



Peer Community In Ecology

Investigate fine scale sex dispersal with spatial and genetic analyses

Sophie Beltran-Bech  based on peer reviews by **Sylvine Durand** and 1 anonymous reviewer

August Sevchik, Corina Logan, Melissa Folsom, Luisa Bergeron, Aaron Blackwell, Carolyn Rowney, Dieter Lukas (2019) Investigating sex differences in genetic relatedness in great-tailed grackles in Tempe, Arizona to infer potential sex biases in dispersal. Missing preprint_server, ver. Missing article_version, peer-reviewed and recommended by Peer Community in Ecology.

<http://corinalogan.com/Preregistrations/gdispersal.html>

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The preregistration "Investigating sex differences in genetic relatedness in great-tailed grackles in Tempe, Arizona to infer potential sex biases in dispersal" [1] presents the analysis plan that will be used to genetically and spatially investigate sex-biased dispersal in great-tailed grackles (*Quiscalus mexicanus*). Several hypotheses implying mating systems, intrasexual competition or sex-related handicaps have been proposed to explain the diversity of dispersal patterns between or within species according to their ecological requirements, environmental factors such as seasonality [2], or individual characteristics such as age [3] or sex [4]. In birds, females are classically the dispersing sex, while males remain close to the place they were hatched [5], with potential benefits that males derive from knowing the local environment to establish territories [6]. In great-tailed grackles the males hold territories and the females choose which territory to place their nest in [7]. In this context, the main hypothesis is that females are the dispersing sex in this species. The authors of this preregistration plan to investigate this hypothesis and its 3 alternatives ((i) the males are the dispersing sex, (ii) both sexes disperse or (iii) neither of the two sexes disperse), investigating the spatial distribution of genetic relatives. The authors plan to measure the genetic relatedness (using SNP markers) and geographic distances among all female dyads and among all male dyads in the fine geographic scale (Tempe campus, Arizona). If females disperse away from relatives, the females will be less likely to be found geographically close to genetic relatives. This pre-registration shows that the authors are well aware of the possible limitations of their study, particularly in relation to their population of 57 individuals, on a small scale. But they will use methods that should be able to detect a signal. They were very good at incorporating the reviewers' comments

and suggestions, which enabled them to produce a satisfactory and interesting version of the manuscript presenting their hypotheses, limitations and the methods they plan to use. Another point I would like to stress is that this pre-registration practice is a very good one that makes it possible to anticipate the challenges and the type of analyses to be carried out, in particular by setting out the working hypotheses and confronting them (as well as the methods envisaged) with peers from this stage. I therefore recommend this manuscript and thank all the contributors (authors and reviewers) for their work. I look forward to seeing the outcomes of this study.

References:

- [1] Sevchik A., Logan C. J., Folsom M., Bergeron L., Blackwell A., Rowney C., and Lukas D. (2019). Investigating sex differences in genetic relatedness in great-tailed grackles in Tempe, Arizona to infer potential sex biases in dispersal. In principle recommendation by Peer Community In Ecology. [corinalogan.com/Preregistrations/gdispersal.html](<http://corinalogan.com/Preregistrations/gdispersal.html>)
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- [3] Marva, M., and San Segundo, F. (2018). Age-structure density-dependent fertility and individuals dispersal in a population model. *Mathematical biosciences*, 300, 157-167. doi: [10.1016/j.mbs.2018.03.029](<https://dx.doi.org/10.1016/j.mbs.2018.03.029>)
- [4] Trochet, A., Courtois, E. A., Stevens, V. M., Baguette, M., Chaine, A., Schmeller, D. S., Clobert, J., and Wiens, J. J. (2016). Evolution of sex-biased dispersal. *The Quarterly Review of Biology*, 91(3), 297-320. doi: [10.1086/688097](<https://dx.doi.org/10.1086/688097>)
- [5] Greenwood, P. J., and Harvey, P. H. (1982). The natal and breeding dispersal of birds. *Annual review of ecology and systematics*, 13(1), 1-21. doi: [10.1146/annurev.es.13.110182.000245](<https://dx.doi.org/10.1146/annurev.es.13.110182.000245>)
- [6] Greenwood, P. J. (1980). Mating systems, philopatry and dispersal in birds and mammals. *Animal behaviour*, 28(4), 1140-1162. doi: [10.1016/S0003-3472(80)80103-5]([https://dx.doi.org/10.1016/S0003-3472\(80\)80103-5](https://dx.doi.org/10.1016/S0003-3472(80)80103-5))
- [7] Johnson, K., DuVal, E., Kiehl, M., and Hughes, C. (2000). Male mating strategies and the mating system of great-tailed grackles. *Behavioral Ecology*, 11(2), 132-141. doi: [10.1093/beheco/11.2.132](<https://dx.doi.org/10.1093/beheco/11.2.132>)

Reviews

Evaluation round #3

DOI or URL of the preprint: <http://corinalogan.com/Preregistrations/gdispersal.html>
Version of the preprint: 1.7

Authors' reply, 28 November 2019

Dear Dr Beltran-Bech and members of the PCI Ecology managing board,
Thank you for the positive reply and for carefully checking our latest revisions!

We have added a reference to Figure 3 in the text in Methods > Planned Sample: "Females were caught at all but one site, such that comparisons are possible of the genetic relatedness of pairs of females trapped at various distances from each other. Males were not caught at all trap sites, but there are several sites at which multiple males were caught and sufficient sites for comparisons of males that were caught close to each other, at intermediate, and at long distances (Figure 3)."

As before, the version-tracked version of this preregistration is in rmarkdown at GitHub. In this document, we have now also included the reference to PCI Ecology at the top: <https://github.com/corinalogan/grackles/blob/master/Files/Preregistrations/gdispersal.Rmd>

We really appreciate all the support from the reviewers and editor to help us set up a good study!

All our best, Gus, Corina, Melissa, Luisa, Kelsey, Aaron, Carolyn, and Dieter

Decision by **Sophie Beltran-Bech** , posted 27 November 2019

Requested revisions

Dear colleagues, thank you for considering our comments. Can you integrate Figure 3 into the text of the manuscript? I await your return and submit my recommendation. Best regards, Sophie BELTRAN-BECH

Evaluation round #2

DOI or URL of the preprint: <http://corinalogan.com/Preregistrations/gdispersal.html>

Version of the preprint: 1.7

Authors' reply, 26 November 2019

Decision by Sophie BELTRAN-BECH, 2019-11-22 16:28 Manuscript: <http://corinalogan.com/Preregistrations/gdispersal.html> version 1.7 Requested revisions

The authors responded satisfactorily to the comments raised by the 2 reviewers. One of the reviewer provides other useful comments that should help authors to improve the manuscript. I suggest to follow these asked corrections carefully. In addition to responses to reviewer's comments, some typos require corrections (see below). Following all these suggested corrections, I will recommend your pre-print.

Dear Dr.'s Beltran-Bech and Durand, Thank you for checking our revised preregistration again in such detail and for providing this helpful feedback! We made these changes to our preregistration and associated files at <http://corinalogan.com/Preregistrations/gdispersal.html>, and we responded to your comments below. As before, the version-tracked version of this preregistration is in rmarkdown at GitHub: <https://github.com/corinalogan/grackles/blob/master/Files/Preregistrations/gdispersal.Rmd>. In case you want to see the history of track changes for this document at GitHub, click the previous link and then click the "History" button on the right near the top. From there, you can scroll through our comments on what was changed for each save event and, if you want to see exactly what was changed, click on the text that describes the change and it will show you the text that was replaced (in red) next to the new text (in green). We think the revised version is much improved due to your generous feedback! All our best, Gus, Corina, Melissa, Luisa, Kelsey, Aaron, Carolyn, and Dieter

My minor comments and suggestions: Comment 1: - In your predictions (after alternative 5): there is a mistake in the typo of "individuals" in the sentence : "the first two aim to detect...among individuals" [but some modifications are proposed by the reviewer and perhaps, my proposition will be obsolete.

Response 1: Thank you. Following the reviewer's advice, we removed Alternative 5 (see comment 9).

Comment 2: - For the figures 1 and 2: can you add an orientation (compass showing north for example) to your figures?

Response 2: Good idea. We added compass roses to figures 1 and 2 to indicate orientation.

Comment 3: - Figure 1 legend: do you mean "than females" (and not than males)? Please correct.

Response 3: Thank you, we changed this to: "males will have higher levels of genetic relatedness than females because females likely have larger dispersal distances"

Comment 4: - You compare the sex ratio 26 females / 11 males using mist nets (2.36 females per male) to your study but there is no reference cited with this 26/11 sex ratio. Where does this sex-ratio come from? Clarify please.

Response 4: To estimate the population sex ratio for the first revision, we went back to our records and counted the number of male and female grackles that were trapped using mist nets at our site since the beginning of our study. From these records, we calculated the ratio of 26 females / 11 males specifically for this preregistration. We modified the respective paragraph to clarify this. Methods > Planned Sample: "To estimate the sex ratio at the field site, we counted the number of females and males that were trapped in mist nets since the beginning of our study (September 2017 - October 2019). This trapping method likely does not elicit a sex bias in terms of which sex is caught because the nets are invisible. Therefore, if one sex is more neophobic than the other, both sexes are likely to be trapped using this method. A total of 26 females and 11 males were trapped using mist nets (a ratio of 2.36 females per 1 male), which is very similar to the sex ratio in our sample consisting of 40 females and 17 males (2.35 females per 1 male)."

Comment 5: - For the "Sampling size rational" section, could you replace "This was the largest sample size possible by July 2019 when the DNA were sequenced using ddRADseq." by "The sample size presented was the largest one possible by July 2019 when the DNA were sequenced using ddRADseq.".

Response 5: Thank you, we changed this accordingly.

Comment 6: - For the "data collection stopping rule" section, could you formulate it in sentence form (with a verb)? For example: "We decide to analyse all blood..."

Response 6: We reformulated the "data collection stopping rule" section to a full sentence. Methods > Data collection stopping rule: "We analyzed all blood samples that were collected through June 2019, which was the end of the trapping season."

Comment 7: - The reference "Coulon et al." dates from 2006 and not 2005. Can you correct this point in all the preprint?

Response 7: There was confusion with this article because an 'online early' version of it was published in November 2005 and was available for citing at this point, but the final publication date was from an issue in March 2006. We now revised this to 2006 throughout the preregistration.

Reviews Reviewed by Sylvine Durand, 2019-11-22 15:08 Comment 8: The authors have done a great job responding to both reviewers. They addressed each comment with care, which lead to clarify several aspects. In particular, I am very happy with the new way the authors present their alternatives and the corresponding predictions, this is much clearer and easier to visualize! The Mantel correlogram analysis is better described. I have one remaining major comment about alternatives (plus very few minor comments).

Response 8: Thank you for checking our edits and for your positive feedback!

Comment 9: Alternatives 1 and 5: Globally, I am confused with the new alternatives 1 and 5 which involve both dispersal rates and dispersal distances. This makes the predictions a bit hard to follow, and raises the question of why you did not include the other possible alternatives (eg dispersal rate males > females and dispersal distance males < females). Alt 1 and 5 may rather be seen as limitations of the study; ie the negative

relationship you expect to observe between relatedness and distance in males under your main hypothesis may be weakened by the strong dispersal of few males (alt1) or the weak dispersal of all males (alt5). Moreover, I am unsure about the predictions for alt 1 and 5. Maybe this reflects the difficulty to make accurate predictions when introducing more variables (rate and distance). Alternative 1: - analysis i: if only very few males disperse, I do not expect mean relatedness in males to decrease to the point of being similar to females, because there would still be many male relatives in the sampled area. - analysis iii: I do not expect a U-shaped relationship for males. In high distance classes, a majority of philopatric males would still be associated to unrelated males. Moreover, the few males that disperse would be associated to some related males at high distance, but mostly to many unrelated males, thus positive autocorrelation should not be expected for high distance classes. Alternative 5: - analysis iii: I do not expect an inverted U-shaped relationship for males. Males are not expected to be very unrelated at short distance if they do not move very far, so I don't think there will be a negative autocorrelation at short distance classes. Moreover, you will not find only relatives at intermediate distances, but also unrelated individuals, so there will be no positive autocorrelation for intermediate distance classes. To conclude, in my opinion, it would be better to keep things simple. It may be clearer to remove alternatives 1 and 5, and maybe include more information on signal weakness in the limitations of the study.

Response 9: Thank you for these considerations and explanations! We thought that we might be able to differentiate dispersal rate from dispersal distance, but your feedback helps us understand that this isn't feasible. We agree that not knowing the actual distances that either philopatric or dispersing individuals might move is better framed as a potential limitation to our ability to detect effects. We accordingly removed the previous Alternatives 1 and 5, and added: Analysis Plan > Ability to detect actual effects: "However, we do not know the average distance that either philopatric or dispersing individuals move. The scale of our sampling area might be so small that individuals of the sex that disperses the least are likely to have hatched outside of this area. In addition, there could be variation among either females or males in the distances individuals move, with potentially also a small proportion of individuals of the predominantly philopatric sex dispersing, which could obscure patterns in the small sample of individuals in our study. Accordingly, we might not be able to detect differences in average relatedness between females and males (analysis i), but we still might expect a sex bias in the geographic distances among relatives (analysis ii)."

Minor comments Comment 10: Reply 11 from the response to reviewers: I did not mean to suggest a new alternative; rather I was trying to understand the sentence I quoted. I think there has been a misunderstanding, because on the previous version of the manuscript the sample area was described as within a 500m circle (contrary to a 1000m radius circle in the present version). I was referring to the possibility that males move outside the campus area, but I was just trying to describe how I understood the sentence of the authors. Sorry for any confusion.

Response 10: We are sorry for the confusion! This resulted because we did not explain the change in the radius for the circle in the previous revision. We previously stated this as a circle with a 500m radius based on our experience in the field. As we added information on the sampling locations during the previous revision, we realized that the sample also included individuals from a site further away, which expanded our actual sample area to have a 1000m radius.

Comment 11: B. Hypothesis "the third [analysis] aims to determine separately for each sex whether individuals are likely to have dispersed or not": autocorrelation analysis does not provide the likelihood of dispersal for individuals, but describes the genetic structure in the population in regards with relatedness. Please reformulate.

****Response 11: Thanks for clarifying that we need to be more explicit about what spatial autocorrelation analyses provide (rather than what we infer from their results). We changed this to: Hypotheses: "the third aims to describe the genetic structure separately for each sex and whether relatives are predominantly found within certain distances from each other or whether relatives are not structured***

in geographic space (analysis iii: spatial autocorrelation).” *

Comment 12: Figure 1: please remove the box on the top left corner mentioning genetic diversity, or adapt its content in regards to relatedness. In the legend, change “higher levels of genetic relatedness than males” to “higher levels of genetic relatedness than females”

Response 12: We replaced the text in the box of the top left corner of Figure 1 to indicate that we are focusing on levels of average relatedness. We changed the text in the legend to “males will have higher levels of genetic relatedness than females because females likely have larger dispersal distances”.

Comment 13: Ability to detect actual effects: “the signal might be too inclusive in either or both sexes” I don't understand what you mean with “inclusive”... Weak?

Response 13: Yes, we meant weak. We now replaced ‘inclusive’ with ‘weak’ in: Analysis Plan > Ability to detect actual effects: “the signal might be too weak in either or both sexes to make inferences about sex differences”

Comment 14: Analysis Plan. Analysis iii: Given the modification of the sample area size from the first version of the manuscript, the authors have modified the bin size from 50 to 200m. I suggest being careful with this change: a too big bin size may lead to group both related and unrelated males in the smallest class, which will prevent detection of the expected positive autocorrelation. This is especially relevant here as males are expected to move around 50m because of the distance between trees if I understand correctly. Maybe a way to resolve this issue is to try several bin sizes as suggested in Peakall et al 2003: “Sampling at intervals greater than the scale of genetic structure will fail to detect the genetic structure at all, whereas sampling at intervals well below the scale of genetic structure may be associated with unnecessarily small sample sets, and therefore limited statistical power. Because there is no a priori way to predict the extent of genetic structure from a single autocorrelation analysis, we have calculated r (along with associated errors about r and the null hypothesis), for increasing distance class sizes that span the minimum distance between samples to the maximum distance of sampling (10–800 m). »

Response 14: Thank you! We planned to adjust both the size and number of bins for the autocorrelation analyses, but realize that the text in the analysis plan only described plans to reduce the number of bins (and accordingly increase the distances covered by each bin). We now amended the text. Analysis Plan > Analysis iii: “In case the pairwise distances among individuals are too small in each bin to detect a signal, then we will reduce the number of bins. In addition, we might adjust the distances covered by each bin to have shorter distances for the first few bins to increase the chance to detect relatives within the smallest bins (changing from 11 equally sized 200m bins to, for example, 9 bins at varying distances such as 0-50m, 50m-100m, 100m-150m, 150m-200m, 200m-500m, 500m-750m, 750m-1000m, 1000m-1500m, 1500m-2000m) (following @peakall2003spatial).”

Decision by [Sophie Beltran-Bech](#) , posted 22 November 2019

Requested revisions

The authors responded satisfactorily to the comments raised by the 2 reviewers. One of the reviewer provides other useful comments that should help authors to improve the manuscript. I suggest to follow these asked corrections carefully. In addition to responses to reviewer's comments, some typos require corrections (see below).

Following all these suggested corrections, I will recommend your pre-print.

My minor comments and suggestions:

- In your predictions (after alternative 5): there is a mistake in the typo of “individuals” in the sentence : “the first two aim to detect...among individuals” [but some modifications are proposed by the reviewer and perhaps, my proposition will be obsolete.

- For the figures 1 and 2: can you add an orientation (compass showing north for example) to your figures?
- Figure 1 legend: do you mean "than females" (and not than males)? Please correct.
- You compare the sex ratio 26 females / 11 males using mist nets (2.36 females per male) to your study but there is no reference cited with this 26/11 sex ratio. Where does this sex-ratio come from? Clarify please.
- For the "Sampling size rational" section, could you replace "This was the largest sample size possible by July 2019 when the DNA were sequenced using ddRADseq." by "The sample size presented was the largest one possible by July 2019 when the DNA were sequenced using ddRADseq."
- For the "data collection stopping rule" section, could you formulate it in sentence form (with a verb)? For example: "We decide to analyse all blood..." - The reference "Coulon et al." dates from 2006 and not 2005. Can you correct this point in all the preprint?

Reviewed by **Sylvine Durand**, 22 November 2019

[Download the review](#)

Evaluation round #1

DOI or URL of the preprint: <http://corinalogan.com/Preregistrations/gdispersal.html>

Authors' reply, 01 November 2019

Dear Dr.'s Beltran-Bech, Durand, and an anonymous reviewer, We greatly appreciate the time you have taken to give us such useful feedback! We are very thankful for your willingness to participate in the peer review of preregistrations, and we are happy to have the opportunity to revise and resubmit. We revised our preregistration and associated files at <http://corinalogan.com/Preregistrations/gdispersal.html>, and we responded to your comments below. Note that the version-tracked version of this preregistration is in rmarkdown at GitHub: <https://github.com/corinalogan/grackles/blob/master/Files/Preregistrations/gdispersal.Rmd>. In case you want to see the history of track changes for this document at GitHub, click the previous link and then click the "History" button on the right near the top. From there, you can scroll through our comments on what was changed for each save event and, if you want to see exactly what was changed, click on the text that describes the change and it will show you the text that was replaced (in red) next to the new text (in green). We think the revised version is much improved due to your generous feedback! All our best, Gus, Corina, Melissa, Luisa, Kelsey, Aaron, Carolyn, and Dieter

Round #1 Decision by Sophie BELTRAN-BECH, 2019-09-27 13:49

Manuscript: <http://corinalogan.com/Preregistrations/gdispersal.html> Requested revisions

The preregistration "Investigating sex differences in genetic diversity in great-tailed grackles in Tempe, Arizona to infer potential sex biases in dispersal" presents the analysis plan that will be used to genetically investigate sex-biased dispersal in great-tailed grackles. One of the reviewer and I agree that this preregistration submitted by August Sevchik et al. is a very good practice. However, the 2 reviewers suggested interesting ways to reinforce some of the authors' assumptions, as well as different methods for analyzing the data that I suggest to explore. A change in the term "genetic diversity", in some citations, and a better description of the sampling site to better understand the spatial analyses are rightly requested by the reviewers. Finally, reviewers provide useful corrections that should help authors to improve the manuscript. I suggest to follow these corrections carefully. Pending these changes, I would be glad to read a new improved version of the manuscript to eventually recommend it.

Reply to the editor: We are grateful for the constructive advice on our preregistration! We feel that the feedback has helped us to set up a stronger project. In response to the comments, we have included additional planned methods, provided more details on the sampling, clarified explanations for critical terms,

and added the code for the analyses. We provide detailed responses below (in italics; we refer to the section where we made the change at the end of each reply).

Reviews

Reviewed by anonymous reviewer, 2019-09-14 14:51 This preregistration presents the methods that will be used to genetically investigate sex-biased dispersal in great-tailed grackles in a small study site in Arizona. The authors sampled 53 adult birds (38 females, 15 males) which are being genotyped at SNP loci. Intended analyses include comparing relatedness within males vs. within females, and comparing how genetic relatedness decreases with geographic distances, in males vs. in females. I have major reservations about the second analysis, plus a few other comments/suggestions, all described below. Prediction 2: Given the spatial scale of the study (maximum distance between two individuals = 500m, if I understood well), I am very skeptical that a signal of decrease of genetic relatedness with distance can be found. Plus the sample size is very limited, which even decreases the power of the analysis. The analysis can be done, but I would be surprised if the result was informative for the study question. However, another test that can be done is to compare the geographic distance of related vs. unrelated individuals in both sexes. Lower geographic distances are expected for related individuals of the less dispersive sex. See for example Coulon et al. (2006).

Reply 1: Thank you very much for your feedback on our preregistration! We realize that the power of our approach is limited by the small sample size and small scale of the study area. Any signal we find would potentially be caused by the presence of first- or second-degree relatives among spatially close individuals of one sex but not the other, rather than a continuous change in relatedness in one sex but not the other. Accordingly, the method you suggested appears potentially better suited to detect any sex bias. We have now added these analyses, investigating whether the distances among any closely related males ($r \geq 0.25$ or $r \geq 0.125$) are shorter than the distances among any closely related females: Analysis Plan - Analysis ii: "Based on the calculations of pairwise genetic relatedness, we will select the subset of pairs who are estimated to be more closely related than cousins ($r \geq 0.125$) or half-siblings ($r \geq 0.25$). For this subset of individuals, we will determine whether the pairwise geographic distances are shorter for the males or the females in the sample (@coulon2005dispersal). We will perform 10,000 random draws of pairs of males and of females matching the numbers of inferred closely related dyads, and calculate the difference between the average geographic distances for each sex. We will assess 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."

A section describing the sampling site and design (esp., spatial distribution of samples of the two sexes) should be added. This is important for such a study that will partly rely on spatial analyses (test of prediction 2). Some details are provided in the preregistration but they are dispersed throughout the text. For example, if it impossible to know if the spatial distribution of individuals is even and hence allows an even repartition of distances among the 11 bins for the Mantel correlogram (but see above my reservations about this test anyway).

Reply 2: Thanks for pointing out that we needed these extra details. We now added a section on sampling, describing the location of the sampling locations and the number of individuals of either sex trapped at each location. We added two figures: Figure 2 now includes a map and the number of individuals from each sex that were trapped in each location, and Figure 3 shows a distribution of geographic distances between pairs of individuals). We also added the following: Methods - Planned Sample: "Males were not caught at all trap sites, but there are several sites at which multiple males were caught and sufficient sites for comparisons of males that were caught close to each other, at intermediate, and at long distances."

Prediction 1: This prediction can be made even without involving the notion of "populations", i.e. genetic relatedness between individuals can vary even within one population, so there is no need to refer to the fact

that females may come from other populations.

Reply 3: Yes, you are correct - our writing made it sound like we thought this was a closed population. We changed the text throughout to reflect that our sampling sites might not reflect a closed population and that we are referring to the movement of individuals from outside of the sampling area to inside our sampling area. In particular, with regards to the Hypothesis and Alternatives, we now state whether we expect either males or females to remain or disperse away from where they hatched.

I think the term “genetic diversity” used at several places in the preregistration (sentence above predictions, first sentence prediction 1 alternative 1, prediction 1 alternative 2, legend figure 1) is not appropriate, because the authors will actually work on genetic relatedness. Both are related, of course, but the use of the term genetic diversity is confusing because usually understood as, for example, the number of alleles per locus, or heterozygosity, when those metrics will not be used in this study.

Reply 4: Great point! We replaced all instances of the term ‘genetic diversity’ with the indeed more accurate term of ‘genetic relatedness’ (Title, Abstract, Hypothesis, Figure 1 legend, Analysis Plan - Analysis i, Analysis Plan - Analysis iii).

Dependent variable: average relatedness between all pairs of individuals WITHIN ONE SEX

Reply 5: We changed this accordingly: Methods - Dependent variable: “Average relatedness between all pairs of individuals within one sex”

Reviewed by Sylvine Durand, 2019-08-28 11:22 This preregistration presents the detailed analysis plan to test for the presence of female-biased dispersal in a bird population. The authors will compare average relatedness between males and females, and use individual geographic locations to look for different patterns of isolation-by-distance between sexes. The results will help the authors to contextualise their findings on behavioural flexibility. First of all, I commend the authors for submitting a preregistration, this is very good practice! The aim and hypothesis are clearly stated. However, I have several comments, most of them requiring more precision about the population genetics concepts underlying the analyses and about the cited literature. I would like more emphasis on the (likely) possibility that the sample may not allow detecting sex-biased dispersal even if it exists. I would also like the authors either to reassure me about the Mantel correlograms they plan to use, or to switch to another suggested analysis. I hope that the authors will not be annoyed by the amount of (overly?) detailed comments, and that they will find them helpful. Title: Genetic diversity (number of alleles) and genetic relatedness (allele sharing) are two distinct concepts. Since genetic diversity is only mentioned in the Hypothesis section, the authors should change “diversity” into “relatedness”, especially as they do not plan to investigate diversity.

Reply 6: Thank you very much for your support of preregistrations and for all of your (not overly!) detailed feedback! This is a good point and we replaced all instances of the term ‘genetic diversity’ with the indeed more accurate term ‘genetic relatedness’ (see Reply 3 above).

Abstract: “... and to potentially infer the distance that individuals move”: how do you plan to estimate this? The mentioned analyses do not allow such estimation, and I don’t think it is possible to infer an absolute dispersal distance by using genetic relatedness. At best, one could estimate the distance below which individuals are genetically more similar to each other than to the average individual, using spatial autocorrelation analyses for example. However this distance is very dependent on the sampling scheme, and cannot be considered as an absolute dispersal distance (for more information, see Peakall et al. (2003)).

Reply 7: We realize that with our approach we are not able to determine the actual distance that individuals move, only whether individuals of one sex might on average move further distances than individuals of the other sex. Given the limited sample size and spatial range of our sampling scheme it is highly unlikely that we will have a sufficient number of pairs of individuals with an inferred close relationship (i.e. parent-offspring)

to potentially even begin to estimate dispersal distances. We have removed this from the preregistration. (Abstract)

B. Hypothesis I would have liked to see some kind of “mirror” between P1 and P2. For instance, the situation where males disperse more than females is only mentioned for P1 but not P2, and the possibility of a too low genetic diversity is only mentioned for P2. Also, the authors chose to present two alternatives (P1 alt2 and 3) leading to the same expected result (no male-female difference), whereas P2 alt2 presents a single expected result (no relationship between distance and relatedness) with several explanations. Please homogenize the predictions and alternatives.

Reply 8: We realize that we had mixed predictions about the behavior of individuals with predictions about the signal we are expecting in the analyses we are performing. We now made this systematic: we first have the hypothesis about sex bias in dispersal and the potential alternative scenarios, and we next list the specific predictions for the hypothesis and all five alternatives for each of the three types of analyses we run (sex bias in average levels of genetic relatedness; sex bias in geographic distances between close genetic relatives; and correlogram determining spatial autocorrelation in genetic relatedness for each sex) (Hypothesis).

I am generally concerned about the use of the terms “genetic diversity” and “genetic variants” that the authors use several times throughout this section. Genetic diversity refers to the number of alleles in a population, and I guess that authors use “genetic variants” for “variants with new alleles”. It is true that immigrants from outside populations can bring new alleles. However, this is not always the case, and it is in my opinion not so expected for this population. If all females disperse before breeding at each generation, surrounding populations should be connected by an important gene flow. As such I do not expect surrounding populations to be strongly differentiated from the focal population; they should present a rather similar allelic composition. The argumentation should be more based on genotypes than on diversity. Of course, I may be misinterpreting the text, but I think that the hypotheses and predictions deserve a clear statement and that the authors should rephrase with less emphasis on diversity. In particular, Fig 1 (which should be called in the text in the P1 section) is misleading and really conveys the idea that the authors heavily rely (only?) on allelic diversity to explain the expected patterns of relatedness.

Reply 9: We realize that our sampling sites might not reflect a closed genetically differentiated population, where immigrants would be likely to contain alleles not usually found among the individuals living in the area. We removed these statements about allelic composition and genetic diversity and replaced them with language that reflects that the focus of the investigation is on differences in genetic relatedness. (Title, Abstract, Hypothesis, Figure 1 legend, Analysis Plan).

P1 alt3: Please explicitly state the expected result (no male-female difference). Besides, this alternative is not entirely clear to me, especially with regards to what the authors call “the broader population”. Even if individuals move a distance smaller than the area, we can still consider the campus as one (viscous) population, the consequence being that all levels of relatedness (close relative as well as unrelated pairs) will be similarly included in the sample for both sexes, independently of “new genetic variants”. If the distance moved is broader than the scale of the campus, then the sample can be seen as only a part of a bigger population from which all individuals (including males) originate. In my opinion, it would be safer to simply say that the area is too small to host clustered relatives (whatever the sex), rather than hypothetically evocating multiple populations.

Reply 10: As a result of this and the other comments above about populations, we now removed this prediction because we realized that our study site is not a separate population. All of our predictions now refer to the distance individuals move and the likelihood of their being geographically close to genetic relatives (Hypothesis).

P2 alt1 1): "the distances between trap sites are not large enough to detect a decline in relatedness": I do not understand how increasing distance between traps could help to detect a decline; on the contrary, the analysis would lose resolution. Unless the authors mean that the maximum distance between traps is not enough, ie males move more than 500m, but this goes back to P2 alt1 2) "males move away from their hatch sites to breed".

Reply 11: Good point! We incorporated your comment into the new Alternative 5. Hypothesis - Alternative 5: "Males remain not on the exact site they hatched but within the general area, while females disperse farther distances."

I can also think of other P2 alternatives: - alt3: There is a negative correlation between distance and genetic relatedness in females and no correlation in males, if males move more than females. - alt4: There is a negative correlation for both sexes, if both sexes have a dispersal distance shorter than the scale of the area. This alternative can be subdivided: 1) males present a stronger signal than females, suggesting female-biased dispersal, or 2) there is no difference between males and females, either because dispersal is not sex-biased, or because sex-biased dispersal cannot be inferred from this analysis because of a too small difference in distance moved between males and females (see later comment about limitations of the study).

Reply 12: Thank you for the suggestions of these alternatives! We now included them. Hypothesis - Alternative 2: "Males disperse away from where they hatched, while females remain where they hatched." Hypothesis - Alternative 3: "Individuals of both sexes remain close to where they hatched."

C. Methods Planned sample: Just a question out of curiosity: your sample is clearly female-biased; does it represent the population sex-ratio? If yes, then another (complementary) explanation to female-biased dispersal could be a higher intrasexual competition for females, leading to their dispersal (local mate competition hypothesis (Dobson 1982)). If the overall sex-ratio is balanced, then there could be a bias in sampling due to a higher female mobility if they disperse more.

Reply 13: This is a great question, which caused us to analyze our trapping data. We found that the larger number of females in our sample appears to reflect the population sex ratio and we added new text as follows: Planned sample: "The larger number of females than males in our sample appears to reflect the adult sex ratio at this study site. We estimated the sex ratio based on grackles that were trapped in mist nets, which is a trapping method that likely does not elicit a sex bias in terms of which sex is caught because the nets are invisible. Therefore, even if one sex is more neophobic than the other, both sexes are likely to be trapped using this method. A total of 26 females and 11 males were trapped using mist nets (a ratio of 2.36 females per 1 male), which is very similar to the sex ratio in our sample consisting of 40 females and 17 males (2.35 females per 1 male)."

D. Analysis plan Ability to detect actual effects: I believe that some papers are cited inappropriately: - Relatedness "has been shown to be as effective as Fst measures ...": in the cited paper, relatedness is calculated among populations, as explicitly shown by the formula linking relatedness to Fst (which can only be calculated between pairs of populations, not pairs of individuals). Their whole argumentation and simulations are performed over several populations with an island model for dispersal. They do not argue in favour of individual-based relatedness to infer sex-biased dispersal, but of population-based relatedness, which is not applicable for the present manuscript focusing on a single population.

Reply 14: As mentioned in Reply 3, we now realize that the conceptualization of our study site as a closed population is unlikely to be accurate. We have therefore removed this statement and revised the text as follows: Ability to detect actual effects: "We only have individuals from within a single site, so we cannot 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). We therefore rely on measuring genetic relatedness between pairs of individuals."

Relatedness “can detect even modest sex biased dispersal in fine-scale spatial distribution (Banks & Peakall 2012)”: this paper investigates the power of spatial autocorrelation analyses, not the pertinence of relatedness as an estimator of genetic distance.

Reply 15: We now changed this statement to specifically refer to the autocorrelation analysis we are using to detect sex biased dispersal. Ability to detect actual effects: “Approaches relying on spatial analyses of multi-locus genotypes have been shown to be able to detect even modest sex biased dispersal in fine-scale spatial distribution, in particular analyses of spatial autocorrelation (@banks2012genetic). However, our sample size is small, meaning that we might have only limited power to detect potential differences between females and males (@goudet2002tests). For the spatial distribution of relatives (Analysis ii), the number of related individuals in our sample might be too small to detect a strong pattern of the relatives of one sex being more geographically closer to each other than relatives of the other sex. For the isolation-by-distance leading to a change in relatedness within the range of our sampling locations, the signal might be too inclusive in either or both sexes to make inferences about sex differences (Analysis iii). However, for the comparison of average relatedness (Analysis i), given that we have a large number of SNP loci, we expect that we should have sufficient power to obtain a qualitative assessment of whether relatives are present in our sample (@wang2009parentage) and accordingly whether dispersal is more prevalent in either females or males (examples of empirical studies that detected a signal with small sample sizes include @hofmann2012evidence, @quaglietta2013fine, @gour2013philopatry, @botero2017variation).”

“We restrict our sample to adults [...] to avoid patterns of sex-bias in relatedness that arise from differential reproductive skew or mortality”: adult-only sampling is an imperative requirement to detect sex-biased dispersal because genomes of both sexes are mixed at each generation after reproduction, not because of other sex-related bias in relatedness. Besides, I do not understand how reproductive skew or differential mortality could bias relatedness according to the sex of offspring.

Reply 16: We realize that we did not express this clearly. The rationale for only including adults in the sample is indeed the explanation you provided, that our aim is to detect sex biases in natal dispersal. The reference to reproductive skew and differential mortality came from imagining an extreme situation in which there would only be a single male surviving and in turn having offspring with multiple females. In this scenario, when including the offspring, all males would be closely related to each other (either as father-son or siblings) while many of the females would not be closely related to each other. We removed this from the Ability to detect actual effects section to avoid any confusion.

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An additional factor may limit the ability of this study to detect sex-biased dispersal. Since genes are mixed at each generation, sex-biased dispersal can only be detected if there is a strong difference in dispersal between sexes. A low difference between sexes may then prevent the detection of a signal, especially given the low sample size. This should be mentioned, and preferably included in the predictions, similarly to what the authors have already done by including P1 alt3, or P2 alt1. Or, to avoid overloading the prediction section, the authors can also limit themselves to a “no difference between sexes due to power limitation” prediction, and later detail the potential reasons for this limitation in the appropriate section (including current P1 alt3 and P2 alt1). These are just suggestions, I do not know which is best, but I would appreciate some homogeneity when mentioning limitations across the study.

Reply 17: The limited power would indeed affect all analyses. We have now clarified how the small sample size and low power might influence each of the three analyses. (Ability to detect actual effects - see our Reply 14 for the revised text)

“the sample should be sufficient to obtain a qualitative assessment of whether dispersal is more prevalent in

either females or males (Banks & Peakall 2012)”: I disagree with this statement. On the contrary, Banks and Peakall conclude that sample size should be maximized (even at the expense of loci number). The fact that other studies detected a signal with a small sample size does not guarantee that this size will be sufficient to detect any pattern of sex-biased dispersal; rather, it suggests that sex-biased dispersal can be detected with low sample size under some conditions (maybe the signal was particularly strong in these populations, maybe sample sex ratio was more balanced...). As stated by the authors, male sample size is particularly low, so actual isolation by distance may easily go undetected for this sex, especially if male spatial structure is weak. This should be clearly stated in the limitations of this study. On the other hand, maybe ddRADseq can generate a number of SNPs high enough to compensate for low sample size, but I suppose this number has to be very high since allelic diversity (max 4 alleles per locus) is very low for these markers (though this remains very speculative!!).

Reply 18: We have changed this section to be clearer about the limitations of our approaches, and we now mention that the small sample size might prevent us from detecting signals of spatial structure. Based on the data we received from the ddRadSeq processing in September 2019, it appears that we will have >400 SNP loci where both alleles occur at high frequencies and >3600 SNP loci in total. (Ability to detect actual effects - see our Reply 14 for the revised text)

P1: average relatedness and sex This analysis seems sound, even though few studies have explored the power of this method. I suppose that “higher than the majority” is higher than in 95% of the permutations?

Reply 19: Yes, in addition to a qualitative assessment of how the observed values of average relatedness compare to the simulated ones, we will also assess whether this passes a 95% threshold for comparison with alternative approaches. The revised text is as follows: Analysis Plan - Analysis i: “We will compare the mean and variance in average relatedness among all females to that among all males. Since average relatedness tends to decrease as the number of individuals in the sample increases (regression to the mean), we will perform a permutation analysis to investigate whether the average relatedness among the males or among the females in our sample is higher than what would be expected for a random sample of the same number of females or of individuals of both sexes. We will perform 10,000 random draws of 17 individuals either from among the females or from among all individuals and of 40 individuals from among all individuals, and generate distributions of average relatedness among these samples. We will assess whether the observed average relatedness among the 17 males or the 40 females in our sample is higher than what is observed in the majority of random samples. We will report the proportion of samples lower than the actual values and, for comparison with other approaches, assess whether the observed values are higher than 95% of all random draws.”

P2: average relatedness and trap site distance I am not familiar with Mantel correlograms, and I find that Aguillon et al (2017) were not really clear in their explanations, so I have some questions about the interpretation of this analysis. How will you assess that there is a “stronger negative correlation” in one sex compared to the other? Do you expect to see a significant association spanning over more distance classes in the philopatric sex? Do you plan to compare the magnitude of the association (=comparing r values between sexes)? After a quick search, I did not find many studies using Mantel correlograms to test for sex-biased dispersal. Moreover, Legendre and Fortin (2010) conclude that Mantel correlograms are not very powerful and should be avoided when other methods are available. Of course, if the authors find more support for Mantel correlograms and can argue that this method is relevant to detect sex-biased dispersal, I will have no objection to this analysis. I nevertheless suggest to consider a different way to analyse the data. In particular, Legendre and Fortin (2010) mention spatial autocorrelation analyses which are easy to perform using the program Genalex (Peakall and Smouse 2006) and have successfully detected sex-biased dispersal in a variety of species (Peakall et al. 2003; Neville et al. 2006), including some studies already cited by the authors (Singh Gour et al. 2013, Delgadillo et al. 2017). Maybe the authors should consider the possibility of performing such analyses (and adapt the

manuscript in consequence, as the method is based on spatial autocorrelation and not on isolation by distance).

Reply 20: We realize that our preregistration, and the paper by Aguillon et al. 2017 that we referred to, did not provide sufficient information on our planned approach. The method we are intending to use is a correlogram and not a single Mantel test. It was established by Legendre & Legendre (Numerical Ecology 2012 Elsevier), and is a follow up to the manuscript you mentioned (Legendre & Fortin 2010), where, in the final paragraph, the authors suggested to determine the presence of spatial autocorrelation with correlogram analysis. We added more details about the approach to the manuscript. In brief, the approach relies on partitioning the geographic locations into a series of discrete distance classes. For each distance class, a separate matrix is generated coding whether a given distance in the matrix falls within that range or not. Each value is tested for significance using permutations. The result of this set of analyses is a Mantel's correlogram, completely analogous to an autocorrelation function but performed on a set of distance matrices. The permutation statistics, plotted against distance classes, produce a multivariate correlogram. As in the spatial autocorrelation analyses in the program Genalex, these analyses are performed separately for each sex to determine whether isolation-by-distance might occur and indicate dispersal of the individuals of that sex. We opted for the correlogram approach over the use of that implemented in the program Genalex as it can be implemented with the open source software R and does not rely on proprietary software (Microsoft Excel). The revised text is as follows: Analysis Plan - Analysis iii: "For males and females separately, we will link the matrices of average relatedness and of geographic distance between all pairs of individuals by first plotting genetic relatedness against geographic distance and next by assessing the strength of their association using Mantel correlograms. We will use the function 'mantel.correlog' in the vegan package (@oksanen2013package) in R, performing 10,000 permutations to assess the strength of the association. This approach relies on the establishment of the multivariate Mantel correlogram by @legendre2012numerical. The approach relies on partitioning the geographic locations into a series of discrete distance classes. The result of this set of analyses is a Mantel's correlogram, analogous to an autocorrelation function but performed on a set of distance matrices. For each distance class, a separate matrix is generated and codes whether a given geographic distance between a pair of individuals falls within that range or not. A normalized Mantel statistic is calculated using permutations for each distance class. The permutation statistics, plotted against distance classes, produce a multivariate correlogram. These analyses are performed separately for each sex to determine whether isolation-by-distance might occur and indicate dispersal of the individuals of that sex. A stronger negative correlation between genetic relatedness and spatial distance for males than for females would indicate that males disperse shorter distances than females, and in particular we expect that males captured at the same trapping site will be much more closely related to each other than females captured at the same trapping site."

Decision by [Sophie Beltran-Bech](#) , posted 27 September 2019

Requested revisions

The preregistration "Investigating sex differences in genetic diversity in great-tailed grackles in Tempe, Arizona to infer potential sex biases in dispersal" presents the analysis plan that will be used to genetically investigate sex-biased dispersal in great-tailed grackles. One of the reviewer and I agree that this preregistration submitted by August Sevchik et al. is a very good practice. However, the 2 reviewers suggested interesting ways to reinforce some of the authors' assumptions, as well as different methods for analyzing the data that I suggest to explore. A change in the term "genetic diversity", in some citations, and a better description of the sampling site to better understand the spatial analyses are rightly requested by the reviewers. Finally, reviewers provide useful corrections that should help authors to improve the manuscript. I suggest to follow these corrections carefully. Pending these changes, I would be glad to read a new improved version of the manuscript to eventually recommend it.

Reviewed by anonymous reviewer 1, 14 September 2019

This preregistration presents the methods that will be used to genetically investigate sex-biased dispersal in great-tailed grackles in a small study site in Arizona. The authors sampled 53 adult birds (38 females, 15 males) which are being genotyped at SNP loci. Intended analyses include comparing relatedness within males vs. within females, and comparing how genetic relatedness decreases with geographic distances, in males vs. in females. I have major reservations about the second analysis, plus a few other comments/suggestions, all described below.

1. Prediction 2: Given the spatial scale of the study (maximum distance between two individuals = 500m, if I understood well), I am very skeptical that a signal of decrease of genetic relatedness with distance can be found. Plus the sample size is very limited, which even decreases the power of the analysis. The analysis can be done, but I would be surprised if the result was informative for the study question. However, another test that can be done is to compare the geographic distance of related vs. unrelated individuals in both sexes. Lower geographic distances are expected for related individuals of the less dispersive sex. See for example Coulon et al. (2006).
2. A section describing the sampling site and design (esp., spatial distribution of samples of the two sexes) should be added. This is important for such a study that will partly rely on spatial analyses (test of prediction 2). Some details are provided in the preregistration but they are dispersed throughout the text. For example, if it impossible to know if the spatial distribution of individuals is even and hence allows an even repartition of distances among the 11 bins for the Mantel correlogram (but see above my reservations about this test anyway).
3. Prediction 1: This prediction can be made even without involving the notion of "populations", i.e. genetic relatedness between individuals can vary even within one population, so there is no need to refer to the fact that females may come from other populations.
4. I think the term "genetic diversity" used at several places in the preregistration (sentence above predictions, first sentence prediction 1 alternative 1, prediction 1 alternative 2, legend figure 1) is not appropriate, because the authors will actually work on genetic relatedness. Both are related, of course, but the use of the term genetic diversity is confusing because usually understood as, for example, the number of alleles per locus, or heterozygosity, when those metrics will not be used in this study.
5. Dependent variable: average relatedness between all pairs of individuals WITHIN ONE SEX

Full cited reference: Coulon, A., J.-F. Cosson, N. Morellet, J.-M. Angibault, B. Cargnelutti, M. Galan, S. Aulagnier, and A. J. M. Hewison. 2006. Dispersal is not female-biased in a resource-defence mating ungulate, the European roe deer. *Proceedings of the Royal Society of London B* 273:341-348.

Reviewed by [Sylvine Durand](#), 28 August 2019

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