Dear Dr. Fronhofer, reviewers, and the PCI Ecology Managing Board,

Thank you for providing this helpful feedback! We made the changes listed below (in blue) in response to your and the reviewers' comments. The revised manuscript and associated files can be found at

(pdf) https://ecoevorxiv.org/t6beh/

(html): http://corinalogan.com/Preregistrations/gdispersal.html

Round #2

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by Emanuel A. Fronhofer, 2021-01-12 14:48

Dear Mr. Sevchik, Dear Dr. Lukas,

thank you for your revisions. As you will see both referees and I appreciate your efforts very much. Nevertheless, both referees have some major and some minor points that you should take into account when revising your manuscript.

E1: Besides these points, I would like to draw you attention especially to one minor comment that may represent some additional work. One referee notes: "Will this preregistration mention appear in the final ms? Are details in this preregistration essential for the good understanding of this ms? If so I feel that it would be better to have them included in the present ms rather than for the reader to have to download also the preregistration document. Reading the methods section, it feels like in fact it has been updated, hence I think it would be clearer to simply remove the preregistration mention, but I will let the Editor decide on this." PCI does not (yet) have guidelines regrading how preprints that have preregistrations should be formatted. Nevertheless, one important aim, regardless of any guidelines, should be clarity and it seems that in its current format the manuscript could gain clarity by some restructuring. I would like to encourage you to make the manuscript read more like a self-sufficient entity and avoid the impression that the reader should check the preregistration. This being said, you should definitely refer to the preregistration and include the section on deviations as you are currently doing.

Reply E1: We have restructured the manuscript to turn it more into a self-sufficient article that builds on the preregistration. For this purpose, we have moved the methods from the end of the article to the section between introduction and results, and now explain the deviations from the preregistration directly at the relevant points in the methods (rather than explaining them in detail in the section titled Deviations from the Preregistration).

The section Deviations from the Preregistration now includes a only bulleted summary of these changes to facilitate comparisons with the preregistration.

Deviations from the Preregistration
Methods -> "Deviation from the preregistration"

E2: Minor points: The links included in the manuscript should all be DOIs, otherwise they might not be stable and the manuscript may not be reproducible in some years time (e.g., line 102-103, code after lines 277). You could use Zenodo, for instance, to generate DOIs. Zenodo also allows for a nice integration with GitHub. Please check all of your links throughout the manuscript.

Reply E2: We replaced the links to the data and to the code that are needed to reproduce our findings with DOIs. In addition, we mention that copies are also available on GitHub, which some readers might prefer for ease of access.

E3: Line 168: symbol seems to be missing after "...many (R"

Reply E3: The symbol we had previously chosen (representing 'about equal to') could not be rendered by markdown and we have now replaced this with an equal sign.

Results -> Genotyping: "as many (R=0) or fewer"

Reviewed by anonymous reviewer, 2021-01-06 15:00

This revised version of the manuscript takes into account all comments made on the first round, and I find that the new outline of the main and alternative hypotheses is much clearer and faithful to what the authors have explored and tested. I do have a some remaining comments however, and overall, I felt that important statements should be supported by literature, while the reference list is presently very thin.

R1.1: In the introduction, the very simplified version of hypotheses outline avoids several confusing predictions that were present in the initial manuscript. I do find it very dry now, with even less biological insight on the species allowing one to formulate reasonable predictions, yet I understand that perhaps the knowledge is lacking preventing clear elaborate predictions.

Reply 1.1: Thank you again for your excellent feedback! We appreciate how much you have helped improve this manuscript. We expanded the introduction (additions below in bold) to include more background information on the natural history of grackles, which informed the formulations of the predictions, including why some of their differences from commonly studied bird species might make them an interesting system to investigate dispersal decisions in females and males.

*Introduction: Here, we investigate SNP (single nucleotide polymorphism) genotype data for a sample of great-tailed grackle (*Quiscalus mexicanus*) females and males at a single site. Great-tailed grackles* ***differ in several aspects from the majority of bird species in which dispersal has been investigated thus far, which might make them a relevant study system to gain further insights into the factors shaping the dispersal decisions of females and males. Great-tailed grackles*** *are a highly social passerine bird found in the Americas.* ***Individuals forage year-round in small fission-fusion groups in areas that are not obviously defended against other individuals, and at night they roost in large associations [Johnson & Peer 2001], unlike most other bird species where, at least during the breeding season, pairs or families defend foraging territories (Cockburn 2001). This could indicate that resource competition might be lower in great-tailed grackles, potentially reducing pressure to remain in or move to high quality areas. Essentially everywhere they occur, great-tailed grackles live in human-modified environments (MacGregor-Fors et al. 2009) and their wide range of foraging habits routinely, includes exploiting human foods (King 2012). In these environments, they can occur in large numbers and at high densities (Escobar-Ibáñez et al. 2020). Great-tailed grackles have recently extensively expanded their geographic range [Wehtje 2003], indicating that they are highly mobile.*** *Great-tailed grackles are sexually dimorphic, with males being larger than females and differing in plumage. During the mating season, some males defend territories around suitable breeding habitats and mate with females who build their nests in these territories. Holding a territory leads to higher reproductive success for these males, but females also mate with roaming males, leading to a polygamous mating system [@johnson2000male].* ***This resembles the mating system observed in many mammalian species, where males disperse to areas with the highest number of potential mates [e.g. Höner et al. 2007].*** *Previously,* ***great-tailed grackle females*** *were assumed to perform all activities related to offspring care, from building the nest through incubating and feeding the hatchlings, but observations indicate that at least some males partake in these activities [@selander1970parental; @Folsom2020malecare]. 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.

R1.2: Related to this comment on natural history insight, is the fact that the focal population is urban, and it might well be that dispersal patterns in such environments are not similar to what happens in wild areas. This is a comment I made earlier (R1.4) on which the authors chose not to include a mention in the ms. I do feel that it may be an important feature to mention since results could have been very different in a wetland or mangrove.

Reply 1.2: Great-tailed grackles appear to be restricted to human-modified environments wherever they occur. Even within their original range in Central America, current sightings are only reported from urban or farm areas. Nevertheless, local conditions might have modified the patterns of dispersal we inferred. We added the following:

Introduction: Essentially everywhere they occur, great-tailed grackles live in human-modified environments, and they have recently massively expanded their range [Wehtje 2003], indicating that they are highly mobile.
Discussion: In addition, information on dispersal patterns from different sites might help to understand how much the sex bias we detect at this site in the city center of Tempe is shaped by local factors or whether it is linked to general features of the biology of great-tailed grackles.

R1.3: Also, I am somewhat uncomfortable with the use of the term 'predict'. Usually predictions are tested and following the results, they are confirmed or invalidated. Here, some of the 'predictions' are rather assumptions that are essential to make further predictions, but they are not themselves predictions. For ex., take this prediction from line 88 "We predict that the movement of individuals will influence the spatial distribution of genetic relatives", testing this prediction would require to compare the distribution of genetic relatives between groups of individuals that move or do not move, in order to show that movement is related to genetic relatedness. This is not what the authors have done, and in fact they probably don't need to do that as this relationship is already well described for many species. So in this sentence, I would not use the term 'predict', but rather 'we assume', or simply 'Our study is based on the fact/assumption that..', ideally supported by a few references. I am not including this comment in the minor details section below as I do not think it is 'just' one word to change in the text, I think the authors need to rethink what they consider a prediction.

Reply 1.3: Thank you for noticing this. Yes, the word "predict" should in this context only be used for those statements that our analyses can assess. We changed the sentence accordingly:

Hypotheses: "We expect that the movement of individuals will influence the spatial distribution (Aguillon et al. 2017)"

R1.4: Finally, I was struck by the mention line 234 that the authors consider they have a "large number of SNP loci": although 635 SNPs might have been a common genomic sample size 10 years ago, it is now in the low end of sample sizes used, so I wouldn't call it a large number of SNPs. In fact, I am surprised that this low SNP number and the potentially low power to assess relatedness is not discussed, and that generally the ms contains very few insight and references on genetic/genomic approaches to estimate relatedness since this is an important topic for the authors' aims. See for example:- A comparison of 16 microsats vs 4800 RADseq SNPs: https://onlinelibrary.wiley.com/doi/full/10.1002/ece3.4905

Reply 1.4: The statement about a large number of SNP loci was already in our preregistration, before we generated the data. We had included the statement because our genomics approach is directly based on Thrasher et al. https://doi.org/10.1111/1755-0998.12771. Thrasher et al. obtained 411 SNP loci, and through various checks concluded that "our case study, using the variegated fairy-wren, shows that our modified ddRAD-seq method recovers more than enough SNP loci to confidently discern relationships in a species with a complex social system." At the time of writing the preregistration, we expected an even larger number of SNP loci for the great-tailed grackles than what Thrasher et al. obtained because we sequenced all samples to a greater depth. In fact, using their filtering, we obtained >3600 SNPs, so 9 times as many as Thrasher et al. With this larger number of loci, we decided to reduce the noise in our sample by restricting the filters of which loci to include, following advice to select loci with high heterozygosity and with almost complete information for all individuals (see e.g. https://www.molmed.medsci.uu.se/digitalAssets/64/a_64906-f_Morin_TrendsEcolEvo_2004.pdf**). Accordingly, even though the absolute number of loci in our study is smaller than in previous studies, we have high power to detect kin relationships. Lemopolous et al. in the study cited by the reviewer used much less stringent filtering to end up with the 4800 SNPs, which had 30% missing data and an average expected heterozygosity of 0.18 (meaning that for many loci, the rare variant occurs in too few individuals to aid in the reconstruction of relationships). Accordingly, despite the larger number of SNPs, Lemopolous et al. report that**

they have an average exclusion probability of paternity of only 0.98. In our restricted sample of 635 SNPs, we have less than 3% missing data and an expected heterozygosity of 0.48, giving an exclusion probability of 10^{-24}. We added the following:

Methods > SNP processing: The second filter applied more stringent conditions for the loci to be retained. 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 filtering, but still more than in the study by @thrasher2018double because each individual in our sample had been sequenced to a greater depth. 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 an effective number of SNPs for analyses. The more restrictive filtering reduces noise from missing data and retains high power by selecting loci with high heterozygosity (their heterozygosity approaches the maximum of 0.5) [Morin et al. 2004].

R1.5: Santure 2010: https://pubmed.ncbi.nlm.nih.gov/20149098/, see in particular their conclusion that “Our data suggest that using marker information to reconstruct the pedigree, and then calculating relatedness from the pedigree, is likely to give more accurate relatedness estimates than using marker-based estimators directly” - Smouse 2010 - how many SNPs are enough (summarising Santure): https://pubmed.ncbi.nlm.nih.gov/20456228/

- There are probably more recent papers on this topic!

Reply 1.5: On the point of constructing pedigrees: while this method can trace relatedness more accurately, it is not a feasible approach in our case. We added the following explanation:

Methods > Relatedness estimation: Additional note: Our preregistration did not include plans to perform pedigree reconstructions as an alternative way to assess relatedness among the individuals for three reasons. First, we have a cross-sectional sample, which does not contain longitudinal information from tracking individuals seen with their potential parents into adulthood. Second, adults are of unknown age, so for any related individuals who share an allele at (almost) all loci we would not be able to determine which is the parent and which is the offspring. Third, grackles are not expected to have large clusters of siblings [@johnson2000male], as for example in fish species, making it highly unlikely that our sample contains extended families.

Minor details

R1.6: Line 79: I don't understand why there is a 'Hypothesis' term at the start of this line, isn't it the same main hypothesis as in the previous paragraph?

Reply 1.6: Thank you for spotting this. We did not correct this from the earlier formatting in which we had not provided the additional background. We now removed the 'main hypothesis' label from the first paragraph in this section because it describes the rationale for why we declared one hypothesis the main and the others as alternatives.

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, therefore we present these hypotheses simply as alternatives.

R1.7: Line 96: individuals is misspelled.

Reply 1.7: Thanks for catching this. We changed the section where this word appears and made sure to omit the spelling mistake (see Reply 1.8).

R1.8: I do not see the difference between the 'first' prediction outlined lines 93-94 ("higher levels of average relatedness are expected among all individuals of the philopatric sex than among all individuals of the sex that disperses") and the 'second' prediction in lines 96-97 ("finding close genetic relatives in short distances from each other indicates that these individuals have remained philopatric"). I think this might be because of the formulation of the second prediction, which could be rephrased, but they also might be redundant predictions.

Reply 1.8: The predictions are specific to each set of analyses. The patterns that each analysis aims to detect are all shaped by the same underlying mechanisms, the movement of individuals. The first prediction focuses on average relatedness across all same-sex individuals in the full sample (one value per sex). The second prediction focuses on the geographic distances among genetic relatives, linking

all pairwise relatedness estimates to all pairwise distances (one relatedness value and one distance value per pair of individuals for all possible pairs within each sex). We changed the text to make this clearer.

Hypotheses: We will assess these predictions in three analyses. The first analysis (analysis i: average levels of relatedness among individuals in our sample) focuses on whether individuals disperse beyond the trapping area and compares one average value of relatedness per sex. Here, we predict, higher levels of average relatedness among all individuals of the philopatric sex than among all individuals of the sex that disperses. This follows if some dispersing emigrants move outside of the trapping area, away from parents and siblings, while immigrants can come from a variety of areas outside the trapping area and therefore consist of unrelated individuals. The second analysis (analysis ii: geographic distances between individuals that are close genetic relatives) focuses on the distances among close relatives of the same sex that are trapped within our trapping area and investigates the pairwise distances among individuals of the same sex who are closely related. Here,, we predict that there are sex biases in the average distances between trapping sites for relatives compared to non-relatives, because philopatric individuals will remain close to same-sex parents and siblings while individuals that disperse within the trapping area will end up in different locations than their same-sex parents and siblings. The third analysis (analysis iii: spatial autocorrelation) focuses on how relatedness among pairs of same sex individuals changes as the distance between them increases and investigates correlations among all estimates of pairwise relatedness and pairwise geographic distances among individuals of the same sex. Here, we predict a decline in levels of relatedness as distances among individuals increase to indicate that individuals have remained philopatric such that close relatives are found in close geographic proximity. In contrast, we predict no structure of relatedness in geographic space for individuals who disperse because relatives will be found both close and far from each other.

R1.9: Line 101: Will this preregistration mention appear in the final ms? Are details in this preregistration essential for the good understanding of this ms? If so I feel that it would be better to have them included in the present ms rather than for the reader to have to download also the preregistration document. Reading the methods section, it feels like in fact it has been updated, hence I think it would be clearer to simply remove the preregistration mention, but I will let the Editor decide on this.

Reply 1.9: We have changed the structure to have everything included in this manuscript, and we now highlight updates from the preregistration at the specific points in the methods. See also our Reply E.1.

R1.10: Line 158: I would remove “the” in front of “individuals”

Reply 1.10: We changed this because of the new structure, where we present the methods prior to the results.

Moved to Methods > ddRadSequencing: "we excluded the above mentioned 5 individuals later".

R1.11 Results are presented alternatively in past tense (e.g. line 157 and onwards) and present tense (e.g. lines 170 and onwards). Please homogenize.

Reply 1.11: The beginning of the results section included descriptions of the methods, which is why it was written in the past tense. We now moved these sections to the Methods, and checked that all results are presented in present tense.

Results > Genotyping: Our dataset consists of 635 SNPs for 52 individuals. Data is 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.

R1.12: Lines 174-181: what is the criterion to decide that distances are shorter or not different to what is "expected by chance"? This criterion is particularly elusive in the case of males since you have only one closely related male dyad.

Reply 1.12: We assume that this comment refers to lines 191-197 in the section on "distances among close relatives"? We removed the two statements about distances shorter or longer than expected by chance because they were confusing (revised paragraph below). For the females, the permutation analysis provides a comparison with how the distances among the closely related individuals compares to the distances that would be expected by chance. For the males, we cannot perform a permutation because we only have one pair of closely related males. Instead, we compare their distance to the distances among all the remaining pairs of males to indicate that the related pair is fully within the range.

Results > Analysis ii: A median distance as short as or shorter than 340m is observed in less than 6% of all random samples of seven female dyads and a median distance of 360m or shorter is observed in less than 4% of all random samples of twelve female dyads. The distance among the one pair of males related at closer than 0.25 is 670m, and the median distance among the three male dyads related at 0.125 or closer is 1183m (SD=353m). This compares to a median of 972m (SD=569m) among all dyads of males, with about 40% of male dyads being 670m or less apart. The difference in distances among the twelve related females ($r \geq 0.125$, on average 360m apart) compared to the three related males ($r \geq 0.125$, on average 1183m apart) is 823m. This difference in distance (or greater differences in distance) is present in only 2% of 10,000 random draws comparing average distances among 12 random females and three random males.

R1.13: Table 1's title requires more detail to explain what are the corrected probabilities.

Reply 1.13: We changed the legend to explain that the corrected probabilities account for multiple testing.

Table 1: Output of correlogram analyses linking pairwise relatedness to pairwise distances. The values represent the correlations between relatedness and distance for males and females across trapping sites binned into distance classes, with the probabilities of observing the values by chance corrected for the multiple tests across distance classes (based on the Holm-Bonferroni method).

R1.14: Line 224: "to where they hatched" is in fact, if I'm not mistaken, a speculation, since you do not have data on where any of the birds have hatched, right? I think this point should be clarified.

Reply 1.14: Yes, it is possible that other factors lead to closely related females associating in close proximity (e.g. they recognize each other). Based on what is known from other species, the most parsimonious explanation is however that closely related females are found in close proximity because they did not move far from where they hatched such that daughters remain close to mothers and sisters. We clarified this in the text.

Discussion: Our results show that, unlike in most other bird species, the majority of great-tailed grackle males are not philopatric because ***sons are not found in close proximity to fathers or brothers****.* ***In contrast, several*** *female great-tailed grackles* ***are found in close proximity to genetic kin****.* ***The most likely explanation for this assortment of kin in space is that at least some females*** *remain close to where they hatched.*

Reviewed by anonymous reviewer, 2020-12-14 20:31

In this revised version of "Investigating sex differences in genetic relatedness in great-tailed grackles in Tempe, Arizona to infer potential sex biases in dispersal", the authors have addressed most of the comments made on a previous version of the ms; or adequately explained why they have not followed the reviewer or editor's suggestions (except for one very minor one, see below). The different text or figure editions and additions have helped clarifying some points. Only the part about relatedness comparison in different subsamples still needs some work, as I suggest below. Besides this point, I only have a few minor comments.

R2.1: Abstract, l.29-30: « These relatedness results suggest that, unlike most other bird species, female great-tailed grackles appear to have hatched and remained at this site, while males disperse to new areas. » This sentence should be moderated a little, as the results do not show that all females stay and on-site, and all males disperse.

Reply 2.1: Thank you so much for all of your useful feedback on this manuscript! We are very happy that you like the revision. We changed this to clarify that the patterns of philopatry and dispersal are our most likely interpretations for the observed associations among relatives.
Abstract: Our results indicate sex biases in relatedness structure that differ from most other bird species. Female great-tailed grackles associate with close genetic relatives, presumably by remaining close to where they hatched which would lead to them remaining close to their mothers and sisters. Males are not found close to genetic relatives, suggesting that they disperse away from their fathers and brothers.

R2.2: l.96: indivIduals

Reply 2.2: We changed this section (see Reply 1.7).

R2.3: l.158: an extra “the” before “5 individuals”

Reply 2.3: We changed this (see Reply 1.9).

R2.4: l.162: the estimated value of expected heterozygosity should be added

Reply 2.4: We added this information
Results > Genotyping: the observed heterozygosity (individuals carrying one copy each of the two bases) is 0.48, slightly higher than the heterozygosity expected in a population with the same allele frequencies and random mating (0.46).

R2.5: l.164: the authors forgot to use a mathematical expression instead of words in this sentence (editor comment #5)

Reply 2.5: Thank you for catching this. We changed this to the mathematical expression.
Results > Genotyping: 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^{-139}.

R2.6: l.347 to 349: it is still unclear what was compared to what, in that paragraph. I suggest this rewording: “We compared (i) the observed average relatedness among the 37 females in our sample with the relatedness in the 10000 random samples of 37 individuals from both sexes; (ii) the observed average relatedness among the 15 males in our sample with the relatedness in the 10000 random samples of 15 individuals from both sexes; (iii) the observed average relatedness among the 15 males in our sample with the relatedness in the 10000 random samples of 15 females.” Also to clarify these same analyses: l.173: add “from the whole sample” after “we randomly drew 37 individuals”

Reply 2.6: Thank you, we changed this accordingly.

Methods > Analysis i: We compared (i) the observed average relatedness among the 37 females in our sample with the relatedness in the 10,000 random samples of 37 individuals from both sexes; (ii) the observed average relatedness among the 15 males in our sample with the relatedness in the 10,000 random samples of 15 individuals from both sexes; (iii) the observed average relatedness among the 15 males in our sample with the relatedness in the 10,000 random samples of 15 females. We report the proportion of 10,000 random samples with lower relatedness than the observed values and, for comparison with other approaches, assess whether the observed relatedness is higher than the relatedness calculated for 95% of all random draws.

Results > Analysis i: To assess whether the average relatedness among females is higher than expected, we compare it to the average relatedness calculated in random draws of 37 individuals from all 52 individuals. In less than 4% of the draws of random 37 individuals is the level of relatedness as high as or higher than that observed in our sample of females (Figure 1a). Therefore, although the difference in the level of average relatedness among females compared to among all individuals is small (0.004), it is higher than expected by chance. The average relatedness observed among the 15 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).

R2.7: And it would be helpful to the reader to add more explanations of why the authors conclude (as they do at the beginning of the discussion) that “the mean level of average genetic relatedness is lower among males compared to females”, while their test comparing male and female relatedness suggests there is no difference (i.e. explain again the issue of lack of power in the samples made of 15 individuals in the interpretation part of the ms).

Reply 2.7: We changed this to clarify that our focus is more on the levels of relatedness within each of the sexes rather than the comparison between them.

We assess for each sex whether it is more likely that individuals are philopatric or dispersing.

Discussion: We find that the mean level of average genetic relatedness is slightly lower among males compared to females in our sample and that females are more closely related to each other than expected by chance while males are not

Our small sample (we estimate that we trapped ~25%-30% of all grackles within this area, which is continuously connected to other areas in which grackles reside) and the limited number of genetic relatives we found, restrict the inferences we can draw...we cannot infer how substantial this sex bias is (our comparison of average relatedness between sexes is inconclusive), or what percentage of females and males might disperse, how far they might move.

R2.8: I think Fig 2 and 3 are (in part) redundant, but I'll leave it up to the authors to decide whether to remove one or not.

Reply 2.8: Figures 2 and 3 display slightly different information. Figure 2 focuses on how closely individuals are related (which is not displayed in Figure 3), while Figure 3 focuses on how many related or unrelated individuals are at a given distance from each other (which is difficult to make out from Figure 2). We therefore decided to keep both figures.