Investigating sex differences in genetic relatedness in great-tailed grackles in Tempe, Arizona to infer potential sex biases in dispersal

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Abstract

In most bird species, females disperse prior to their first breeding attempt, while males remain close to the place they were hatched for their entire lives. Explanations for such female bias in natal dispersal have focused on the resource-defense based monogamous mating system that is prevalent in most birds. In this system, males are argued to benefit from philopatry because knowing the local environment can help them to establish territories to attract females, while females are argued to benefit from dispersing because they can find suitable unrelated mates. However, theoretical, field, and comparative studies highlight that the exact factors shaping dispersal decisions are often more complex. Studying species with different social and mating systems can help illuminate the relative role of various factors in the evolution of sex biased dispersal. Here, we use genetic approaches to determine whether females and/or males disperse in great-tailed grackles (*Quiscalus mexicanus*), which have a mating system where the males hold breeding territories that multiple females might choose to place their nest in, but females forage independently of these breeding territories across a wider area. First, we find that, for individuals caught at a single site in Arizona, the average relatedness among all female dyads is higher than expected at random, whereas average relatedness among all males dyads is not. Second, we find that female close relatives are found within shorter distances from each other than pairs of unrelated females, whereas male close relatives are found at larger distances from each other than pairs of unrelated males. Third, we find a decline in relatedness with increasing spatial distances for females, but not for males. 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. Our findings show that reduced resource competition might facilitate female philopatry and that prior knowledge of an area does not appear to be a prerequisite for male great-tailed grackles to establish breeding territories.

Introduction

Maturing birds face a decision about where to establish themselves for breeding. In the majority of avian species, the potential costs and benefits of breeding movement decisions appear to differ between the sexes, with males remaining in the area they hatched while females move to breed elsewhere (Greenwood 1980). The main theory proposed to explain this sex bias towards male philopatry has focused on the resource-defense based monogamous mating system found in most bird species (Greenwood 1980; Trochet et al. 2016). In monogamous systems, males tend to stay philopatric to defend an area they know to provide resources to attract females, whereas females disperse to avoid the risk of inbreeding with close relatives who dominate reproduction in the area. However, alternative hypotheses about the benefits and costs of philopatry or dispersal could equally apply to explain the dominant female bias in dispersal among species with resource defense based monogamy. In general, it is likely that, in both sexes, decisions of whether to remain in the area or to move short or substantial distances to new breeding grounds are influenced by an interplay of the potential costs of movement, resource availability and competition, and the potential benefits or costs of interacting with close relatives (Mabry et al. 2013; Trochet et al. 2016; Li and Kokko 2019). One way toward a better understanding of the relative role of the various factors that potentially explain breeding movement decisions of both female and male birds is to study dispersal in species with different social and mating systems.

Studying dispersal outside of well established study systems is difficult, which means that there is only limited information from bird species with unusual social and mating systems. It is challenging to set up studies that span a large geographical area where the identity of many individuals can be established and followed. As such, the fate of individuals who leave the area often remains unknown and it is unclear whether new individuals found in the area have moved to the area or were simply not observed previously (Walters 2000). To overcome these challenges, genetic approaches are now incorporated to identify dispersal patterns (Lawson Handley and Perrin 2007; Banks and Peakall 2012). In particular, to identify potential sex biases in dispersal, two approaches are used. The first approach relies on determining the spatial distribution of variants of genetic markers that have a sex-specific inheritance (Lawson Handley and Perrin 2007). The second approach uses data from a large number of genetic markers spread across the genome to determine how the similarity across these markers changes with increasing spatial distances among males and females (Banks and Peakall 2012). Studies based on the second approach have increased in recent years because the costs of generating genotypes for a large sample of individuals have rapidly decreased (Harrison, York, and Young 2014; Weinman, Solomon, and Rubenstein 2015; and Thrasher et al. 2018).

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 are a highly social passerine bird found in the Americas. Great-tailed grackles have a wide range of foraging habits, including exploiting human foods. Individuals forage in small fission-fusion groups in ranges that are not obviously defended against other individuals, and at night they roost in large associations. 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 (Johnson et al. 2000). Previously, 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 (Selander 1970; Folsom et al. 2020). Both the mating and the social system are accordingly different from the resource-defense based monogamous system found in the majority of birds, offering an opportunity to determine if and how these differences might influence the dispersal behavior of both males and females.

Hypotheses

Main hypothesis: Based on the argument that males are expected to be philopatric when they defend resources beneficial to females, and that, in response, females disperse to avoid mating with relatives, our main hypothesis predicts that there are sex differences in the natal disperal rate and distance among individuals in great-tailed grackles, with males remaining close to where they hatched and females moving away from where they hatched.

To determine whether other factors play a larger role in shaping dispersal, we assess three alternative hypotheses:

Alternative hypothesis 1: Based on observations that great-tailed grackle males only compete during a short period to gain access to small, distinct breeding territories, without defending resources for females for an extended period of time, males might move to areas where mating opportunities are higher or competition is lower. In this case, inbreeding risk might not be high for females, and we predict sex differences in the opposite direction with males dispersing away from where they hatched and females remaining where they hatched.

Alternative hypothesis 2: The polygamous mating system of great-tailed grackles, where females might be able to choose among potential males, might reduce a female's risk of mating with their father or brother. In this case, individuals of both sexes can remain close to relatives, and we predict that individuals of both sexes remain close to where they hatched.

Alternative hypothesis 3: Given that great-tailed grackle individuals of both sexes do not establish territories around resources (food or mates), they might move multiple times throughout their lives to areas where competition is the lowest. In this case, we predict that individuals of both sexes disperse away from where they hatched.

We predict that the movement of individuals will influence the spatial distribution of genetic relatives. Individuals of the sex who remain close to where they hatched are expected to be close to genetic relatives while individuals of the sex who disperse are expected not to be close to genetic relatives. We also expect that the further the distance an individual moves, the less likely they are to be even distantly related to another individual within the study area. Our hypotheses generate specific predictions about contrasts in the levels of relatedness and the spatial distribution of genetic relatives according to whether individuals are philopatric or disperse. We will assess these predictions in three analyses: first, higher levels of average relatedness are expected among all individuals of the philopatric sex than among all individuals of the sex that disperses (analysis i: average levels of relatedness among individuals in our sample); second, we predict that there are sex biases in levels of average genetic relatedness among indivduals found within a certain distance of each other, where finding close genetic relatives in short distances from each other indicates that these individuals have remained philopatric (analysis ii: geographic distances between individuals that are close genetic relatives); and third, a decline in levels of relatedness as distances among individuals increase, indicating that individuals have remained philopatric, whereas no structure of relatedness in geographic space, indicating that individuals disperse (analysis iii: spatial autocorrelation).

Associated Preregistration

Our hypotheses, methods, and analysis plans are described in the peer-reviewed preregistration of this article. Details on the final methods, including all data and code, are listed the methods below.

Deviations from the preregistration

Analyses began in March 2020 after the preregistration passed pre-study peer review at *Peer Community In Ecology* in November 2019. During the preparation of the analyses, we noticed that we made a mistake when calculating the sex composition in the sample: different from what was written in the preregistration, the sample for our genetic analyses

consists of 41 (not 40) females and 16 (not 17) males. In addition, we realized that the sample included some juvenile individuals (<1 yr of age). We excluded these 4 juveniles from the main analyses because they might have been pre-dispersal at the time of capture. The dataset for the relatedness analyses therefore consisted of 37 adult females and 15 adult males.

We made the following changes and additions to the analyses, all of which test existing predictions and rely on approaches described in the preregistration:

- **ddRadSeq single nucleotide polymorphism (SNP) filtering:** In addition to using the parameters of (Thrasher et al. (2018); loci only considered if they were present in 80% of the samples ® and had a minimum frequency of the minor allele of 0.05 (min maf); e.g., the rare variant at a loci is present in at least 5% of the samples), resulting in 3647 SNPs), we repeated the filtering with more stringent conditions (loci only considered if they were present in 95% of the samples (r), minimum minor allele frequency of 0.05 (min maf), resulting in 635 SNPs). We decided to use the resulting genotypes from the second, more restrictive setting for the relatedness analyses because of our small sample size (e.g., if some individuals had a lower quality sample, their relatedness to other individuals might consistently be misclassified) and because these settings still provided a large number of SNPs for analyses.
- **Exclusion of genotypes:** We noticed that one individual, female A053PS, was indicated to be related to all other individuals in the sample. When we checked the genotype of this individual, we noticed that it had much higher levels of heterozygosity, independent of how we filtered the data. The genotype did not show signs of shifting of alleles between loci (e.g., showing a genotype that lists allele 2 from locus 1 with allele 1 from locus 2 and so on) or issues with missing data or the dropout of alleles. Instead, the increase in heterozygosity was always close to the square of the heterozygosity observed among the remaining individuals, suggesting that this genotype might be constructed from two different individuals. We were not able to retrace where such an error might have been introduced, and accordingly we decided to exclude individual A053PS from the analyses.
- **Relatedness estimator:** The 'compareestimator' function in the R package 'related' caused fatal errors on multiple computers. We therefore calculated pairwise relatedness using two estimators: 1) the estimator by (Wang 2002), following the observation in (Thrasher et al. 2018) of the suitability of this estimator for ddRadSeq data, and 2) the estimator by (Queller and Goodnight 1989), which has been the standard for multiple studies. With both sets of relatedness estimates, all of our inferences (high levels of average relatedness among females, shorter distances among closely related females, spatial structure among female genotypes) were similar. We only present the results based on the estimator by Queller & Goodnight because we noticed that, with our data, the estimator by Wang appeared to be more influenced by missing data in the genotypes.
- **Analysis i average relatedness and sex:** We adjusted the permutations to reflect the actual sex composition in our sample.
- **Analysis ii distances among genetic relatives:** We did not perform the permutation to assess whether the difference in the average distance among closely related females and the average distance among closely related males was different than expected because of the very low number of closely related male dyads in our sample (only a single dyad). Instead, we performed a permutation to assess whether the average distance among closely related female dyads (r>0.2499) was shorter than the average distance among a random sample of the same number of female dyads.
- **Analysis iii relatedness and geographic distance:** For the correlogram analyses with set distance classes, we added a configuration where we set the distance classes using information on the average distance among close genetic relatives from analysis ii. We spaced the distance classes such that the observed average distance among close female kin (~330m) and among close male kin (~670m) fell about halfway between the breakpoints for the distance classes (set at 0-150m, 150-450m, 450-900m, 900-1500m, 1500-2000m).

Results

Genotyping

We generated SNP genotypes for 57 individuals from our study site in Arizona (we excluded the 5 individuals later, see State of the Data for details). We retained 635 SNPs. Data was missing for 2.7% of all alleles, with no individual or locus showing a particular underrepresentation of information. All loci had 2 alleles and the observed heterozygosity was 0.48, slightly higher than the heterozygosity expected in a population with the same allele frequencies and random mating. The probability of identity for siblings, the chance that two siblings will show the same genotypes given the allele frequencies across these 635 loci, is less than 10 to the power of minus 139.

Analysis i: average relatedness and sex

The average relatedness among the 37 adult females (666 dyads) is -0.013 (standard deviation, SD=0.07), the average relatedness among the 15 adult males (105 dyads) is -0.015 (SD=0.08), and the average relatedness among all 52 adult individuals in our sample (1326 dyads) is -0.017 (SD=0.07). To assess whether the average relatedness among females is higher than expected, we randomly drew 37 individuals and calculated their relatedness. In less than 4% of the draws did we observe a level of relatedness as high as or higher than what we found in our sample of females (Figure 1). 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 among males is not different from that expected by chance among 15 randomly drawn individuals (40% of random samples give a value as low as or lower than what we found in our sample of males). Of the eight close genetic relatives (relatedness of 0.25 or higher), seven are female dyads and one is a male dyad.

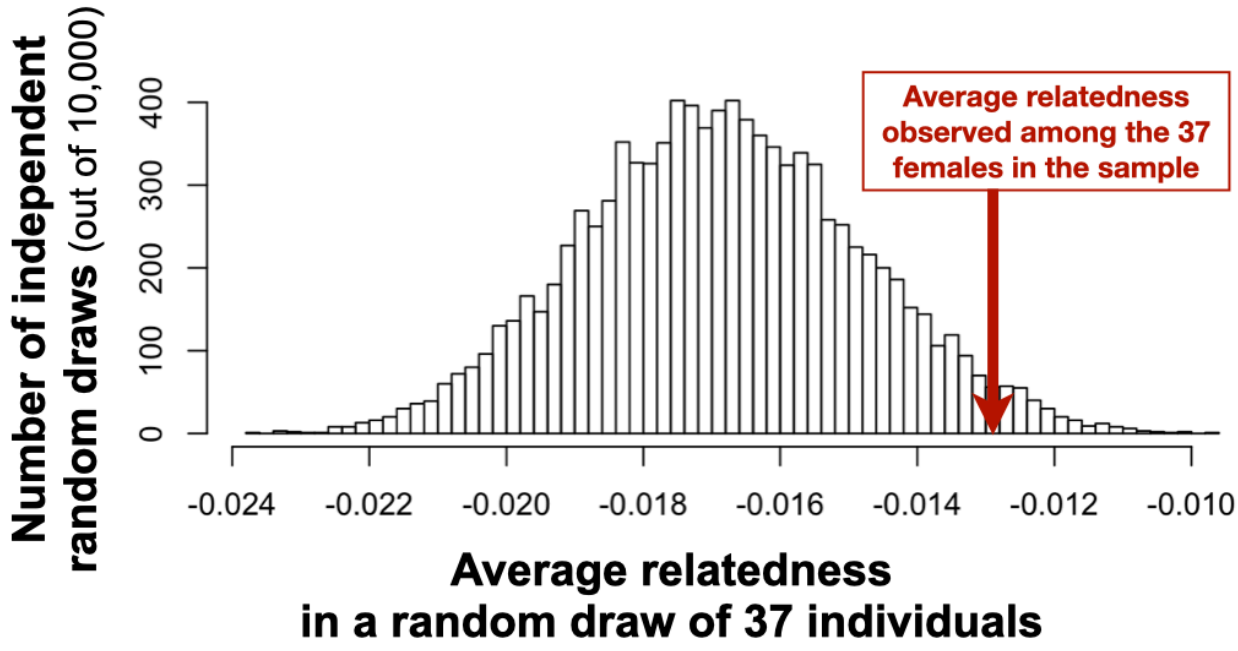

Figure 1: **Figure 1.** In only a small number of random draws is the average relatedness among 37 individuals as high or higher than the observed relatedness among the 37 females in our sample.

Analysis ii: distances among genetic relatives

Close female genetic relatives were found to have been trapped in close spatial proximity to each other (Figure 2). The median distance between the eight female dyads related at 0.25 or closer is 340m (SD=440m) and between the twelve female dyads related at 0.125 or closer is 360m (SD=354m), compared to a median of 620m (SD=464m) among all dyads of females. A median distance as short or shorter than 340m is observed in less than 6% of all random samples of 7 female dyads and a median distance of 360m or shorter is observed in less than 4% of all random samples of 12 female dyads. Therefore, the closely related female dyads were found at shorter distances than expected by chance. 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 fewer apart. Therefore, the closely related male dyads were found at distances that would be expected by chance.

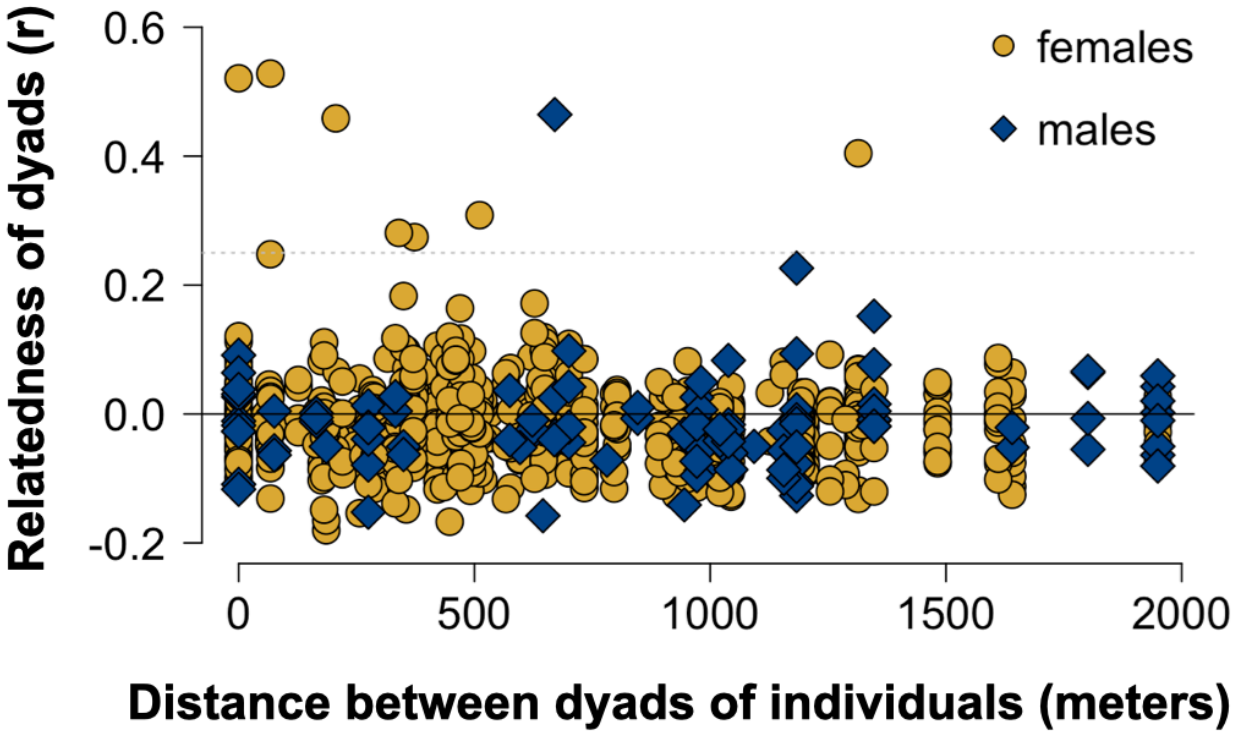

Figure 2: **Figure 2.** Change in genetic relatedness as geographic distance among dyads increases. Each dot reflects a single dyad, a pair of female individuals (yellow) or a pair of male individuals (blue). There are very few close male relatives who are found at larger distances. The small number of close female relatives are all found within relatively short distances of each other. The dotted horizontal line indicates the level of relatedness for half-siblings (r=0.25).

Analysis iii: spatial autocorrelation

Correlogram analyses linking genetic relatedness and spatial distance for females showed negative values when females are in close spatial proximity and positive values when they are far apart (the corrected probability values for females are different than expected by chance in two of the five distance classes), suggesting that as spatial distance among females increases the relatedness among them decreases (Table 1). Correlogram analyses for males showed no consistent relationships between genetic relatedness and spatial distance, with values fluctuating around zero (none of the corrected probability values for males are different than expected by chance in any of the five distance classes; Table 1).

Table 1: Correlogram analyses: the correlation between relatedness and distance.

Distance class	Females: correlation	Females: corrected probability	Males: correlation	Males: corrected probability
0-150m	-0.10	0.01	-0.01	0.39
150-450m	0.02	0.32	0.09	0.37
450-900m	-0.05	0.25	-0.13	0.21
900-1600m	0.10	0.04	0.09	0.55
1600-2000m	0.01	0.66	-0.05	0.73

Discussion

Our results support the first alternative hypothesis that males disperse more than females. We find that the mean level of average genetic relatedness is lower among males compared to females in our sample (analysis i); the mean geographic distance between pairs of individuals that are close genetic relatives is higher among males compared to females (analysis ii); and there is no spatial relationship between genetic relatedness and geographic distance for males, while there is a negative spatial autocorrelation signal indicating a negative relationship between genetic relatedness and geographic distance for females (analysis iii).

The consistency of the results across the three types of analyses supporting female philopatry and male dispersal is reassuring given our small sample size and additional limitations. Previous studies relying on spatial analyses of multi-locus genotypes have also been able to detect even modest sex biased dispersal in fine-scale spatial distribution (examples of empirical studies that detected a signal with small sample sizes include Hofmann et al. (2012), Quaglietta et al. (2013), Gour et al. (2013), Botero-Delgadillo et al. (2017)). In particular, the large number of SNP loci we have for each individual likely increased our power to obtain a qualitative assessment of whether relatives are present in our sample and, accordingly, whether dispersal is more prevalent in either females or males based on spatial autocorrelation (Banks and Peakall 2012). However, because we only have information for a small number of individuals from within a single site, we could not use methods that rely on assigning individuals to a source population or measure the relative distribution of genetic variation within versus among populations (Fst or similar measures). We also do not know whether there is a proportion of females who do disperse or the distances that individuals might disperse.

Our findings indicate that great-tailed grackles are a species that might help us better understand the factors influencing dispersal decisions of female and male birds. The reversal of the sex bias in great-tailed grackles compared to what is observed in most other avian species is in line with the main hypothesis that has been put forward to explain the contrast in sex biases in dispersal between birds and mammals: that in polygynous species, males disperse to search for mating opportunities, while in monogamous species, males remain philopatric to defend resources for high-quality partners. However, given that the link between the mating system and dispersal is much less clear-cut than sometimes assumed (Li and Kokko (2019)) and the limitations of our study, we cannot determine the exact reasons underlying why males disperse or why females apparently remain close to where they hatched. We only observe a general pattern of bias, but we do not have sufficiently detailed information on the experiences of particular individuals that might have shaped their dispersal behavior. Additional individual-based studies are needed to investigate resource and mating competition and whether the patterning of relatives in space relates to kin-based social interactions and inbreeding.

Methods

The methods below are based on the preregistration, with small changes as described in the deviations from the preregistration section above.

Sample

DNA from 57 great-tailed grackles was obtained from wild individuals caught in Tempe, Arizona, USA (see Figure 3 for a map showing the trap locations and sample sizes for the individuals included in the analyses). These individuals were either immediately released, or temporarily brought into aviaries for behavioral testing and then released back to the wild.

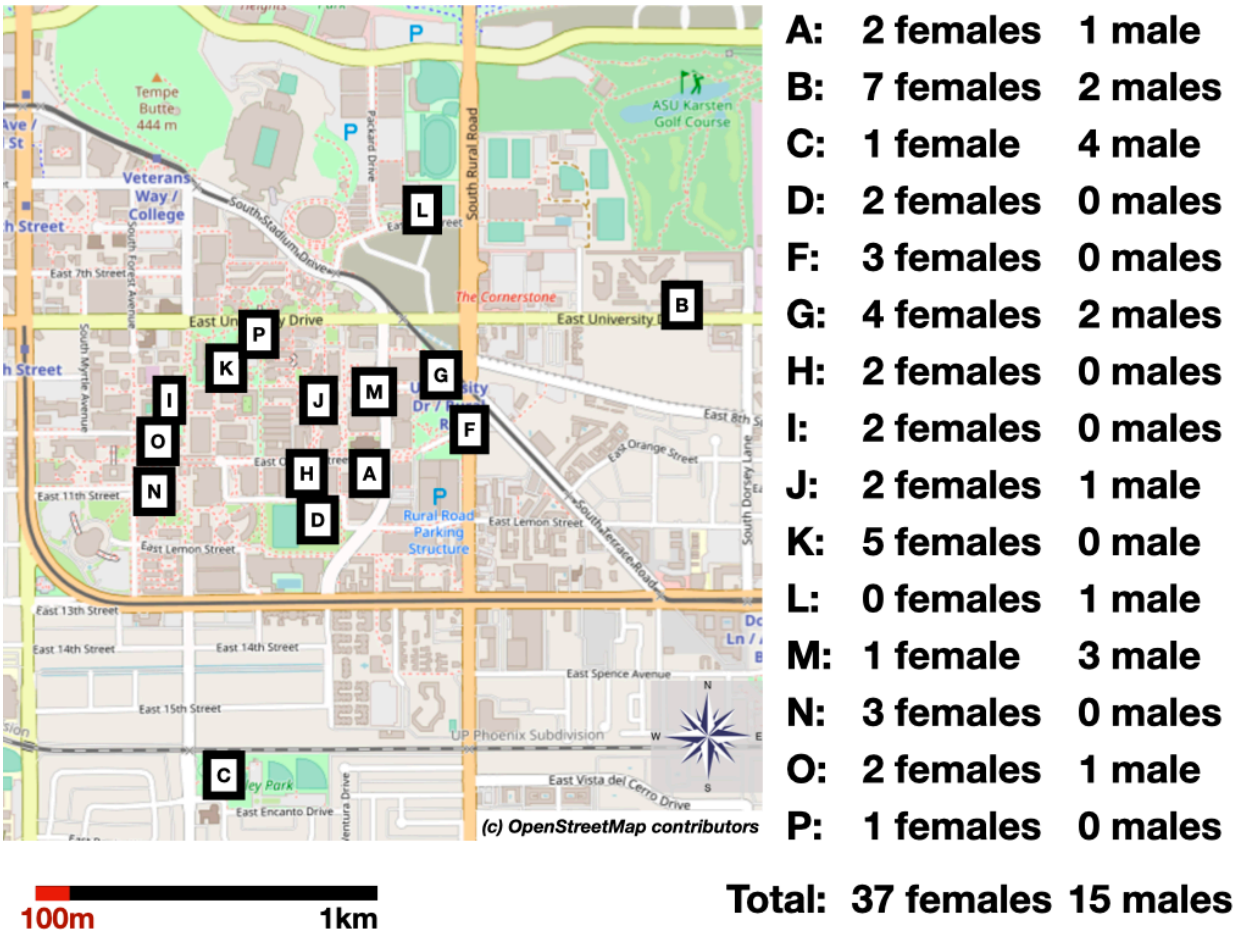

Figure 3: **Figure 3.** Map displaying the sampling locations of grackles on the Arizona State University campus and the number of great-tailed grackles trapped at each location as part of this research.

The larger number of females than males in our sample appears to reflect the adult sex ratio at this study site. 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 37 adult females and 15 adult males (2.47 females per 1 male).

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, and at intermediate and long distances apart.

Sample size rationale

The sample size presented was the largest one possible by July 2019 when the DNA were sequenced using ddRADseq.

Data collection stopping rule

We analyzed all blood samples that were collected through June 2019, which was the end of the trapping season.

Open data

All data necessary for the analyses are available at https://doi.org/10.5063/F1W66J48 and at github (the provided code will load these files directly from github). The raw genetic data is available at http://ncbi.nlm.nih.gov/

```
options(width = 60)

library(related)
library(tidyr)
library(dplyr)
library(vegan)
library(geosphere)
library(DataCombine)
library(data.table)
library(readr)

# SNP data, processed to calculate pairwise relatedness
input <- readgenotypedata("https://raw.githubusercontent.com/
                          corinalogan/grackles/master/Files/Preregistrations/
                          gDispersal_GrackleGenotypesForRelatedness.txt")

# Individual level data, listing the sex (M ale or F emale),
# age (A dult or J uvenile), and latitude and longitude of
# the capture location
gracklelocations <- read_csv(url("https://raw.githubusercontent.com/
                                 corinalogan/grackles/master/Files/Preregistrations/
                                 gDispersal_GrackleIndividualInformationForRelatedness.csv"))
gracklelocations <- data.frame(gracklelocations)
```

Randomization and counterbalancing

No randomization or counterbalancing is involved in this study.

Blinding of conditions during analysis

Experimenters were blind to the sex of the bird when processing samples using ddRADseq (only the alphanumeric bird ID was visible on the tube and no team member has memorized which ID goes with which bird because we give the birds names).

Blood collection

Whole blood samples were collected from individual birds by brachial or medial metatarsal venipuncture. Blood was collected and stored in one of two ways until DNA extraction:

1) At the beginning of the project (2018), 70uL of whole blood was added to silicone-coated micro-blood collection tubes containing 280uL of lysis buffer (White and Densmore (1992), pp. 50-51) and stored at room temperature for up to a year before DNA extraction.

2) In 2018 a different method was implemented, using DNA from packed red blood cells: 150uL of blood was collected from trapped great-tailed grackles and stored for a minimum of 30 minutes and a maximum of 60 minutes at room temperature or 3 hours on ice. Samples were then centrifuged at 15x gravity for 10 minutes to separate the serum from the cellular fraction. After the serum layer was removed and stored, 600uL lysis buffer (White and Densmore (1992), pp. 50-51) was added to the remaining packed cells. Tubes containing packed cells and lysis buffer were stored at room temperature for up to 1 year before extraction.

DNA extraction and quantification

Some samples were extracted at Arizona State University by Rowney (samples through Dec 2018), while others were shipped with ice packs to Washington State University for extraction by Blackwell and his lab (samples collected Jan-Jun 2019). DNA was extracted from the above samples using the DNeasy Blood and Tissue kit (Qiagen) with slight modifications from the manufacturer's protocol (see details in Thrasher et al. (2018) Supporting Information, page 7; our slightly modified protocol is available at https://cryptpad.fr/pad/#/2/pad/edit/4eLjZYSBPsIwUC42BTqWczBJ/. Approximately 100ul of blood/lysis mixture was mixed with 20ul Proteinase K, 150ul PBS, and 200ul buffer AL, then incubated overnight at 64C while shaking. Samples were mixed with 200ul ethanol and added to spin columns. Columns were centrifuged and washed according to kit protocol using buffers AW1 and AW2. DNA was eluted into 50ul of RNAse and DNAse free water at 64C after a 5-10 min incubation on columns. DNA quantification was then performed on a Qubit 4.0 Fluorometer (Fisher Scientific) following the manufacturer's protocol for broad range dsDNA. The average yield of samples used for sequencing was 34ng/ul. Extracted DNA samples were shipped with ice packs to the Cornell Lab of Ornithology for ddRAD sequencing in July 2019.

ddRAD sequencing

The DNA was processed using ddRADseq by Sevchik and Bronwyn Butcher (Cornell University) following methods in Thrasher et al. (2018). Each of the samples' DNA concentrations was measured using the Qubit dsDNA BR Assay Kit and the Qubit Fluorometer following the manufacturer's protocol. For this particular experiment, the necessary DNA concentrations were between 5-50ng/ul and so any sample outside of this range needed to be normalized. Those samples with a concentration higher than 50ng/ul were diluted to approximately 25ng/ul with nuclease-free water. For those samples with concentrations lower than 5ng/ul, both elutions were pooled and the DNA concentrated by evaporation using an Eppendorf Vacufuge. The DNA extracts are then run through a PCR thermocycler where the fragments are digested with a combination of two restriction enzymes (SbfI-HF and MspI) and 20 different adapters attached to the end of the DNA pieces. A 1% agarose gel is run to ensure the proper digestion and ligation of the DNA samples. The samples are then cleaned up using MagNA beads and size selected using BluePippin for a prespecified length (between 400-700 base pairs). After the samples return from size selection, they are amplified using a low-cycle

PCR process and pooled together to be sent in to be sequenced. Sequencing was performed on an Illumina NextSeq500 (using a mid-output kit and run with Illumina PhiX control (15%) to aid sequence alignment) to generate 150 bp single end reads at the Core Facilities of the Cornell Institute of Biotechnology. These data were post-processed to generate SNP data for relatedness analyses as in Thrasher et al. (2018). After filtering reads for quality and demultiplexing to assign sequences back to specific individuals, genetic loci were assembled *de novo* because no reference genome exists for great-tailed grackles. We followed the cut-offs described in Thrasher et al. (2018) for single nucleotide polymorphism filtering, but in addition adjusted the settings to only consider loci that were present in 95% of samples.

Relatedness analyses

Genetic relatedness between all pairs of individuals was calculated using the package "related" (Pew et al. (2015)) in R, following methods in Thrasher et al. (2018). We estimated relatedness using the approaches of (Queller and Goodnight (1989)), a widely used and relatively straightforward estimator, and of (Wang (2002)), an estimator that accounts for small sample sizes and skewed allele distributions.

Dependent variable

Average relatedness between all pairs of individuals within one sex: the arithmetic mean of the estimated relatedness based on sharing of SNP alleles among either all female or all male dyads

Independent variables

1) Sex (female, male): the sex of the individuals assigned based on morphological features

```
options(width = 60)
input$gdata$V1 <- as.character(gracklelocations$Individual)
gracklelocations <- filter(gracklelocations, Individual != "AF_053PS")
adults <- filter(gracklelocations, Age %in% "A")[, ]$Individual
adultgracklelocations <- filter(gracklelocations, Individual %in%
    adults)
```

2) Distance between trap sites (meters): straight line distance (assuming earth as an ellipsoid) between all pairs of trapping locations based on the longitude and latitude of each site.

```
options(width = 60)

# Plot pairwise distances among all females and among all
# males in the sample Calculate all pairwise distances
all_pairwise_distances <- distm(adultgracklelocations[, c("Lon",
    "Lat")], adultgracklelocations[, c("Lon", "Lat")], fun = distVincentyEllipsoid)
rownames(all_pairwise_distances) <- adultgracklelocations$Individual
colnames(all_pairwise_distances) <- adultgracklelocations$Individual
diag(all_pairwise_distances) <- NA

# Calculate pairwise distances among all the females
female_pairwise_distances <- distm(adultgracklelocations[adultgracklelocations$Sex ==
    "F", c("Lon", "Lat")], adultgracklelocations[adultgracklelocations$Sex ==
    "F", c("Lon", "Lat")], fun = distVincentyEllipsoid)
```

```
rownames(female_pairwise_distances) <- adultgracklelocations[adultgracklelocations$Sex ==
    "F", ]$Individual
colnames(female_pairwise_distances) <- adultgracklelocations[adultgracklelocations$Sex ==
    "F", ]$Individual
diag(female_pairwise_distances) <- NA

# Calculate pairwise distances among all the females
male_pairwise_distances <- distm(adultgracklelocations[adultgracklelocations$Sex ==
    "M", c("Lon", "Lat")], adultgracklelocations[adultgracklelocations$Sex ==
    "M", c("Lon", "Lat")], fun = distVincentyEllipsoid)
rownames(male_pairwise_distances) <- adultgracklelocations[adultgracklelocations$Sex ==
    "M", ]$Individual
colnames(male_pairwise_distances) <- adultgracklelocations[adultgracklelocations$Sex ==
    "M", ]$Individual
diag(male_pairwise_distances) <- NA

# plot distributions of pairwise distances
hist(all_pairwise_distances, col = "grey75", border = "black",
    breaks = 10)
hist(female_pairwise_distances, col = "grey75", border = "black",
    breaks = 10)
hist(male_pairwise_distances, col = "grey75", border = "black",
    breaks = 10)
```

Analyses

We did not plan to exclude any data. We did not have to exclude individuals because more than half of their genotype is unknown. However, after receiving the genotypes, we did exclude one individual whose genotype showed inexplicably high levels of variation across the loci. Analyses were conducted in R (current version 3.6.1; R Core Team (2017)).

Analysis i: average relatedness and sex

We compared the average and variance in 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 performed 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 performed 10,000 random draws of 15 individuals either from among the females or from among all individuals and of 37 individuals from among all individuals, and generated distributions of average relatedness among these samples. We assessed whether the observed average relatedness among the 15 males or the 37 females in our sample is higher than what is observed in the majority of random samples. We report the proportion of 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.

```
options(width = 60)

# Analysis 1: Assess whether average relatedness is higher
```

```
# among females or among males Calculate pairwise
# relatedness, here choosing the relatedness method developed
# by Wang and Queller & Goodnight
outfile <- coancestry(input$gdata, wang = 1, quellergt = 1)

# extract the relevant information from the file
pairwise_r <- outfile$relatedness

# We now exclude the individual with the dubious genotype and
# the juvenile individuals
pairwise_r <- filter(pairwise_r, ind1.id != "AF_053PS")
pairwise_r <- filter(pairwise_r, ind2.id != "AF_053PS")

# Next, we exculde all juvenile individuals
pairwise_r <- filter(pairwise_r, ind1.id %in% adults)
pairwise_r <- filter(pairwise_r, ind2.id %in% adults)

# This leaves us with 1326 pairwise relatedness values among
# the 52 remaining individuals



# identify which individuals are female and which are male
females <- filter(gracklelocations, Sex %in% "F", Age %in% "A")[,
    ]$Individual
males <- filter(gracklelocations, Sex %in% "M", Age %in% "A")[,
    ]$Individual

# Calculate average of and variance in relatedness among all
# individuals, all females, and all males First using the
# relatedness estimates based on the method by Wang
mean(filter(pairwise_r, ind1.id %in% females, ind2.id %in% females)$wang)
mean(filter(pairwise_r, ind1.id %in% males, ind2.id %in% males)$wang)
mean(pairwise_r$wang)

var(filter(pairwise_r, ind1.id %in% females, ind2.id %in% females)$wang)
var(filter(pairwise_r, ind1.id %in% males, ind2.id %in% males)$wang)
var(pairwise_r$wang)

# Next using the relatedness estimates based on the method by
# Queller and Goodnight
mean(filter(pairwise_r, ind1.id %in% females, ind2.id %in% females)$quellergt)
mean(filter(pairwise_r, ind1.id %in% males, ind2.id %in% males)$quellergt)
mean(pairwise_r$quellergt)

var(filter(pairwise_r, ind1.id %in% females, ind2.id %in% females)$quellergt)
```

```r
var(filter(pairwise_r, ind1.id %in% males, ind2.id %in% males)$quellergt)
var(pairwise_r$quellergt)


# Perform a simulation to assess whether average relatedness
# among males is different from what we would expect in a
# random subset of the same number of individuals First based
# on the relatedness estimates based on the method by Wang
simulatedrelatedness <- matrix(ncol = 1, nrow = 10000)
for (i in 1:10000) {
    currentset <- sample(adults, length(males))
    simulatedrelatedness[i, 1] <- mean(filter(pairwise_r, ind1.id %in%
        currentset, ind2.id %in% currentset)$wang)
}
hist(simulatedrelatedness)
# This value is similar to a p-value, it reflects the
# probability that the average relatedness observed among
# males would be expected in a random subsample
sum(simulatedrelatedness > mean(filter(pairwise_r, ind1.id %in%
    males, ind2.id %in% males)$wang))/10000


# Perform a simulation to assess whether average relatedness
# among females is different from what we would expect in a
# random subset of the same number of individuals
simulatedrelatedness <- matrix(ncol = 1, nrow = 10000)
for (i in 1:10000) {
    currentset <- sample(adults, length(females))
    simulatedrelatedness[i, 1] <- mean(filter(pairwise_r, ind1.id %in%
        currentset, ind2.id %in% currentset)$wang)
}
hist(simulatedrelatedness)
# This value is similar to a p-value, it reflects the
# probability that the average relatedness observed among
# males would be expected in a random subsample
sum(simulatedrelatedness > mean(filter(pairwise_r, ind1.id %in%
    females, ind2.id %in% females)$wang))/10000


# Next based on the relatedness estimates based on the method
# by Queller & Goodnight Perform a simulation to assess
# whether average relatedness among males is different from
# what we would expect in a random subset of the same number
# of individuals
```

```
simulatedrelatedness <- matrix(ncol = 1, nrow = 10000)
for (i in 1:10000) {
    currentset <- sample(adults, length(males))
    simulatedrelatedness[i, 1] <- mean(filter(pairwise_r, ind1.id %in%
        currentset, ind2.id %in% currentset)$quellergt)
}
hist(simulatedrelatedness)
# This value is similar to a p-value, it reflects the
# probability that the average relatedness observed among
# males would be expected in a random subsample
sum(simulatedrelatedness > mean(filter(pairwise_r, ind1.id %in%
    males, ind2.id %in% males)$quellergt))/10000


# Perform a simulation to assess whether average relatedness
# among females is different from what we would expect in a
# random subset of the same number of individuals
simulatedrelatedness <- matrix(ncol = 1, nrow = 10000)
for (i in 1:10000) {
    currentset <- sample(adults, length(females))
    simulatedrelatedness[i, 1] <- mean(filter(pairwise_r, ind1.id %in%
        currentset, ind2.id %in% currentset)$quellergt)
}
hist(simulatedrelatedness)
# This value is similar to a p-value, it reflects the
# probability that the average relatedness observed among
# males would be expected in a random subsample
sum(simulatedrelatedness > mean(filter(pairwise_r, ind1.id %in%
    females, ind2.id %in% females)$quellergt))/10000
```

Analysis ii: distances among genetic relatives

Based on the calculations of pairwise genetic relatedness, we selected the subset of pairs who are estimated to be more closely related than cousins (r≥0.125) or half-siblings (r≥0.25). For this subset of individuals, we determined whether the pairwise geographic distances are shorter for the males or the females in the sample (Coulon et al. (2006)). We performed 10,000 random draws of pairs of males and of females matching the numbers of inferred closely related dyads, and calculated the difference between the average geographic distances for each sex. We assessed whether the observed difference in geographic distances is higher than the majority of random samples and, for comparison with other approaches, determine whether the observed distance is higher than that calculated for 95% of all random draws.

```
options(width = 60)

# Analysis 2: Assess whether distances among closely related
# females are shorter than distances among closely related
```

```r
# males First define close relatives as all pairs of
# individuals who are related by a level of 0.25 or higher
# (half-siblings or higher) using the Wang estimator
close_relatives_females <- filter(pairwise_r, wang > 0.2499,
    ind1.id %in% females, ind2.id %in% females)
close_relatives_females_individuals <- c(close_relatives_females$ind1.id,
    close_relatives_females$ind2.id)

# Alternatively, select close relatives as pairs of
# individuals who are related at a level of 0.25 of higher
# using the Queller & Goodnight estimator
close_relatives_females <- filter(pairwise_r, quellergt > 0.2499,
    ind1.id %in% females, ind2.id %in% females)
close_relatives_females_individuals <- c(close_relatives_females$ind1.id,
    close_relatives_females$ind2.id)

# Pick one of the two estimators before proceeding with the
# following analyses

# Next subset the the distance matrix to only include these
# individuals

females_pairwise_distances_matrix <- as.data.frame(female_pairwise_distances)
close_relatives_females_pairwise_distances <- matrix(nrow = nrow(close_relatives_females),
    ncol = 1)

for (i in 1:nrow(close_relatives_females)) {
    ind1 <- close_relatives_females[i, ]$ind1.id
    ind2 <- close_relatives_females[i, ]$ind2.id
    pair_distance <- females_pairwise_distances_matrix[ind1,
        ind2]
    close_relatives_females_pairwise_distances[i, ] <- pair_distance
}

median(close_relatives_females_pairwise_distances)

hist(close_relatives_females_pairwise_distances)


# repeat the same for the males
close_relatives_males <- filter(pairwise_r, wang > 0.2499, ind1.id %in%
    males, ind2.id %in% males)
close_relatives_males_individuals <- c(close_relatives_males$ind1.id,
    close_relatives_males$ind2.id)
```

```
# Again, the alternative with the Queller & Goodnight method,
# pick only one of the two
close_relatives_males <- filter(pairwise_r, quellergt > 0.2499,
    ind1.id %in% males, ind2.id %in% males)
close_relatives_males_individuals <- c(close_relatives_males$ind1.id,
    close_relatives_males$ind2.id)


# Next subset the the distance matrix to only include these
# individuals

males_pairwise_distances_matrix <- as.data.frame(male_pairwise_distances)
close_relatives_males_pairwise_distances <- matrix(nrow = nrow(close_relatives_males),
    ncol = 1)

for (i in 1:nrow(close_relatives_males)) {
    ind1 <- close_relatives_males[i, ]$ind1.id
    ind2 <- close_relatives_males[i, ]$ind2.id
    pair_distance <- males_pairwise_distances_matrix[ind1, ind2]
    close_relatives_males_pairwise_distances[i, ] <- pair_distance
}

median(close_relatives_males_pairwise_distances)

hist(close_relatives_males_pairwise_distances)

# calculate difference between the distances among males and
# among females
observeddifferenceindistances <- median(close_relatives_males_pairwise_distances,
    na.rm = T) - median(close_relatives_females_pairwise_distances,
    na.rm = T)

# perform simulation to generate random draws of matching
# numbers of individuals to assess whether the sex-difference
# in the distance is more or less than what would be expected
# by chance
number_close_relatives_females <- nrow(close_relatives_females)
number_close_relatives_males <- nrow(close_relatives_males)

simulateddifferencesindistances <- matrix(ncol = 1, nrow = 10000)
simulateddfemaleindistances <- matrix(ncol = 1, nrow = 10000)
simulateddmaleindistances <- matrix(ncol = 1, nrow = 10000)

for (i in 1:10000) {
    simulated_close_relatives_females <- sample_n(pairwise_r,
```

```
        number_close_relatives_females, replace = TRUE)

    subset_relatives_females_pairwise_distances <- matrix(nrow = nrow(simulated_close_relatives_females),
        ncol = 1)

    for (j in 1:nrow(simulated_close_relatives_females)) {
        ind1 <- simulated_close_relatives_females[j, ]$ind1.id
        ind2 <- simulated_close_relatives_females[j, ]$ind2.id
        pair_distance <- all_pairwise_distances[ind1, ind2]
        subset_relatives_females_pairwise_distances[j, ] <- pair_distance
    }

    simulated_close_relatives_males <- sample_n(pairwise_r, number_close_relatives_males,
        replace = TRUE)

    subset_relatives_males_pairwise_distances <- matrix(nrow = nrow(simulated_close_relatives_males),
        ncol = 1)

    for (k in 1:nrow(simulated_close_relatives_males)) {
        ind1 <- simulated_close_relatives_males[k, ]$ind1.id
        ind2 <- simulated_close_relatives_males[k, ]$ind2.id
        pair_distance <- all_pairwise_distances[ind1, ind2]
        subset_relatives_males_pairwise_distances[k, ] <- pair_distance
    }

    simulateddfemaleindistances[i, 1] <- median(subset_relatives_females_pairwise_distances,
        na.rm = T)
    simulateddmaleindistances[i, 1] <- median(subset_relatives_males_pairwise_distances,
        na.rm = T)
    simulateddifferencesindistances[i, 1] <- median(subset_relatives_males_pairwise_distances,
        na.rm = T) - median(subset_relatives_females_pairwise_distances,
        na.rm = T)
}

sum(simulateddfemaleindistances < median(close_relatives_females_pairwise_distances))/10000
sum(simulateddmaleindistances > median(close_relatives_males_pairwise_distances))/10000
sum(simulateddifferencesindistances > observeddifferenceindistances)/10000
```

Analysis iii: spatial autocorrelation

To test whether males and females show different patterns of genetic isolation by geographic distance, we followed analyses as in Aguillon et al. (2017). For the analysis, we initially created 11 distance bins separated by 200m between 0m-2000m (the maximum distance between trapping sites). The 200m bin size was chosen because there are roosting trees that are ~50m apart suggesting that dispersal might be occurring below this scale and also to maximize the number of pairs in each distance class. The individuals in our sample were caught at one of 15 trap sites, and the

resulting 105 pairwise distances among individuals will be assigned to one of the 11 bins. In addition, we adjusted 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 Peakall, Ruibal, and Lindenmayer (2003)). Finally, we adjusted the distances to have five bins that reflected the distances among genetic relatives detected in analysis ii (0-150m, 150-450m, 450-900m, 900-1400m, 1400-2000m). For males and females separately, we linked 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 used the function 'mantel.correlog' in the vegan package (Oksanen et al. (2013)) 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 Legendre and Legendre (2012). 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.

```
options(width = 60)

# Analysis 3: Correlogram to assess change of relatedness
# with distances

# have each value only once in the distance matrix
for (i in 1:ncol(all_pairwise_distances)) {
    all_pairwise_distances[i, i:ncol(all_pairwise_distances)] <- NA
}



# turn pairwise_r$wang into a matrix
all_relatedness <- select(pairwise_r, ind1.id, ind2.id, wang)
relatedness_matrix <- spread(all_relatedness, "ind1.id", "wang")
relatedness_matrix <- cbind(relatedness_matrix, AF_061PR = "NA")
relatedness_matrix <- arrange(relatedness_matrix, ind2.id)
relatedness_matrix <- InsertRow(data = relatedness_matrix, NewRow = rep("NA",
    53), RowNum = 1)
relatedness_matrix[1, 1] <- "AF_001YP"
rownames(relatedness_matrix) <- relatedness_matrix[, 1]

# turn pairwise_r$quellergt into a matrix
```

```
all_relatedness <- select(pairwise_r, ind1.id, ind2.id, quellergt)
relatedness_matrix <- spread(all_relatedness, "ind1.id", "quellergt")
relatedness_matrix <- cbind(relatedness_matrix, AF_061PR = "NA")
relatedness_matrix <- arrange(relatedness_matrix, ind2.id)
relatedness_matrix <- InsertRow(data = relatedness_matrix, NewRow = rep("NA",
    53), RowNum = 1)
relatedness_matrix[1, 1] <- "AF_001YP"
rownames(relatedness_matrix) <- relatedness_matrix[, 1]

relatedness_matrix <- relatedness_matrix[1:52, 2:53]

female_relatedness_matrix <- relatedness_matrix[rownames(relatedness_matrix) %in%
    females, colnames(relatedness_matrix) %in% females]
male_relatedness_matrix <- relatedness_matrix[rownames(relatedness_matrix) %in%
    males, colnames(relatedness_matrix) %in% males]

# perform the correlogram analysis first way, defining the
# distance classes
female_correlogram_setdistances <- mantel.correlog(D.eco = female_relatedness_matrix,
    D.geo = female_pairwise_distances, break.pts = c(0, 100,
        200, 300, 400, 500, 750, 1250, 1550, 2000, 2500), cutoff = FALSE,
    nperm = 10000)
male_correlogram_setdistances <- mantel.correlog(D.eco = male_relatedness_matrix,
    D.geo = male_pairwise_distances, break.pts = c(0, 100, 200,
        300, 400, 500, 750, 1250, 1550, 2000, 2500), cutoff = FALSE,
    nperm = 10000)
# second way, setting the number of distance classes
female_correlogram_classes <- mantel.correlog(D.eco = female_relatedness_matrix,
    D.geo = female_pairwise_distances, n.class = 5)
male_correlogram_classes <- mantel.correlog(D.eco = male_relatedness_matrix,
    D.geo = male_pairwise_distances, n.class = 5)

# additional way, with the distance classes based on the
# inferred distance among relatives from analysis ii
female_correlogram_setdistances <- mantel.correlog(D.eco = female_relatedness_matrix,
    D.geo = female_pairwise_distances, break.pts = c(0, 150,
        450, 900, 1600, 2000), cutoff = FALSE, nperm = 10000)
male_correlogram_setdistances <- mantel.correlog(D.eco = male_relatedness_matrix,
    D.geo = male_pairwise_distances, break.pts = c(0, 150, 450,
        900, 1600, 2000), cutoff = FALSE, nperm = 10000)

female_correlogram_setdistances
male_correlogram_setdistances
```

Ethics

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

1) US Fish and Wildlife Service (scientific collecting permit number MB76700A-0,1,2)
2) US Geological Survey Bird Banding Laboratory (federal bird banding permit number 23872)
3) Arizona Game and Fish Department (scientific collecting license number SP594338 [2017], SP606267 [2018], and SP639866 [2019])
4) Institutional Animal Care and Use Committee at Arizona State University (protocol number 17-1594R)
5) University of Cambridge ethical review process (non-regulated use of animals in scientific procedures: zoo4/17 [2017])

Author contributions

Sevchik: Hypothesis development, sample processing, data analysis and interpretation, write up, revising/editing.

Logan: Hypothesis development, data analysis and interpretation, write up, revising/editing, materials/funding.

Blackwell: Hypothesis development, DNA extraction, revising/editing.

Rowney: Blood collection, DNA extraction, sample processing, write up, revising/editing.

Lukas: Hypothesis development, data analysis and interpretation, write up, revising/editing, materials/funding.

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Conflict of interest disclosure

We, the authors, declare that we have no financial conflicts of interest with the content of this article. Corina Logan and Dieter Lukas are Recommenders at PCI Ecology and Corina Logan is on the Managing Board at PCI Ecology.

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