

Review of the preregistration “Investigating sex differences in genetic diversity in great-tailed grackles in Tempe, Arizona to infer potential sex biases in dispersal” by Sevchik et al., submitted to PCI Ecology

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.

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)).

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.

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.

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.

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”.

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).

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.

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 F_{st} measures [...] (Goudet et al. 2002)”: in the cited paper, relatedness is calculated among populations, as explicitly shown by the formula linking relatedness to F_{st} (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.

- 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.

“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.

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.

“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!!).

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?

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).

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