild in GTGR using behavioral observations, which provided us with a way to collect comparable data in BTGR in the wild. Therefore, we **added a measure of flexibility**: switching between food types during focal follows. Before we started collecting this data using focal follows, we received permission to make the addition in Sep 2023 (before we started collecting this data) from the recommender, Esther Sebastián González, who incorporated it into our in principle acceptance.

Sample

GTGR were caught in the wild in Woodland and Sacramento, California, and Tempe, Arizona; and BTGR were caught in the wild in Venus and Lake Placid, Florida. We aimed to bring adult grackles, rather than juveniles, temporarily into the aviaries for behavioral choice tests to avoid the potential confound of variation in cognitive development due to age, as well as potential variation in fine motor-skill development (e.g., holding/grasping objects; early-life experience plays a role in the development of both of these behaviors; e.g., Collias & Collias (1964), Rutz et al. (2016)) with variation in our target variables of interest. After switching away from the aviary tests in BTGR (see above), we continued to focus our study on adult individuals, however, it was not usually possible to discern female adults from juveniles. Adult GTGR were identified from their eye color, which changes from brown to yellow upon reaching adulthood (Johnson and Peer 2001). Juvenile male BTGR were identified by their dark brown feathers (rather than shiny black as in the adult males), and we were not able to distinguish between adult and juvenile females because they both have light brown feathers and brown eyes. We applied colored leg bands in unique combinations for individual identification. For some BTGR individuals, one leg band contained an RFID/PIT tag (Eccel Technology Ltd, https://eccel.co.uk/). GTGR were trapped in the wild using mist nets, walk-in traps, and bownets, and BTGR were trapped using walk-in traps. The bird was then processed by collecting biometric measurements, and, in the case of GTGR, also feathers and blood. After processing, the bird was released back into the wild either immediately (all BTGR and many GTGR), or after

participating in behavioral tests in aviaries (many GTGR; these data are reported in other publications).

We first collected data on the GTGR populations [@logan2024flexfor], where we had a large sample because we were collecting data for many other purposes as well. Whereas, for the BTGR, we were collecting data for this one purpose of comparing food type switch rates. Therefore, we set the minimum sample size (n=6) for the BTGR to a known sample size in the GTGR in which we were able to distinguish differences between populations.

Focal follow protocol

To calculate the probability of switching between food types in BTGR, wWe used the GTGR foraging behavior focal follow protocol fromdescribed in a previous study [see @logan2024flexfor] for the data collection for both GTGR and BTGR, where we measured flexibility as the probability of switching between food types [see @altmann1974observational for a general description of the focal follow method]. 10 min focal follows were conducted between 9:00 and 16:00 in which all food types were recorded (both species), as well as the foraging bout start and end times (BTGR; Table 1). BTGR focals could only begin if the individual was in the foraging state (whereas, GTGR focals could begin any time because we collected non-foraging data as well). This meant that we maximized our focal time by ensuring foraging behavior would be present in the focal (rather than starting a focal when they were sitting in a tree and maybe would not forage at all for the next couple of hours). We were careful to keep a respectable distance of at least 10m unless the bird approached us [using flight initiation distances in @moller2008flight and @eason2006factors as a baseline]. If the focal individual showed alarm behaviors due to the observer (e.g., alarm calling at the observer), the focal was stopped and began again on a different day. We determined which individual to follow next by using the order in which the birds were caught. Of the color marked individuals at a given location that were currently in a foraging state, we conducted the focal on the one who was next on the list. If some individuals already had focal follows, we prioritized following those individuals next on the list who had fewer focal follows. If a focal was conducted in a particular location on a given day, no further focals could be conducted at that location on that day to avoid pseudoreplication of foraging behavior among individuals. The BTGR field site consisted of two distinct areaslocations: one at Lake June Park, a large 16 hectare park that we divided into five sections such that each counted as a separate location (NE, SE, SW, NW, and center), and the other was at a crossroads where a BP gas station was on one side of the road and counted as a location and a Circle K gas station was on the other side of the road and counted as a separate location. All BTGR and most GTGR data were collected using a voice recorder (Voice Memos app on an iPhone) and later transcribed into the datasheet in Google Sheets (https://www.google.com/sheets/about/). Some GTGR data were first entered into the program Prim8 Software (https://www.prim8software.com/), and then transcribed into the Google Sheet.

The GTGR focal follow protocol was modified in three ways to accommodate that we were only interested in collecting foraging behavior in BTGR, in comparison towhereas, in GTGR where, we collected data on other behaviors as well. The BTGR protocol differed in the following ways:

 - End each focal when the bird goes out of view and start a new focal when they come back in view (rather than allow "out of view" for up to 5 min per focal). This omits the problem of trying to account for what might have happened in the time out of view when running the analysis.

- No minimum of 3 weeks between separate focal follows because we are only looking at food type switch rates and these are, by definition, less autocorrelated with time because a switch could only occur within the same focal follow. This will still match what occurred with the GTGR data because, while the aim was to conduct a 10 min focal follow, many ended early because the bird went out of view and was not findable again that day. In these cases, the observer would continue to seek the bird out on consecutive days to obtain the rest of the 10 min to ~~and~~ finish this one focal follow. A study on dolphins (*Tursiops* cf. *aduncus*) found that focal follow data that was separated by 10.5 min was functionally independent [@karniski2015comparison]. Therefore, changing the 3 week spacing minimum to 1 day (until 4 focal follows were reached and then spacing them 1 week apart after) should be enough temporal distance to assume independence of the data points.

- Collect data only on the~~the How (how did the bird obtain the food, e.g., dunk, dig, gape) and~~ "What" (what did the bird eat, e.g., fries, grains, insect) categories that were found in the GTGR and add more food types ~~(the What category) and/or foraging techniques (the How category)~~ for the BTGR as needed. We also recorded the latitude and longitude of the location of the grackle at the end of the focal, as in the GTGR protocol.

We set the BTGR minimum sample size to the average number of focal follows obtained from GTGR in @logan2024flexfor: we aimed for a minimum of 4 focal follows per bird (conducted on separate days between 09:00 and 16:00, attempting to counterbalance mornings and afternoons). The GTGR in the flexibility manipulated condition in @logan2024flexfor had~~We set the minimum sample size at 6 BTGR with~~ an average of 3.2 focal follows per individual. Therefore, we used a minimum of 4 focal follows per BTGR individual (we rounded 3.2 up to the next whole number), ~~which was the number of GTGR in the flexibility manipulated condition in @logan2024flexfor and their average number of focals per bird~~. When we analyzed food type switching in the two GTGR populations in @logan2024flexfor, we were able to detect differences in food type switching between individuals in the flexibility manipulated (n=6) and control (n=7) conditions. Therefore, we set the BTGR minimum sample size at 6 for each population.~~Using this sample size, we were able to detect differences between the manipulated and control conditions in GTGR~~ [@logan2024flexfor]. This matched with a power analysis in @logan2024flexfor (in the section: Ability to detect actual effects > Scenario 2) on a very similar model, where we found that we could reliably detect large and medium effects with a sample size of 8 in the smallest population. The full experimental protocol is online at: https://docs.google.com/document/d/1WK6oR04LR1Q2CGXgICvUNN28MWlM-3o2U66lanDv-lc/edit?usp=sharing.

While methods for BTGR focal follows used the same protocol as for GTGR, there were 8 BTGR focal follows that were exceptions: they either occurred less than a week after the last focal follow (for those birds who had at least 4 focal follows, focal follow numbers 5+ should have been spaced apart by at least 1 week), or occurred on a day when another focal follow was also conducted at the same location. Across all of these 8 exceptions, only 1 food item was taken and no food type switches occurred. The data from these 8 focal follows therefore did not match the filter criteria, and are not part of the analyses.

Only those focal follows that contained data on food types, including unknown food types, were included in the analyses. This made it so focal durations were balanced between the species. In

BTGR, focals only started if a bird was foraging, whereas data in addition to foraging data were collected for GTGR and their focals could start no matter what behavior they were engaging in.

Table 1. The foraging section of the GTGR ethogram that was used for (and added to) BTGR data collection. Two new food categories were added that were uniquely seen being eaten by BTGR.

Foraging behavior	Behavior	Description
HOW is the bird searching for food? (foraging technique)	Flip	Flipping over objects
	Lift / nudge	Lifting or nudging objects with bill
	Pick up	Pick up object
	Dig	Digging in ground with bill or feet
	Sweep	Sweeping head back and forth (i.e., actually sweeping the bill across the substrate)
	Gape	Using gaping bill to search through substrate
	Extract	Extracting from a substrate (didn't show up in the GTGR techniques, but was on their ethogram)
	Stalk / catch	Lowers body posture to be parallel to ground to stalk/catch prey from air, from ground, from tree, etc.
	Share	Food is shared with the focal bird by another bird
	Break	Break object into pieces
	Dunk	Dunk object in water or other liquid substance
	Theft	Steal object from another bird's bill/feet or near its body
	NEW for BTGR: Drink	When the item they are foraging on is liquid (e.g., oil water, soda, etc.)
	NEW for BTGR: Pull	Pull on an item until it breaks off or is otherwise obtained (e.g., a leaf off of a branch)
WHAT are they eating?	23 categories (add more if needed)	Fry, lizard, unknown, grains (pizza, noodles, bread, rice, chips, rice krispies, crackers, pretzel, muffin, cookie, popcorn), insect, rock, cat food, worm, seed, food crumbs, vegetation (leaf, grass, branch, flower, moss), fruit (flesh, peel), bird poop, candy, vomit, misc. trash (paper, condiment packet), soil (clay, dirt), condiment, carcass, chicken (bone, skin), peanut, mulch. New for BTGR: oil, cheese
FORAGING (state)	Record the	When the bird is searching for food (have to be touching

	start and stop times for each foraging bout	what they are searching through), pecking in the ground, and/or eating food. If the bird pauses foraging behavior for up to 10s, keep this state going. If it pauses for >10s, end the foraging state

Open data

The data and code are published in the Knowledge Network for Biocomplexity's data repository [@logan2024xpopbtgrdata].

Analyses

We did not **exclude** any data. When there were **missing** data (e.g. if a bird did not have any food type or food switching data), then it was not included in the analyses. Following procedures in @mcelreath2020statisticalrethinking2020, we constructed a **hypothesis-appropriate mathematical model** for the response variable that examines differences in the response variable between sites. The single population of BTGR in Venus, Florida, wasere considered a site and we examined differences between BTGRthem and each of the two GTGR sites. For each focal follow, we calculated the number of switches between food types that occurred and the total amount of time that the bird was observed (using seconds as the unit of time because that was the resolution of data that we collected). We used a rate to be able to directly compare the results among individuals, regardless of any differences in how much time they were followed for. We summed both measures across focal follows to have one data point per bird. This model takes the form of:

$switches_{i}$ ~ Binomial($totaltime_{i}$, p),

logit(p) ~ a_{i}[$site$],

where $switches_{i}$ is the sum of the number of times individual, i, changed food types within focal follows that contained food type data, $totaltime_{i}$ is the number of seconds individual, i, was observed across all of its focal follows that contained food type data, p is the probability of switching to a different food type per second, and a_{i} is the intercept (one per level of $site$: GTGR Arizona, GTGR California, and BTGR).

The model was the same for analyzing the variance in switch rates except b[individual] and an extra prior were added as follows:

logit(p) ~ a_{i}[$site$] + b[individual]

which gives the probability of switching food types per bird. We specified the priors for this as:

d[individual] ~ dnorm(0,σ[site]),

σ[site] ~ dexp(1),

where σ[site] gives the average variance across individuals per site.

We then performed pairwise contrasts to determine whether there were differences between sites, concluding that there is a difference if 89% of the difference between two sites is on the same side of zero [following @mcelreath2020statistical~~rethinking2020~~]. The Bayesian approach first estimates for each population the most likely distribution of values given the observed sample, and only in a second step do we compare these estimated distributions. The important part here is that the sample size in each population exceeds a given minimum to reliably estimate the distribution for this population. Differences in sample size across populations do not matter with this approach [@mcelreath2020statistical].

We ran these analyses in R (current version 4.0.3; R Core Team (2017)) and used the following R packages: rethinking [@mcelreath2020statistical~~rethinking2020~~], cmdstanr [@cmdstanr], and dplyr [@dplyr]. Our code is available at @logan2024xpopbtgrdata.

Study design table

Table 2. The Stage 1 did not have a study design table because it was written before PCI RR existed and was only transferred to PCI RR in 2025. Therefore, we include a study design table for Stage 2.

Question	Hypothesis	Sampling plan	Analysis plan	Rationale for deciding the sensitivity of the test for confirming or disconfirming the hypothesis	Interpretation given different outcomes	Theory that could be shown wrong by the outcomes	Result
Are there differences in flexibility between boat-tailed and great-tailed grackles?	Reversal learning average and variance: GTGR > BTGR	Bespoke Bayesian analysis in Logan et al. 2024 showed that we were able to detect differences between	Bayesian model: Response: Number of food type switches per total number of seconds of	Contrasts will determine whether the populations differ from each other. We will conclude that there	GTGR are more flexible than BTGR, and this could be a main facilitator of their range expansion	Non-behavioral traits are primary facilitators of rapid range expansions (Summers et al. 2023)	

	Reversal learning average and variance: GTGR = BTGR	populations with sample sizes of 6 and 7. We are using this exact analyses in the current study	observation time for each bird Explanatory: Population (BTGR, GTGR Arizona, GTGR California) *(See Analyses section for more details)*	is a difference if 89% of the difference between two sites is on the same side of zero	The original flexibility exhibited by these species happens to be suited to the uniformity of human-modified landscapes	Flexibility facilitates adapting to environmental change (see references in introduction)	There were no population or species differences in reversal learning
	Reversal learning average and variance: GTGR < BTGR				Flexibility is not the primary facilitator of the GTGR range expansion	Flexibility facilitates adapting to environmental change (see references in introduction)	

Results

The dataset consisted of 8 BTGR and 76 GTGR (54 in Arizona, 22 in California) who had data that involved eating at least one food type. We met our minimum sample size of at least 6 individuals in each population and can therefore assess whether a given population is different from the others. A total of 36 food types across all sites were documented. The BTGR took a total of 14 food types, including cheese and oil, which the GTGR did not have. The Arizona GTGR took 20 food types, including lizard, bird poop, candy, vomit, condiment, and carcass, which the other populations did not have. The California GTGR took 15 food types, including mulch, which the other populations did not have.

There were **no strong differences in the AVERAGE probability of switching** among food types per second between BTGR and either population of GTGR, nor between the two GTGR populations (Figure 1; contrasts: GTGR AZ-BTGR: mean difference per second=-0.0004, sd=0.0003, 89%CI=-0.001-0.0001; GTGR CA-BTGR: mean=-0.0002, sd=0.0004, 89%CI=-

0.001-0.0004; GTGR AZ-GTGR CA: mean=-0.0002, sd=0.0003, 89%CI=-0.0007-0.0002). This supports Prediction 6 alternative 1.

There were also **no strong differences in the VARIANCE of the probability of switching** among food types per second between BTGR and either population of GTGR, nor between the two GTGR populations (Figure 1; contrasts: GTGR AZ-BTGR: mean difference per second=-0.16, sd=0.61, 89%CI=-0.99-0.76; GTGR CA-BTGR: mean=0.13, sd=0.73, 89%CI=-0.94-1.28; GTGR AZ-GTGR CA: mean=-0.29, sd=0.45, 89%CI=-0.97-0.37). The variances from the raw data for food switches per minute for each site were 0.01 for BTGR, 0.12 for GTGR Arizona, and 0.14 for GTGR California (note that the Bayesian model accounts for any potential differences in variance because it compares the distributions of data points between all populations). This supports Prediction 6 alternative 1.
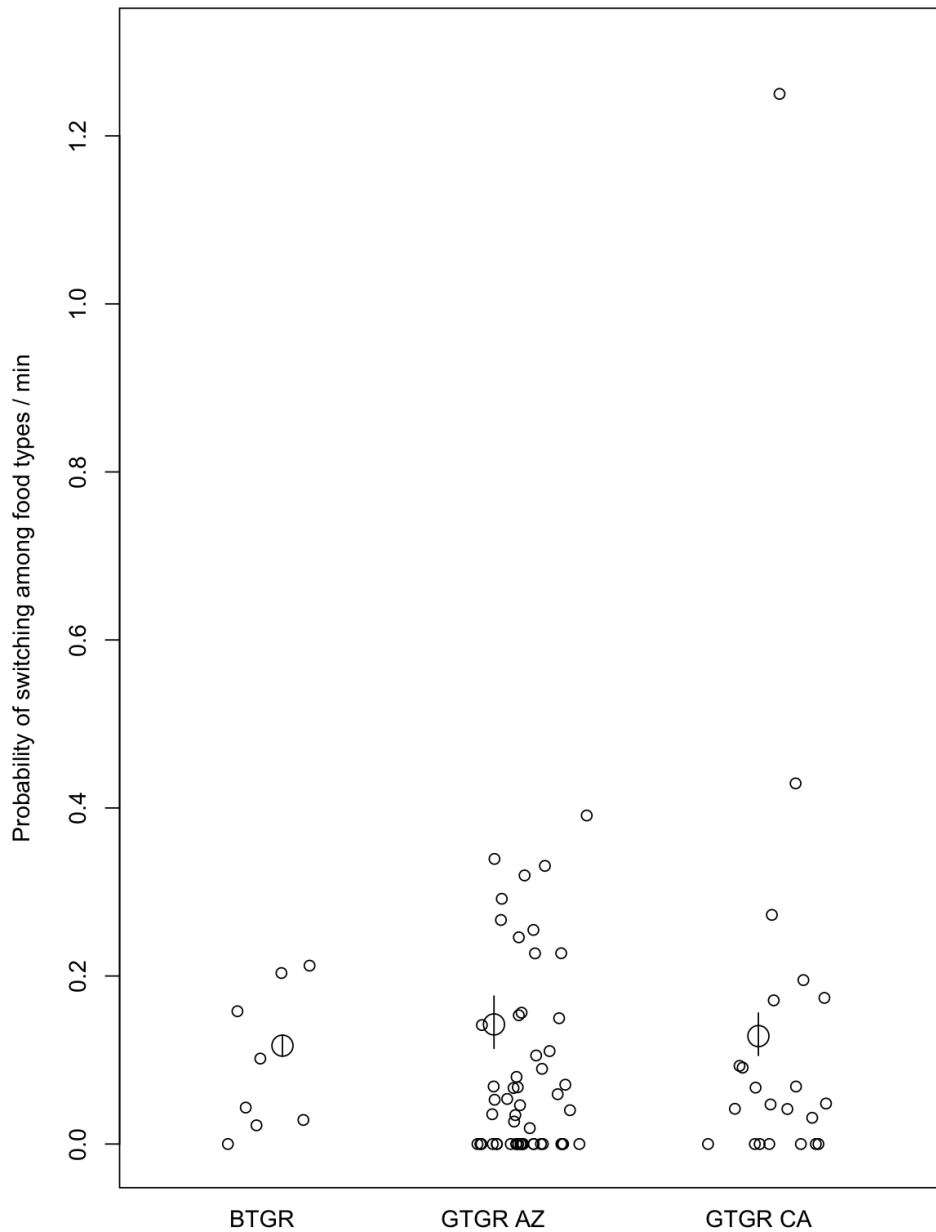
Figure 1. The probability of switching among food types per minute for the BTGR (n=8), and GTGR in Arizona (n=54) and California (n=22). The small circles are the data points per individual and the large circles are the estimated means with their 89% compatibility intervals represented by the vertical lines.

Discussion

There were no strong differences in flexibility (averages or variances), measured as food type switching during focal follows, between BTGR and GTGR. This converges with the small amount of evidence we were able to obtain from the reversal learning tests in the aviary from

two BTGR individuals: their reversal learning speeds were well within the range of the GTGR reversal learning speeds [@logan2023xpop]. We know that GTGR are highly flexible relative to other species [@logan2016behavioral], therefore the similar levels of flexibility between the two species indicates that BTGR are also highly flexible.

We found~~That there was~~ no strong difference~~s~~ in flexibility, measured as food type switching, between the two GTGR populations, which supports our finding that there was also no difference in the average flexibility based on reversal learning between these populations [@logan2024flexfor]. This provides additional evidence that ~~further validates the use of~~ food type switching is likely measuring the same trait as reversal learning and therefore~~as~~ a valid measure of flexibility ~~is valid because there was also no difference in the average flexibility based on reversal learning [@logan2024flexfor]~~. @logan2024flexfor did find a difference in the reversal learning flexibility variance between the GTGR populations and, while we found no strong difference in the variance when using food type switching probabilities, the California population, ~~which is~~ close~~st~~r to the northern edge of the range, had higher variances, which is in the same direction as what @logan2023xpop found.

Unfortunately, we were not able to obtain comparable exploration, innovativeness, and persistence data on the BTGR to understand whether one or more of these behaviors could relate to species differences in range expansion rates. The few data we were able to obtain from BTGR in the aviaries were analyzed in @logan2023xpop and suggest that BTGR are less innovative (n=4) and less persistent (n=5) than both GTGR populations, while having similar levels of exploration (n=5) as the California GTGR and being less exploratory than the Arizona GTGR. Indeed, the California GTGR on the northern edge of their range are more persistent than the Arizona GTGR population, though there were~~with~~ no strong differences in innovation or exploration. This lends more support to persistence as the behavior that might play a larger role in the range expansion of the already highly flexible GTGR. However, this is speculative due to the small BTGR aviary sample sizes. More research is needed to make robust comparisons between the two species on these other behaviors.

The lack of a difference in flexibility between the two species suggests that the original behaviors exhibited by these species happen to be suited to the similar challenges~~uniformity~~ of human-modified landscapes where both species are found. This supports the hypothesis that, because these species are closely associated with human-modified environments [@wehtje2003range; @summers2023xpop; @post1992dominance; @post2000boat], which may be similar across the geographic range, individuals in new areas may not need to learn much about their new environment. They can eat familiar foods and access these foods in similar ways across their range. For example, fast food restaurant chains likely make the same food and package it in the same packaging across North America, and outdoor cafes and garbage cans also look similar across their range. It is possible that environmental, rather than behavioral, variables play a larger role in restricting the BTGR range expansion. @summers2023xpop found that, between 1979 and 2019, BTGR were present in primarily warm, wet, coastal habitats and this did not change over time. The range they expanded into during this time was made suitable to them likely due to climate change. In contrast, GTGR

shifted to using more urban, arid habitats over this time, which suggests that behavior could be a key facilitator involved in their range expansion. @maclean2017 found a similar result using a metaanalysis: range shifts were associated with habitat breadth. Because urban environments represent a rapidly increasing category of environmental change [@liu2020high; @wu2011quantifying; @goldewijk2001estimating], our observations highlight the importance of differentiating between whether a behavior might be linked to the ability to live in urban environments versus the ability to expand into new habitats. Flexibility is potentially linked to living in urban environments, but not necessarily to the ability to expand into novel habitats.

In conclusion, the evidence that two closely related species with similar levels of flexibility, but different range expansion rates does not support the hypothesis that flexibility is the primary facilitator of rapid geographic range expansions into new areas. This does not rule out that flexibility might be a needed variable to rapidly expand a range, as shown by GTGR who have high average levels of flexibility on and away from the edge, but just because it is present, does not mean that the species will expand their range, as shown by the BTGR. However, Iit appears that other behaviors, such as persistence [@logan2023xpop], innovation, food neophobia [@cohen2020innovation; @martin2005taste; @liebl2014living], exploration [@chuang2021personality], and risk aversion [@eccard2022timid] may play a more primary role in expanding the edge of the range further. This indicates the importance of investigating multiple behaviors in each species of interest to determine what, if any, role they play and in what direction.

Ethics

This research is carried out in accordance with permits from the:

1. US Fish and Wildlife Service (scientific collecting permit number MB76700A-0,1,2)
2. US Geological Survey Bird Banding Laboratory (federal bird banding permit number 23872)
3. Arizona Game and Fish Department (scientific collecting license number SP594338 [2017], SP606267 [2018], SP639866 [2019], and SP402153 [2020])
4. Institutional Animal Care and Use Committee at Arizona State University (protocol number 17-1594R)
5. Institutional Animal Care and Use Committee at the University of California Santa Barbara (protocol number 958)
6. Institutional Animal Care and Use Committee at Archbold Biological Station (protocol number ABS-AUP-033-R)
7. California Department of Fish and Wildlife (scientific collecting permit [specific use] number S 192100001 19210 001)
8. RegionalSan (access permit number AP 2021-01)
9. Florida Fish and Wildlife Conservation Commission (scientific collecting permit number LSSC-21-00050)

Author Contributions

Logan: Hypothesis development, data collection (BTGR focal follows and field work), data analysis and interpretation, write up, revising/editing, materials/funding.

McCune: Method development, data collection (Arizona and California focal follows and field work), data analysis and interpretation, revising/editing.

Rowney: Data collection (Arizona field work), revising/editing.

Lukas: Hypothesis development, data collection (BTGR field work), data analysis and interpretation, write up, revising/editing.

Conflict of Interest Disclosure

We, the authors, declare that we have no financial conflicts of interest with the content of this article. CJ Logan and D Lukas are Recommenders at PCI Registered Reports, and CJ Logan is also on the Managing Board.