Round #1

Dear Mr. Sevchik, Dear Dr. Lukas,

thank you very much for submitting your preprint "Investigating sex differences in genetic relatedness in great-tailed grackles in Tempe, Arizona to infer potential sex biases in dispersal" to PCI Ecology. It is great to see preregistrations leading to preprints.

Two referees have reviewed your work and I agree very much with their points which you will find below. I would like to highlight that both referees have issues with some of the presentation, for example, the end of the abstract. I agree very much and would suggest that you reformulate as suggested. This holds for the entire text.

Both referees feel that there is some need for more biological and behavioural context and some methodological questions are also raised.

I suggest revising your preprint in light of the referees' comments, accompanied by a detailed response to their criticism. I am looking forward to receiving a revised version of your preprint.

Sincerely yours, Emanuel A. Fronhofer

Reply: Thank you for checking our article in such detail and for providing this helpful feedback! The suggested changes have helped us improve our manuscript. We reply to each comment below. In particular, we changed the presentation to focus on what can be answered with these analyses, rather than speculating about the potential broader implications.

Comment E.1: The first referee also has some points related to visualization and I find myself in agreement. I suggest replacing Fig. 1 with a 2-panel figure that shows males and females, for example. The figure caption should also include a quantitative and not qualitative statement ("small number of random draws").

Reply E.1: We changed figure 1 according to the suggestions. The revised version is:

>Figure 1: Females are more related than expected by random chance, whereas males are not. a) In less than 4% of 10,000 repetitions is the average relatedness among 37 randomly drawn individuals (of both sexes) as high as or higher than the observed relatedness among the 37

females in our sample. b) In contrast, average relatedness among 15 randomly drawn individuals (of both sexes) is higher than the observed relatedness among the 15 males in our sample in 38% of 10,000 draws.

Comment E.2: Along similar lines of thought, I was wondering whether Fig. 2 could be adapted to actually show what is described in the paragraph above.

Reply E.2: We added a new figure (Fig. 3) to show the result that closely related females are found closer to each other than random pairs of females, while the few closely related males are not found near each other.

>Figure 3: The geographic distance among dyads of closely related individuals (relatedness of 0.125 or higher; light circles) compared to the distance among dyads of unrelated individuals (coloured bars). a) Among females, closely related individuals were trapped at locations near to each other (median distance indicated by dotted grey line), with eleven of the twelve closely related female dyads at distances as near as or nearer than the median of unrelated female dyads (vertical black line). b) In contrast, only one of the three closely related male pairs was trapped at locations that were as near as or nearer than the median distance among the unrelated males (vertical black line). The distances among the closely related males were about three times larger (median indicated by dotted grey line) than the distances among closely related females.

Comment E.3: Finally, for readers who are less familiar with the methods you use, I would suggest explaining why relatedness can be negative.

Reply E.3: We added the explanation that, with this estimator, individuals are classified as **related** if they share more genetic variants (alleles) than expected by chance given the frequencies of variants in the population (relatedness: R>0) and as **unrelated** if they share as many ($R \approx 0$) or fewer genetic variants than expected (R<0). We realized that, in general, it might be helpful to add more information on the particular approaches and their interpretation because the methods are at the end of the manuscript and some readers might not be familiar with these techniques. We therefore expanded the first paragraph of the Results section to address this.

>Results > Genotyping: We generated single-nucleotide polymorphism (SNP; where at a given position in the genome two different bases, alleles, can occur) genotypes for 57 individuals from our study site in Arizona (we excluded 5 individuals later, see Deviations from the Preregistration for details). We retained 635 SNPs. Data was missing for 2.7% of all alleles (individuals missing information for either one or both of their chromosomes for that particular

position), with no individual or SNP showing a particular underrepresentation of information. All SNPs had 2 alleles and the observed heterozygosity (individuals carrying one copy each of the two bases) was 0.48, slightly higher than the heterozygosity expected in a population with the same allele frequencies and random mating. The increased heterozygosity potentially reflects that inbreeding is rare, likely because individuals of one sex disperse prior to breeding. The probability of identity for siblings, the chance that two siblings will show the same genotypes given the allele frequencies across these 635 loci and random mating among individuals, is less than 10 to the power of minus 139. We used the frequencies of the alleles at these SNPs to calculate relatedness among pairs of individuals, with individuals being classified as related if they share more alleles than expected by random chance given the frequencies of variants in the population (relatedness: R>0) and as unrelated if they share as many (R≈0) or fewer genetic variants than expected by chance (R<0).

Comment E.4: Minor points: line 15: "were hatched" should be "hatched"

Reply E.4: Thanks for catching this! We made the change.

Comment E.5: lines 136-143: Please correct brackets for references (See also lines 302-305.).

Reply E.5: Another good catch! We made them consistent now.

Comment E.6: line 164: Please use a mathematical expression for this. figures: "Figure X" is always repeated twice. Please correct this.

Reply E.6: Sorry for the formatting error - this only occurs with the PDF version, which is why we missed it. We now corrected the error.

Reviews

Reviewed by anonymous reviewer 1

The study of avian life history traits has fuelled many investigations in the field of evolutionary ecology, providing invaluable data and analyses on many topics such as evolution of trade-offs, parent-offspring conflicts, evolution of senescence, adaptation to a changing environment, etc..

There is one fundamental trait however, for which we still know relatively little in birds, and that is dispersal and in particular natal dispersal. This is because measuring the distance between place of birth and place of reproduction is quite another challenge, on the field, than counting the number of eggs or monitoring nestling survival. In many species, capture-mark-recapture analyses encompassing nestlings and breeding birds is not feasible at a relevant scale to study natal dispersal, and therefore, we still have very poor knowledge on the distribution of natal dispersal in both sexes. In this context, it is interesting to collect new data on dispersal in a variety or birds, with different mating and social systems, as this will help, in the end, in understanding the factors influencing dispersal.

This study aims at gaining insight on dispersal in male and female great-tailed grackles, by comparing the genetic relatedness among males and females in a breeding site of Arizona.

The manuscript is very clearly written and makes an enjoyable read. I do however have two major concerns that could heavily influence the data interpretation.

Reply: Thank you for your feedback! We realize that some of the interpretations we made were beyond the scope of what we can address within this particular setup, and that we did not include information that was relevant to explain our study fully. We explain below the changes we made.

Comment R1.1: The authors choose to place their study in the general context of the resource-defence based monogamous mating system in many bird species which predicts females are more philopatric than males. Since the mating and social system of the focal species are different from this mating system described in many bird species and leading to this prediction of higher philopatry in females, the authors believe that their study can 'offer an opportunity to determine if and how these differences might influence the dispersal behaviour of both males and females', and this is where I think the authors are getting a bit carried away. With empirical data on one species in one population, the authors will at best be able to provide a useful example on a different mating and social system which will provide insight into whether dispersal in the focal species provides a counter-example to the classic prediction for monogamous species. I appreciated the effort to outline 4 different hypotheses in the introduction, leading to four different patterns of comparative dispersal in this species, however, an important caveat preventing the simple test of these four hypotheses is that the genetic relatedness data gathered in this study, although very valuable, does not allow to decipher between the effects of natal dispersal and breeding dispersal, yet the hypotheses outlined concern one or the other. In particular, the main hypothesis clearly states a prediction about sex differences in natal dispersal while Alternative hypothesis 3 relies on the expectation of high breeding dispersal. Since relatedness among individuals will result from both natal and breeding dispersal, I do not think that the data obtained can bring clear interpretations on either of the two processes. Note that the predictions made in L92-L105 at the end of the introduction are very clear and logical, yet they do not mention the main and alternative hypotheses, which is in a way a demonstration that these predictions do not have a direct and simple link with the

hypotheses. This does not mean that this study is not interesting, but it does mean that the general framework may need a rethink, and the interpretation and conclusions should be toned down and address this issue of natal versus breeding dispersal. While the discussion is presently cautious, the conclusion of the abstract on the role of reduced resource competition in determining female philopatry is very muchspeculative, and should be presented as such. I would reformulate the sentence L30 about what the results 'show' (at least change to 'suggest').

Reply R1.1: As we were writing up this post-study manuscript, we felt that we needed to add more framing to the hypotheses and predictions we made in the preregistration. We therefore added information on the potential pathways through which various factors might change dispersal patterns. Your comment correctly points out that we actually cannot make any inferences about the factors shaping sex patterns of dispersal in this species. We accordingly removed this framing and returned the hypotheses and predictions back to the focused text in our preregistration. We also made the following changes to the abstract and introduction, to clarify that our results cannot decipher why the males in our sample might have dispersed farther than females:

Final sentence of Abstract > Our findings show that great-tailed grackles offer a relevant study system to further understand the factors shaping natal philopatry and dispersal, given this reversal of the usual sex-bias in dispersal together with their divergent social and mating system.

Final paragraph of introduction > Both the mating and the social system are accordingly different from the resource-defense based monogamous system found in the majority of birds, which might lead to a deviation from female-biased dispersal. Determining patterns of philopatry and dispersal in great-tailed grackles can offer further insights into the potential association between dispersal decisions and the various factors that might shape them.

Hypotheses > Our main hypothesis assumes that great-tailed grackles show a pattern of female-bias in dispersal. It is our main hypothesis because this dispersal pattern predominates across birds and dispersal patterns are often retained from a common ancestor; in addition, the factors that shape this pattern might still operate in great-tailed grackles. Our alternative hypotheses expect that some of the differences in the social and mating system of great-tailed grackles might lead to a deviation from this dispersal pattern. With the setup of our study, we cannot infer why or how dispersal patterns might have changed, and we therefore present these hypotheses simply as alternatives.

Hypothesis: There are sex differences in the natal disperal rate and distance among individuals in great-tailed grackles (*Quiscalus mexicanus*) with males remaining close to where they hatched and females moving away from where they hatched. Males are expected to remain close to the area where they hatched, therefore a large number of the males on the Arizona State University (ASU) campus are expected to have hatched within the area of the study site and stay close to their relatives. In contrast, females are expected to move before their first breeding attempt (@greenwood1980mating), therefore females on campus are likely to come from areas outside of campus in the surrounding area, having moved away from relatives.

Discussion: Our results show that, unlike in the majority of bird species, the majority of great-tailed grackle males are not philopatric and a large number of female great-tailed grackles appears to remain close to where they hatched. Overall, the findings support the first alternative hypothesis that males disperse more than females.

Comment R1.2: My deepest concern regards the mist-net sampling of non-breeding individuals. It is important the authors provide more natural history background about this species, and about the trapped birds. In particular, since trapping and sampling was done across a very large period (September 2017 to October 2019), covering many months that are not during the breeding period (which should be in April May?? this information should be provided) it is not obvious to me at all 1. whether the sampled birds were all breeding birds (if they were roosting birds during the winter, this is an important issue for the interpretation of the data!) and 2. Whether the highly biased sex-ratio for females is really representative of the breeding sex ratio (L233). I have discussed previously the fact that the results are influenced by both natal and breeding dispersal, but I hadn't even realized at first (before reading the methods and understanding that trapping was not limited to the breeding season) that the results could also be influenced by the birds possibly wide wanderings during autumn and winter. This species forms communal roosts during winter, and it is common in social species that roost composition is female biased (although I do not know whether this is the case in the focal species) and also that movements of individuals during winter are different between males and females. In short, since the sample could include transient birds, with a different probability of this for males and females, I do not think the authors can conclude that 1. The sample is representative of breeding birds, which influences the whole interpretation of results, and 2. That the highly biased sex-ratio is representative of the adult sex-ratio at the site, unless they have more information on the sampling that is not provided in the text presently.

Reply R1.2: Birds were trapped and sampled at different times of the year, but (1) we do not think that this will have influenced our inferences because individuals appear to remain in the same area year round. We collected detailed year-round movement data in the Arizona population from focal follows and GPS tracking, which will allow us to empirically show the extent of seasonal changes in where individuals are found and how populations are composed. However, the results have not yet been analyzed because they are part of cross-population preregistrations so we will wait until we have all of the data before conducting these analyses (see details in McCune et al. 2020 http://corinalogan.com/Preregistrations/gspaceuse.html and Logan et al. 2019 http://corinalogan.com/Preregistrations/g_flexforaging.html).

While we are waiting for the results from these other articles, we can say that we focal follow banded individuals and that they are generally found in the same locations regardless of whether it is the breeding season (during which time they are indeed mainly found on breeding

territories) or whether it is the non-breeding season (during which time they congregate at night in communal roosts) (Figures R1 and R2). Accordingly, individuals breed close to where they were trapped.
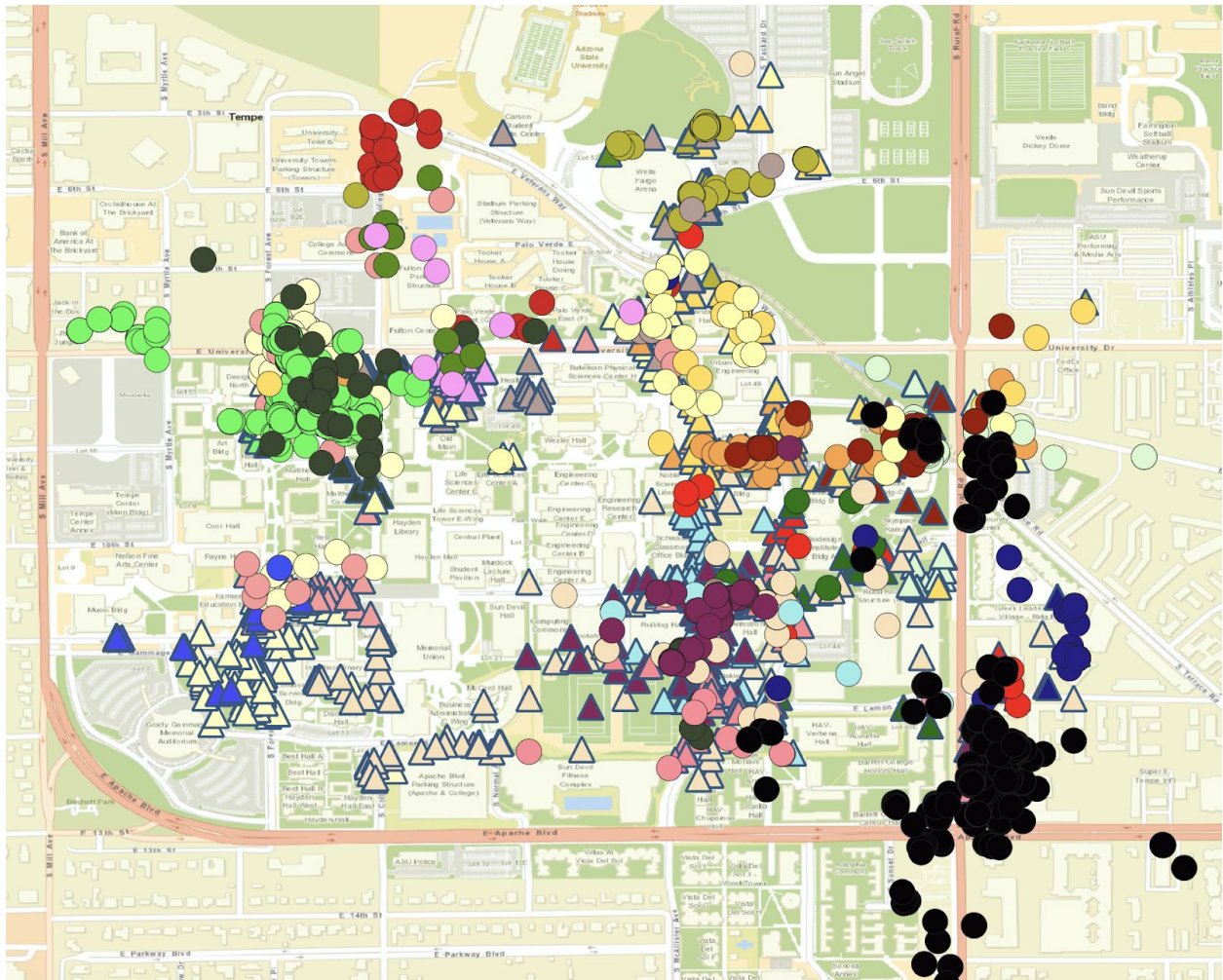

Figure R1. FEMALES in Tempe, Arizona: 23 female grackles (indicated by color) had GPS points during the breeding (triangles) and non-breeding (circles) seasons. There is generally extensive geographic overlap for the same individuals between the two seasons.
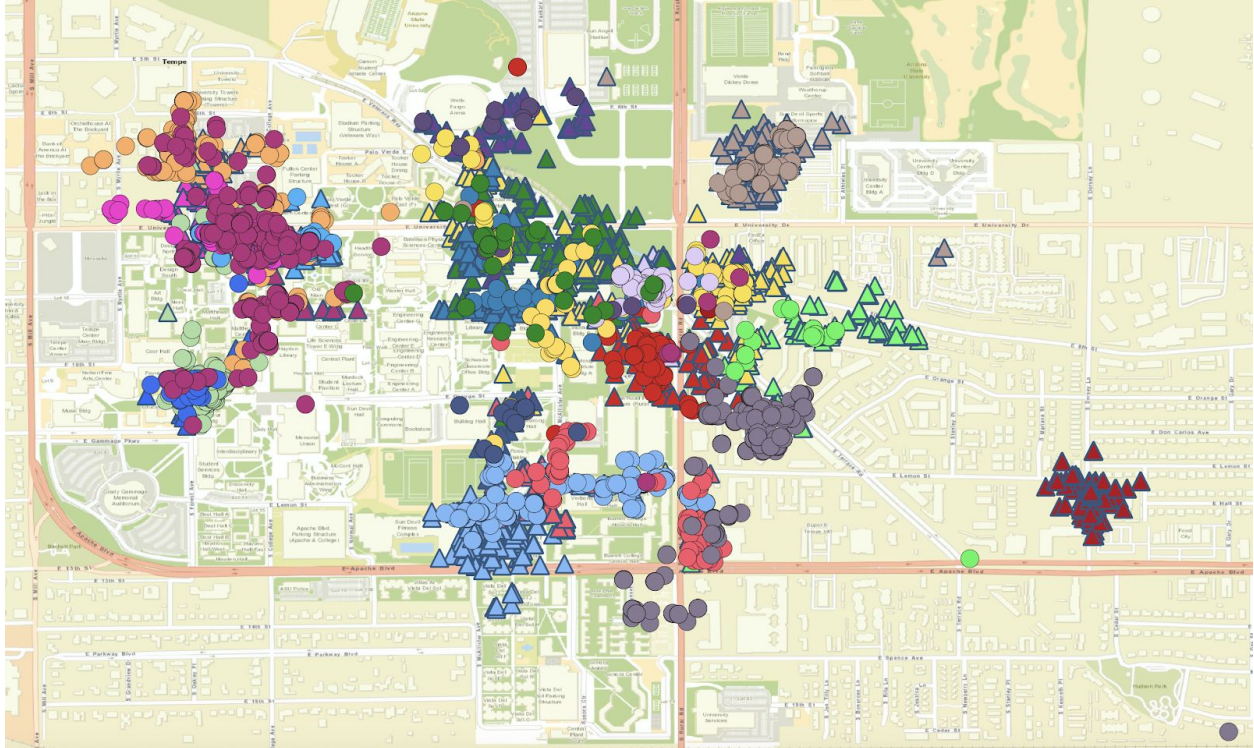
Figure R2. MALES in Tempe, Arizona: 21 male grackles (indicated by color) had GPS points during the breeding (triangles) and non-breeding (circles) seasons. There is generally extensive geographic overlap for the same individuals between the two seasons.

Regarding the sex-ratio (2), the overall sex ratio of those individuals caught with mistnets across all seasons was 2.36 females for every 1 male (reported in the previous version). To address this comment here, we calculated the sex ratio of individuals trapped using mistnets according to season. In both the breeding and the non-breeding seasons, there are many more females than males. There is some variation in the sex ratio, which might also be due to the relatively low sample sizes. Here are the data:

Breeding season (Apr-Aug): 2019: 7 females, 4 males; ratio=1.75 females per 1 male (this data is included in our article)

Non-breeding season (Sep-Mar): average ratio across years=2.59

- 2018-2019: 13 females, 4 males; ratio=3.25 (this data is included in our article)
- 2019-2020: 23 females, 12 males; ratio=1.92 (this data is not included in our article because it was collected after the DNA samples were analyzed, however it gives an idea of variation across years in this population)

Comment R1.3: For analysis i, I was surprised that the average relatedness across females and males was compared by resampling individuals from the same population, rather than

resampling SNPs. Since the resampling is done within a dataset of 52 individuals where females are over- represented, I wonder whether resampling the genetic data is not less biased.

Reply R1.3: If we understand the suggestion to "resample SNPs" correctly, such an analysis would indicate whether the relatedness we detect among the females is a result of genetic similarity across the whole genome or whether it is shaped by particular regions of the genome. This would presumably indicate the robustness of the inference that females share more alleles than expected by chance due to kinship and that this is not a consequence of higher genetic similarity among females resulting from, for example, selection for specific alleles due to local or sex-biased selection. In our view, this does not directly link to the prediction we want to assess, which is whether the resampling of individuals directly assesses whether this set of individuals is different from a random assortment of individuals. In addition, the findings of analysis ii indicate that the increased relatedness we observe among females compared to males results from the inclusion of a number of close female relatives, rather than each female-female dyad being slightly more closely related to each other than each male-male dyad.

We now modified figure 1 (see also Reply E.1) to display the potential effect of resampling different numbers of individuals to reflect the relatedness among females and among males.

Comment R1.4: The sampling area is an urban environment. Although not much is known regarding natal and breeding dispersal in natural versus urbanised environments, it is highly suspected (and documented in some species see e.g. Partecke, J. & Gwinner, E. 2007. Ecology and review in Marzluff 2017 Ibis) that the fragmented landscape of cities will highly influence dispersal. Perhaps you want to mention this in the discussion, and whether this urban effect on dispersal could be sex-specific?

Reply R1.4: We are investigating whether there are population differences in dispersal in this species in a separate preregistration (see Logan et al. 2020 http://corinalogan.com/Preregistrations/gxpopbehaviorhabitat.html#Q2_(dispersal_behavior):_Are_there_differences_in_dispersal_behavior_across_the_great-tailed_grackle's_geographic_range_(Fig_1,_Table_1)). We plan to have the results from this study in 2023 so we cannot answer this question yet, but we can make a suggestion in the Discussion that this is an avenue of future research for the project. However, we have been unable to find a grackle population that is not associated with a human-modified environment across its range (Logan pers. obs. from eBird.org data), therefore we will not be able to examine urbanized vs. non-urbanized differences in our investigation. We changed the manuscript as follows (in bold):

Discussion: "However, because we only have information for a small number of individuals from within a single site, we could not use methods that rely on assigning individuals to a source population or measure the relative distribution of genetic variation within versus among

populations (Fst or similar measures)**, **though we are currently investigating the latter [see @logan2020xpop]**"

Comment R1.5: L14 : replace 'close' by 'closer' as male natal dispersal distance varies greatly across species, what is repeatedly observed is that they have shorter natal dispersal compared to females

Reply R1.5: Good point. We changed this.

Comment R1.6: L19: I would replace the term 'exact' by 'true' or by nothing. Often enough, our field studies measure proxies of these factors and not the 'exact factors' themselves.

Reply R1.6: Good point. We deleted it.

Reviewed by anonymous reviewer 2

In this study, Sevchik and collaborators use genetics to investigate sex-biased dispersal at a local spatial scale in great-tailed grackles. Based on ddRadSeq genotypes, they use three sets of analyses to compare among sexes their degree of relatedness, the geographic distances of related individuals, and the patterns of spatial autocorrelation. These analyses allow them to show that contrary to most bird species, dispersal is male-biased in great-tailed grackles. The sample size is limited but the genetic data are substantial. The methods used are faithful to the description made in the pre-registration, which was recommended. Overall the ms is nicely written, and I appreciated that hypotheses and clear predictions were presented. My comments mostly concern sentences or paragraphs which clarity can be improved, as detailed below.

Reply: Thank you for these constructive comments.

Comment R2.1: Abstract, last sentence, « Our findings show that reduced resource competition might facilitate female philopatry and that prior knowledge of an area does not appear to be a prerequisite for male great-tailed grackles to establish breeding territories": The beginning of the sentence needs to be reformulated, as this study was not designed to assess the effect of resource competition on dispersal.

Reply R2.1: We replaced this sentence (see also Reply R1.1) as follows:

Final sentence of Abstract > Our findings show that great-tailed grackles offer a relevant study system to further understand the factors shaping natal philopatry and dispersal, given this reversal of the usual sex-bias in dispersal together with their divergent social and mating system.

Comment R2.2: Main hypothesis: "Based on the argument that males are expected to be philopatric when they defend resources beneficial to females": In the paragraph describing the species behavior it is suggested that only some males defend territories. So it is surprising for the reader that the formulation of the main hypothesis relies on the idea that males defend resources. It is only when reading the alternative hypotheses that the reader understands that the different hypotheses are based on different aspects of the species behaviour. I suggest slightly reformulating the main hypothesis to make clear that it really means "given that some males defend resources, in which case males are expected to be philopatric and females to disperse to avoid mating with relatives, (…)".

Reply R2.2: As we were writing up this post-study manuscript, we felt that we needed to add more framing to the hypotheses and predictions we made in the preregistration. We therefore added information on the potential pathways through which various factors might change dispersal patterns. We realize that this led to confusion (see also Reply R1.1) and we now removed this framing and returned the hypotheses and predictions back to the focused text from our preregistration.

Hypotheses > Our main hypothesis assumes that great-tailed grackles show a pattern of female-bias in dispersal. It is our main hypothesis because this dispersal pattern predominates across birds and dispersal patterns are often retained from a common ancestor; in addition, the factors that shape this pattern might still operate in great-tailed grackles. Our alternative hypotheses expect that some of the differences in the social and mating system of great-tailed grackles might lead to a deviation from this dispersal pattern. With the setup of our study, we cannot infer why or how dispersal patterns might have changed, and we therefore present these hypotheses simply as alternatives.

Hypothesis: There are sex differences in the natal disperal rate and distance among individuals in great-tailed grackles (*Quiscalus mexicanus*) with males remaining close to where they hatched and females moving away from where they hatched. Males are expected to remain close to the area where they hatched, therefore a large number of the males on the Arizona State University (ASU) campus are expected to have hatched within the area of the study site and stay close to their relatives. In contrast, females are expected to move before their first breeding attempt (@greenwood1980mating), therefore females on campus are likely to come from areas outside of campus in the surrounding area, having moved away from relatives.

Comment R2.3: Alternative hypothesis 2: "The polygamous mating system of great-tailed grackles, where females might be able to choose among potential males, might reduce a female's risk of mating with their father or brother." This argument is valid only if females can discriminate relatives from non relatives. Has this been demonstrated in this species?

Reply R2.3: Our assumption was that the more males a female mates with the more likely it is that at least some of her mating partners are unrelated to her. Potential inbreeding avoidance could in this case be achieved through post-mating mechanisms (e.g. only viable eggs develop), without the necessity for kin recognition mechanisms. However, because we cannot assess any of these factors, we removed the framing from the hypothesis to focus only on the different relatedness patterns that we test for.

Comment R2.4: l.108: IN the methods below.

Reply R2.4: Thank you, we changed this.

Comment R2.5: l.119-124: It is currently difficult to distinguish the parameters from Thrasher et al. 2018 and those specific to the study presented in this ms, because of missing brackets and long sentences with semi-columns. Some rewording would help.

Reply R2.5: We rephrased this section and now have two separate paragraphs for the two separate specifications of the parameters:

Deviations from preregistration > ddRadSeq > For the ddRadSeq single nucleotide polymorphism (SNP) filtering, two sets of restrictions were run to compare the resulting genotypes. The first run was based on the parameters set forth by @thrasher2018double where loci were only considered if they were present in 80% of the samples (r) and had a minimum frequency of the minor allele of 0.05 (min maf). This meant that the rare variant at a loci is present in at least 5% of the samples and it resulted in 3647 acceptable SNPs for analyses.

    For the second run, the filtering was repeated but with more stringent conditions on the loci accepted. Loci were only considered if they were present in 95% of the samples (r) and had a minimum minor allele frequency of 0.05 (min maf). This resulted in 635 acceptable SNPs; 3012 SNPs fewer than in the first, less restrictive run. We decided to use the resulting genotypes from the second, more restrictive setting for the relatedness analyses because of our small sample size (e.g., if some individuals had a lower quality sample, their relatedness to other individuals might consistently be misclassified) and because these settings still provided a large number of SNPs for analyses.

Comment R2.6: Results, l.159-164: It would be informative to add an estimate of the total size of the population, if it is know. That would allow to assess how representative the genetic sample is.

Reply R2.6: We measured population density at the Arizona site, but we have not yet analyzed the data because it is part of our cross-population comparison and we have only collected data from one population so far (for details, see: McCune et al. 2020 http://corinalogan.com/Preregistrations/gspaceuse.html). We will be able to comment on the population density at each population in relation to their dispersal behavior in the manuscript that results from our cross-population dispersal investigation (see Logan et al. 2020 http://corinalogan.com/Preregistrations/gxpopbehaviorhabitat.html). In the meantime, the grackle field site manager, Dr. Kelsey McCune, estimates that about 20% of the population on the Arizona State University campus (where our research was centered) is individually identified with colored bands (color marked). We obtained an additional independent estimate from the grackle field assistant, Melissa Folsom, who estimates that about 30% of the population is color marked. Currently there are about 100 color marked grackles, whereas at the time of the DNA processing for this article, we had samples for 52 color marked grackles. Accordingly, the individuals in the sample for this study represent around 10-15% of the individuals that are regularly encountered at the study site.

Comment R2.7: Results, l.161-164: How was the probability of identity of siblings estimated? (And heterozygosity compared to expected in a population with random mating? – Do the authors simply mean HW equilibrium, here (but they would have forgotten other conditions of HW equilibrium))? This piece of information is missing from the method section.

Reply R2.7: We had added these analyses after we had received the genotype data, but forgot to add an explanation to the manuscript. We now explain how we calculated these measures as follows:

Deviations from the preregistration > **Estimation of expected heterozygosity and probability of identity:** We used functions in the R packages 'adegenet' [@jombart2008adegenet], 'pegas' [@paradis2010pegas], and 'popgenutils' [@toukas2020popgenutils] to edit the genotype data and to calculate, based on the allele frequencies in the data and assuming random mating, the expected heterozygosity (average chance of finding two different alleles across loci across individuals) and probability of identity (chance that two individuals will have the same set of alleles across all loci).

Comment R2.8: Analysis i: l.318-326: "We performed 10,000 random draws of 15 individuals either from among the females or from among all individuals and of 37 individuals from among all individuals, and generated distributions of average relatedness among these samples. We assessed whether the observed average relatedness among the 15 males or the 37 females in our sample is higher than what is observed in the majority of random samples." This description is a bit confusing, as it is difficult to understand what was compared to what. Besides, in the

results section, it seems that in the end only one random distribution was used (10,000 random draws of 37 individuals from among all individuals). I think comparing the observed relatedness to this random distribution is indeed enough, and that the authors should simplify the method description by removing mentions to the other random distributions.

Reply R2.8: Thank you for spotting this. In the previous version, we presented the results from two analyses in the text, but only one of these in the figure (10,000 random draws of 37 individuals from among all individuals). We now added the results from the second analysis (10,000 random draws of 15 individuals from among all individuals) to Figure 1 (see Reply E.1). Your comment made us realize that our preregistration contained a plan for a third analysis, which we forgot to run, and that we now include: we performed 10,000 random draws of 15 individuals from the total 37 females to assess whether differences in average relatedness between males and females are related to the different sample size. We added the code for this, and inserted the following in the results:

Results > Analysis i > The average relatedness among males is not different from that expected by chance among 15 randomly drawn individuals from the total 52 (40% of random samples give a value as low as or lower than what we found in our sample of males)(Figure 1b) or among 15 randomly drawn individuals from the 37 females (61% of random samples give a value as low or lower than the male value). Of the eight close genetic relatives (relatedness of 0.25 or higher), seven are female dyads and one is a male dyad, and the majority of dyads are not related to each other (658/666 female dyads are not close relatives; 104/105 male dyads are not close relatives).

Comment R2.9: Fig.1: I suggest adding arrows showing also the average relatedness of males (and of the whole sample).

Reply R2.9: Good point. We made the change (see also Reply E.1).

Comment R2.10: l.181: Did the authors mean 8 female dyads, instead of 7?

Reply R2.10: The mistake was in the sentence before: there are seven female dyads who are related at 0.25 or higher. We corrected this.

Comment R2.11: l.186: "larger" missing after "were found at".

Reply R2.11: Thanks for catching this. We changed it to "longer".

Comment R2.12: Fig.2: Also add a dotted line to show the 0.125 relatedness level.

Reply R2.12: We added this to the new figure 3.

Comment R2.13: Results of the spatial autocorrelation analysis: They would be much easier to read if they were presented as a figure (spatial autocorrelation coefficient as a function of geographic distance, with standard errors allowing to assess the significance of the autocorrelation coefficients) instead of a table.

Reply R2.13: The approach for the spatial autocorrelation analyses we preregistered based on the feedback of the reviewers of our preregistration does not provide standard errors of the estimates (as far as we can see the function mantel.correlog in the R package vegan only provides the Mantel correlation and estimated p-value for each distance class). Accordingly, a plot would not provide any additional information from the table in this case.

Comment R2.14: Dependent variable, l.307: average relatedness is not the only dependent variable (e.g. analysis ii, geographic distances among relatives).

Reply R2.14: We can see how this was confusing. Actually, there were no dependent and independent variables as such because regressions were not run. Therefore, we relabeled "independent variables" to "additional variables" to clarify.

Methods > Relatedness analyses: Genetic relatedness between all pairs of individuals was calculated using the package "related" [(@pew2015related]) in R, following methods in @thrasher2018double. We estimated relatedness using the approaches of [(@queller1989estimating]), a widely used and relatively straightforward estimator, and of [(@wang2002estimator]), an estimator that accounts for small sample sizes and skewed allele distributions. We calculated average relatedness between all pairs of individuals within one sex: the arithmetic mean of the estimated relatedness based on sharing of SNP alleles among either all female or all male dyads

Methods > Additional variables: sex, distance...

Comment R2.15: Between l.312 and 313, title of the 3rd section of analyses: females should be replaced with males.

Reply R2.15: Thank you. We changed this.

Comment R2.16: Between l.327 and 328, 3rd title: remove "and the juvenile individuals"; 4th title: eXCLude

Reply R2.16: Thank you. We changed this.

Comment R2.17: l.329-336: The explanation of the generation of random distributions of geographic distances is confusing. It is clearer in l.146-151. Maybe it would be better to first explain the process for one sex, and then the other. Indeed, what is making the paragraph confusing is that both procedures are mixed in the same sentence, which makes it difficult to understand what was compared to what in the end.

Reply R2.17: You are correct, the explanation in the methods did not match with the results presented in the manuscript. We adjusted both parts to include the description of the simulation for the separate sexes in the methods and in the section about deviations from the preregistration, and the results from the difference between the sexes in the results section as follows:

Deviations from preregistration > Analysis ii > In addition to the permutation to assess whether the difference in the average distance among closely related females and the average distance among closely related males was different than expected, we performed a permutation to assess whether the average distance among closely related female dyads (r>0.2499) was shorter than the average distance among a random sample of the same number of female dyads.

Results > Analysis ii > The difference in distances among the twelve related females (r≥0.125, on average 360m apart) compared to the three related males (r≥0.125, on average 1183m apart) is 823m. This difference in distance (or greater differences in distance) was found in only 2% of 10,000 random draws comparing average distances among 12 random females and three random males.

Methods > Analysis ii > Based on the calculations of pairwise genetic relatedness, we selected the subset of pairs of individuals who are estimated to be more closely related than cousins (r≥0.125) or half-siblings (r≥0.25). For this subset of closely related individuals, we first determined whether the pairwise geographic distances were shorter than what is observed in a random sample of the same number of same-sex individuals [(@coulon2006dispersal[). We performed 10,000 draws of twelve (reflecting r≥0.125) random pairs of females and calculated the average geographic distance among them. We assessed whether the observed difference in geographic distances is higher than the majority of random samples and, for comparison with other approaches, determine whether the observed distance is higher than that calculated for 95% of all random draws. We repeated these comparisons for the more closely related females (r≥0.25), randomly drawing seven females 10,000 times; and for the males, randomly drawing three (r≥0.125) and randomly drawing one individual (r≥0.25) 10,000 times. Next, we performed 10,000 random draws of pairs of males and pairs of females matching the numbers of closely

related dyads of either sex. We calculated the differences between the average geographic distances among the subset of males and the average geographic distances among the subset of females. We compared this to the observed difference in the distance among closely related males and among closely related females.