

JOURNAL OF AVIAN BIOLOGY

Research article

Extra-pair paternity correlates with genetic diversity, but not breeding density, in a Neotropical passerine, the black catbird

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Journal of Avian Biology

2022: e03034

doi: 10.1111/jav.03034

Subject Editor: Simon Griffith
Editor-in-Chief: Staffan Bensch
Accepted 18 September 2022



The frequency of extra-pair paternity (EPP) varies widely across socially monogamous birds, but the proximate mechanisms driving this variation remain unclear. In this study, we tested two major factors hypothesized to influence extra-pair mating – breeding density and genetic diversity – by comparing genetic mating patterns in two populations of black catbirds *Melanopirila glabrirostris*. This Neotropical songbird is endemic to the Yucatán Peninsula, including eastern Mexico, and its offshore islands. We sampled one mainland (Sian Ka'an Biosphere Reserve) and one island (Isla Cozumel) population and used single-nucleotide polymorphisms (SNPs) to quantify heterozygosity and genetic parentage over two breeding seasons. Moderate levels of EPP occurred in both populations (9.5–35% of offspring and 17–45% of nests). Contrary to predictions, breeding density did not affect EPP: although breeding densities were much higher on the mainland than on the island, EPP rates did not differ between populations, and local breeding density was not correlated with EPP at individual nests. In contrast, partial support emerged for the hypothesis that genetic diversity influences EPP: extra-pair offspring were more heterozygous than within-pair offspring. However, the two populations did not differ in genetic diversity, and neither the heterozygosity of social fathers nor within-pair relatedness predicted EPP. These results are consistent with recent comparative studies suggesting that breeding density is not a critical driver of EPP rates, and that not all tropical songbirds exhibit low rates of EPP.

Keywords: Cozumel, extra-pair mating, mating strategies, multiple mating, temperate zone bias, Yucatán Peninsula

Introduction

Social monogamy is the most common mating system across birds (Lack 1968, Cockburn 2006), yet the use of molecular markers in wild populations has revealed that males often care for nestlings to whom they are unrelated (Gowaty 1985, Ligon 1999). Indeed, extra-pair paternity (EPP) appears to be widespread in birds whereas



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strict genetic monogamy is rare (Griffith et al. 2002, Brouwer and Griffith 2019). Nevertheless, the frequency of EPP varies widely within and among species, and a variety of factors have been proposed to explain the variation at these different levels (Westneat and Stewart 2003, Valcu et al. 2021). Fundamentally, these hypotheses assume that extra-pair mate choice is subject to trade-offs driven by ecological factors influencing relative fitness benefits and costs to the female or male or both (Petrie and Kempenaers 1998). Two major ecological factors proposed to influence the relative costs and benefits of EPP are breeding density and genetic diversity (Westneat et al. 1990).

The breeding density hypothesis posits that EPP is limited by the availability of extra-pair partners. Given that more potential extra-pair mates should be available in populations with higher densities, high-density populations are expected to show higher rates of EPP than low-density populations (Westneat et al. 1990, Westneat and Sherman 1997). Within populations, females with access to more extra-pair partners (those with more local neighbors) should also be more likely to engage in EPP than those with few potential partners (Stewart et al. 2010). Empirical support for the breeding density hypothesis exists both between and within populations (Stewart et al. 2010, Mayer and Pasinelli 2013). However, local breeding density in at least some species has also been found to have the opposite relationship with EPP, which is hypothesized to reflect higher competition for mates and risk of reciprocal cuckoldry (Rätti et al. 2001, Kaiser et al. 2017).

By contrast, the genetic diversity hypothesis posits that females engage in EPP to gain indirect fitness benefits: females paired to males of low genetic quality that mate with extra-pair males of higher genetic quality thereby acquire 'good genes' for their offspring (Petrie and Kempenaers 1998). This hypothesis assumes that the potential for indirect (genetic) benefits should be influenced by the underlying genetic variation of the population (i.e. heritable variance in quality). Across populations, this hypothesis predicts that EPP should increase with overall genetic diversity (Liu et al. 2015, Corregidor-Castro et al. 2022) and with increasing inter-individual variation in male quality (Petrie and Lipsitch 1994). Within populations, females paired to low-quality males, or to genetically similar males, should be more likely to seek extra-pair mates in order to increase the genetic quality or diversity of their offspring (Blomqvist et al. 2002, Richardson et al. 2005). This hypothesis also predicts that extra-pair young should exhibit higher genetic diversity than within-pair young (Foerster et al. 2003, Rubenstein 2007). Although both the breeding density and genetic diversity hypotheses make relatively straightforward predictions, empirical studies have yielded mixed results (Mayer and Pasinelli 2013, Liu et al. 2015, Kaiser et al. 2017).

Comparisons of mainland and island populations of the same species are potentially powerful means of testing both hypotheses because these populations often differ substantially in population size, density and genetic diversity (Petrie and Kempenaers 1998). One comparative analysis showed that rates of EPP were approximately twice as high in

mainland samples than on islands, but this analysis included mostly interspecific comparisons (Griffith 2000). The few intraspecific comparisons available have yielded mixed results, with some studies showing lower EPP in island populations (Griffith et al. 1999, Krokene and Lifjeld 2000) and others finding the opposite pattern (Charmantier and Blondel 2003) or no difference (Conrad et al. 2001). Furthermore, no studies have yet compared mainland and island genetic mating systems in a tropical songbird. This represents a significant gap in our knowledge of EPP because tropical birds often differ from temperate species in ways that might select against EPP (e.g. via longer breeding seasons (Salgado-Ortiz et al. 2008), year-round territoriality (Stutchbury et al. 2007) and/or less synchronous breeding (Stutchbury and Morton 1995)). These differences have led some to argue that while EPP might represent the typical mating strategy for temperate species, genetic monogamy should prevail among tropical species (Stutchbury and Morton 2001, 2008). However, studies of tropical species are too few to detect clear patterns; more estimates of EPP are needed before such generalizations can be made (Macedo et al. 2008, Bonier et al. 2014, Ferretti 2019, Valcu et al. 2021).

In this study, we compared genetic mating patterns in a mainland and an island population of a little-studied Neotropical songbird, the black catbird *Melanoptila glabrirostris*. This mimic thrush (Mimidae) is endemic to the Yucatán Peninsula, including Mexico, Belize and northern Guatemala (Howell and Webb 1995, Cody and Sharpe 2020), and has been described as socially monogamous (Morgenthaler 2003). The species occurs on the mainland and many coastal islands (Miller and Miller 1991, Johnson and Haight 2011), suggesting the potential for differences in genetic diversity across populations. Historical records also indicate large variation in density between populations (Griscom 1926a, b, Bond 1961).

We studied two nesting populations of black catbirds, on the mainland Yucatán Peninsula and on nearby Isla Cozumel. The only previous description of the black catbird's social breeding and mating systems was based on one observed nest (Morgenthaler 2003), yet the Mimidae exhibits a range of sociality, from social monogamy with biparental care (Smith et al. 2020) to occasional polygyny and plural, cooperative breeding (Curry 1989, Curry and Grant 1990). We therefore report the results of extensive nest watches to characterize the social mating and breeding system. Based on these watches, we confirmed that both populations exhibit social monogamy and biparental care. We measured breeding densities at both sites and used single-nucleotide polymorphisms (SNPs) to quantify genetic mating patterns and genetic diversity (heterozygosity). First, we tested the breeding density hypothesis, which predicts that EPP should increase with breeding density (i.e. with the availability of extra-pair mates). We tested this prediction at the population level by comparing breeding densities and EPP rates on the island and the mainland, and at the individual level by asking whether females in areas of locally high breeding density (those with closer nearest-neighbors) were more

likely to engage in EPP. Second, we tested the genetic diversity hypothesis, which predicts that EPP should increase the genetic diversity of offspring. We tested this prediction at the population level by comparing overall levels of heterozygosity and EPP rates on the island and the mainland, and at the individual level by asking: 1) whether the heterozygosity of social fathers predicted their risk of being cuckolded, 2) whether extra-pair young had higher levels of heterozygosity than within-pair offspring (WPO) and 3) whether genetic relatedness of social mates predicted the risk of EPP. These hypotheses and predictions are summarized in Table 1.

Methods

Study area and field methods

We studied black catbirds in Mexico over two breeding seasons (May–July 2008, May–August 2009). The mainland population was south of Tulum, Quintana Roo, in the Sian Ka'an Biosphere Reserve (SKBR), a 651 000-ha federal protected area (Mazzotti et al. 2005). We studied catbirds at two localities along the coastal edge of the reserve: the SKBR Visitors' Center (north site; 20°03'05"N, 87°28'50"W) and San Juan (south site; 19°51'57"N, 87°26'14"W). Habitat in both sites consisted mostly of coastal dune thicket dominated by thatch palm *Thrinax radiata* and various woody shrubs (especially *Pithecellobium* sp.). The island population was located on ~49 000-ha Isla Cozumel (20°26'54"N, 86°57'32"W), which is a UNESCO Biosphere Reserve located ~17 km off the coast of mainland Quintana Roo and separated from it by a deep channel (Cuarón 2009). Cozumel's habitat is mostly semi-deciduous or tropical deciduous forest with, at the time of the study, a low canopy resulting from recent hurricane damage (Perdomo-Velázquez et al. 2017). Dominant vegetation included *Ficus* sp. and *Metopium brownei* and an understory consisting of several Polygonaceae species (Marina Hipolito 2010). We studied catbirds in a small area (~68 ha) on the west side of the island, ~60 km northeast of the north mainland site.

We searched for catbird nests both systematically and opportunistically and used mist-nets to capture adults tending nests and in nearby mist-net lanes (Roldán-Clarà 2009). We marked each captured bird with a unique color-band combination of two plastic color bands and a numbered aluminum band. When possible, we recorded the sex of each adult based

on the presence/absence of a brood patch (female) or cloacal protuberance (male). To confirm the identity of social parents at a nest, we used direct observation of nests, digital video cameras or both, using observation blinds when necessary. More detailed field methods are given in LaPergola (2012) and Roldán-Clarà et al. (2013, 2018). To quantify breeding density, we measured the distance (in m) between each nest and its nearest active neighbor nest (nearest-neighbor distance, NND) using Quantum GIS 1.4.0-Enceladus (<<http://qgis.osgeo.org>>).

Genetic methods

We collected blood samples (50–100 µl) from adults, juveniles and nestlings (7–10 days after hatching) via ulnar venipuncture and collection in heparinized capillary tubes (following recommendations in Fair et al. 2010). We transferred blood samples to 2% SDS blood lysis buffer (100 mM Tris pH8, Na₂ EDTA, 10 mM NaCl, 2.0% SDS) and stored them at room temperature. We extracted genomic DNA for 128 individuals (83 from the mainland and 45 from Cozumel) using QIAGEN DNeasy or Genra PureGene kits, following the manufacturer's protocols. To quantify DNA, we used a Qubit Fluorometer (Life Technologies) dsDNA BR Assay Kit following the manufacturer's protocol. We confirmed the sex of adult birds using a PCR-based protocol (Griffiths et al. 1998).

We used double-digest restriction-associated enzyme sequencing (ddRADseq) to characterize SNPs, following a modified version of the protocols described by Peterson et al. (2012) and Thrasher et al. (2018). In short, we digested genomic DNA with the restriction enzymes *EcoRI* and *MspI*, ligated adapters, ran low-cycle PCR to add index primers (eight index adapters and 16 barcode adapters), size selected fragments between 340 and 410 base pairs (bp) via BluePippin (Sage Science, Beverly, Massachusetts, USA), and pooled index libraries in equimolar ratios into a final library. We submitted the final library for sequencing on an Illumina NovaSeq 6000 (Princeton Univ. Lewis-Sigler Institute for Integrative Genomics), producing 150 bp paired-end reads. For three individuals that produced no usable reads from this run, we re-ran the above protocol and submitted this smaller library to the same facility for sequencing on an Illumina MiSeq Nano, yielding 150 bp paired-end reads. Combining NovaSeq and MiSeq runs, sequencing yielded 425.5 million raw reads across all individuals (NovaSeq: mean ± SE reads per index group = 52.2 ± 1.6 million). We filtered reads using

Table 1. Hypotheses and predictions tested for extra-pair paternity (EPP).

Hypothesis	Prediction
H1. Breeding density	P1.1. The higher breeding density population will have higher rates of EPP than the lower breeding density population. P1.2. Females in areas of locally high breeding density (closer nearest-neighbors) are more likely to have EPP.
H2. Genetic diversity	P2.1. The population with a higher rate of EPP will have higher overall levels of heterozygosity. P2.2. Males of lower heterozygosity are more likely to be cuckolded than males of higher heterozygosity. P2.3. Extra-pair young have higher levels of heterozygosity than within-pair young. P2.4. Social pairs with higher within-pair relatedness are more likely to have EPP than pairs with lower within-pair relatedness.

FASTX Quality Filter to retain only reads with 95% of bases having PHRED score of ≥ 20 and then demultiplexed reads using *process_radtags* in STACKS 2 (Rochette et al. 2019). After demultiplexing, we retained a mean \pm SE of 4.9 ± 0.7 million sequences per individual. Because we lacked a reference genome for the black catbird, we used the STACKS 2 *denovo_map* program to assemble RAD loci (Rochette et al. 2019). Details of the assembly are provided in the Supporting information.

The full panel of SNPs comprised 414 loci, of which our *populations* run retained 311 loci in the mainland population and 314 loci in the island population as candidates for parentage analyses. The mean (\pm SE) percentage of individuals genotyped for each locus was $86.5 \pm 0.3\%$ ($n = 311$ loci) and $86.3 \pm 0.3\%$ ($n = 314$ loci) for the mainland and island, respectively. After filtering for high incidence of null alleles and Hardy–Weinberg equilibrium, we retained 288 loci for mainland parentage analyses and 279 loci for island parentage analyses. The number of loci used for each dyad or trio comparison in CERVUS differed slightly due to the $-r$ threshold we set in the STACKS’s *populations* module (Supporting information). The SNP panels for both mainland and island had similar polymorphic information content, mean observed and expected heterozygosities, and combined non-exclusion probabilities (Supporting information). Of the 414 loci identified, 217 were genotyped in both populations, 95 loci were genotyped only for the mainland and 102 loci were genotyped only for the island. Considering the 217 loci shared between populations, the mainland and island populations had, respectively, five loci and one locus with private alleles.

Parentage and population genetics

We used CERVUS 3.0.7 (Kalinowski et al. 2007) to estimate population genetics metrics (including observed and expected heterozygosity, H_o and H_e , respectively), calculate exclusion probabilities and conduct parentage exclusion analyses. We ran separate parentage analyses for both populations because the island population has been recognized as an endemic subspecies (Paynter 1954) and, not knowing the degree of genetic differentiation a priori, we wanted to retain the largest number of SNPs possible for each population. The distance between the closest mainland locality and the island (~60 km) also precluded parents in one population from contributing offspring to nests in the other. Using CERVUS, we calculated allele frequencies, null allele frequencies and deviation from Hardy–Weinberg equilibrium for each locus using a pool that included all sampled adults and one offspring from each nest where one of the social parents evaded sampling. For the following parentage analyses, we excluded alleles with null allele frequency estimates ≥ 0.2 . We then ran three parentage analyses for each population: 1) maternity exclusion, 2) paternity exclusion given a known mother and 3) paternity exclusion for offspring where the social mother was not sampled and therefore unknown.

CERVUS uses a likelihood-based analysis of genotypes to calculate critical LOD scores (natural logarithm of the

likelihood ratio) for exclusion of parent offspring dyads and trios at 80% and 95% confidence levels (Marshall et al. 1998, Kalinowski et al. 2007). For maternity exclusion analyses, we considered the social mother excluded if her pair LOD score was lower than the critical score at 80% confidence. For paternity exclusion analyses, we considered a social father excluded if his pair LOD score was lower than the critical score at 80% confidence. We also considered a social father excluded if his pair LOD score did not meet this criterion but 1) the social mother had not been excluded and 2) the trio LOD score excluded the parent pair as the true parents. In this scenario, we assume that EPP is a more likely result than quasi-parasitism. Following the above criteria, offspring were coded as WPO if neither social parent could be excluded, and offspring were considered as extra-pair offspring (EPO) if the social father was excluded. We then used two-tailed Fisher’s exact tests to compare our populations for the proportion of offspring that were EPO and the proportion of nests in which we identified ≥ 1 EPO.

To confirm that dyadic genetic relatedness estimates were consistent with assigned relationships, we used the R package ‘related’ (Pew et al. 2015) to calculate pair-wise relatedness estimators for assigned mother–offspring, father–offspring and nestmate pairs. This analysis used the same SNP panels identified for use with CERVUS, and we included all sampled individuals but ran separate analyses for each population. The ‘related’ package can compute relatedness estimates via five different non-likelihood methods (Queller and Goodnight 1989, Li et al. 1993, Ritland 1996, Lynch and Ritland 1999, Wang 2002). As in Thrasher et al. (2018), we used the *compare_estimators* function in ‘related’ to assess the performance of the different estimators with our SNP panels. The five estimators exhibited similarly high correlations (Pearson correlation coefficients > 0.95) between expected and observed values based on 200 simulated pairs. Consequently, we opted to use the Wang (2002) relatedness estimator because it yielded values that most closely approximated those expected for the different relationship categories (Supporting information).

To compare the genetic diversity of populations, social fathers and offspring, we used the full SNP panel, including those loci that had been removed (due to high levels of null alleles or deviation from Hardy–Weinberg equilibrium) for the parentage analyses. We computed standardized multi-locus heterozygosities (sMLH, hereafter ‘heterozygosity’) for individuals using the R package ‘inbreedR’ (Stoffel et al. 2016). Multi-locus heterozygosity (MLH) is the proportion of loci typed for an individual that are heterozygous out of all loci typed for that individual and is considered a proxy for genetic diversity and inbreeding, with lower values of MLH representing a higher degree of inbreeding. In contrast to MLH, sMLH is calculated as a quotient of the number of loci for which an individual is heterozygous over the sum of average H_o for the population across only the subset of loci typed for the focal individual (Stoffel et al. 2016). We used sMLH because it corrects for bias when individuals are typed across different proportions of loci (Coltman et al. 1999), which was the case for our samples.

Hypothesis testing and statistical analyses

We first asked whether the two populations differed in overall breeding density, and whether local breeding density (the NND) was correlated with the frequency of EPP at the population and nest level. To compare populations, we used a linear model (LM) to test for an association between population and the natural log transformation of NND (the non-transformed NND data were over-dispersed). To test the prediction that EPP is negatively related to NND (i.e. positively related to local breeding density), we used a generalized linear model (GLM) fit a with binomial distribution. The binary response variable was whether the nest contained any EPO (1) or not (0). Predictor variables included standardized (z-score transformed) NND and population.

To compare population-level heterozygosity between the mainland and island populations, we used a subset of all sampled individuals (including all known or suspected breeding adults and eight nestlings from nests with only one social parent sampled) to reduce the potential impact of parent-offspring relationships. The set of samples included in this comparison comprised 67 mainland birds (28 adult females, 32 adult males and 7 nestlings) and 28 island birds (16 adult females, 11 adult males and 1 nestling). We used a Wilcoxon rank-sum test to compare heterozygosity between populations; results were qualitatively similar when a linear model was used with transformed sMLH as the response variable (Supporting information). Results were also qualitatively similar to analyses based on a sample of adults only (Supporting information).

We used three different models to test three predictions of the genetic diversity hypothesis, asking 1) whether the genetic diversity of the social father predicts his risk of being cuckolded; 2) whether EPO are more heterozygous than WPO; and 3) whether within-pair relatedness predicts the occurrence of EPP. First, we used a GLM fit with a binomial distribution to test the prediction that EPM is negatively associated with genetic diversity of the social father. The binary response variable was whether any nestlings within the social father's nest were EPO (1) or not (0), and the predictors were population (mainland versus island) and standardized (z-score transformed) heterozygosity of the social father. Second, to test the prediction that EPO are more heterozygous than WPO, we used a linear mixed model (LMM) with sMLH of offspring as the response variable, offspring status (EPO versus WPO) and population as fixed effects, and nest ID as a random effect. Finally, we used one GLM to test for a negative association between within-pair relatedness with paternity loss. The response variable was whether any nestlings sampled from a pair were EPO (1) or not (0), and predictor variables were the population and the standardized Wang relatedness estimator of social pairs.

We conducted all statistical tests using R ver. 3.6.3 (<www.r-project.org>) in RStudio ver. 1.1.463 (<<http://www.rstudio.com/>>). For the Fisher's exact tests, we used the *fisher.test* function, and for Chi-squared test, we used the *chisq.test* function. For LMs and GLMs, we used the *lm* and

glm functions, respectively. We used the *wilcox.test* function in the 'MASS' package (Venables and Ripley 2002) for the Wilcoxon rank-sum test. We report all summary statistics as mean \pm standard error of the mean. Confidence intervals (CI) for count data are 95% and were calculated via the Wald Method.

Results

Sample sizes and identification of social parents

Pooling both years, we found 128 nests (mainland: $n = 107$, island: $n = 21$). Among these, we observed 162.1 h of provisioning behavior at 24 mainland nests and 49.1 h of provisioning behavior at 8 island nests to identify social parents. Human observers collected $\sim 80\%$ of nest-watch hours with video documenting the remainder. Because we were unable to clearly read band combinations for some visits, $\sim 15\%$ of mainland nest visits ($n = 1964$ visits) and $\sim 5\%$ of island nest visits ($n = 553$ visits) involved trips where the attending bird remained unidentified. In both populations, we identified only two adults visiting each nest, confirming that the black catbird exhibits social monogamy and bi-parental care. Only 58 nests reached the nestling stage, and we sampled offspring and one or both parents from 25 of these nests (mainland: $n = 13$ representing 12 social mothers and 12 social fathers; island: $n = 12$, representing 12 social mothers and 12 social fathers). Relative to all nests that we found, the proportion of nests sampled differed between the mainland and island (12% and 57%, respectively; $\chi^2_1 = 19.839$, $p < 0.001$) because a greater proportion of nests failed (especially during incubation) on the mainland.

Genetic parentage and extra-pair paternity (EPP)

Parentage analyses with CERVUS did not exclude the social mother for any nestling in either population. However, the social father was excluded as the genetic father for some nestlings in both mainland and island populations (Table 2). Combining populations, 21% of offspring (CI = 11–37%, $n = 38$) were sired by extra-pair males, and 30% of nests (CI = 15–51%, $n = 23$) contained at least one extra-pair nestling. Patterns of EPP differed slightly between populations, but not significantly so: EPO accounted for 9% of mainland nestlings (CI = 1–30%, $n = 21$) and 35% of island nestlings (95% CI = 17–59%, $n = 17$) (Fisher's exact test, two-tailed: $p = 0.11$). The pattern was similar for nests: 17% of mainland nests (CI = 4–46%, $n = 12$) and 45% of island nests (CI = 21–72%, $n = 11$) contained at least one EPO (Fisher's exact test, two-tailed: $p = 0.19$).

These parentage assignments were generally supported by the Wang relatedness estimator values for the different relationship categories (Supporting information). Full sibling and half sibling relationships were consistent with expectations except in one island nest in which we initially considered two nestlings to be half siblings because they were both extra-pair; however, the pairwise relatedness score for these

Table 2. CERVUS parentage results reported according to the number of nestlings sampled. The number of broods sampled are reported parenthetically.

Parents sampled	Sample sizes		Assigned to social mother		Assigned to social father	
	Mainland	Island	Mainland	Island	Mainland	Island
Both	18 (10*)	17 (11)	18 (10)	17 (11)	16 (8**)	11 (6**)
Mother only	2 (1)	1 (1)	2 (1)	1 (1)	–	–
Father only	3 (2)	–	–	–	3 (2**)	–
Total	23 (13)	18 (12)				

*The 10 mainland broods for which both parents were sampled represent nine unique mothers and nine unique fathers, including one parent pair for which two broods were sampled.

**The number of broods reported are those for which only the social father was assigned.

two nestlings (0.77) fell within the range of a full sibling relationship, suggesting both offspring were sired by the same extra-pair male.

Breeding density and genetic diversity

NND differed significantly between the two populations (Fig. 1a, Table 3a). Mainland nests were closer together (mean NND = 24.0 ± 4.2 m, $n=61$ nests) than island nests (mean NND = 129.5 ± 15.6 m, $n=18$ nests), indicating higher breeding density on the mainland.

However, no significant association between NND and EPP emerged (Table 3b). NND was similar for nests with EPO (mean NND = 108.49 ± 37.1 m, $n=7$ nests) and nests with WPO only (mean NND = 49.93 ± 14.88 m, $n=15$ nests).

Standardized multi-locus heterozygosity did not differ between the two populations ($W=1108$, $p=0.17$; Fig. 1b) although there was a tendency for sMLH to be lower for island birds than for mainland birds. Mean sMLH was 1.01

± 0.04 for the mainland ($n=67$) and 0.93 ± 0.05 for the island ($n=28$).

At the level of the nest, a male's heterozygosity was not a significant predictor of whether he was cuckolded (males with EPO: sMLH = 0.80 ± 0.03 , $n=7$ fathers; males with no EPO sMLH = 1.08 ± 0.10 , $n=15$ fathers; Table 4a). However, the heterozygosity of individual offspring was significantly associated with parentage (Table 4b). Across all offspring, extra-pair nestlings had higher heterozygosity than within-pair nestlings (EPO mean sMLH = 1.36 ± 0.18 , $n=8$ offspring; WPO mean sMLH = 1.00 ± 0.06 , $n=30$ offspring; Table 4b). When analyzing only offspring from mixed parentage nests, sMLH was still significantly associated with parentage (EPO = 1.73 ± 0.02 , $n=5$; WPO = 0.91 ± 0.10 , $n=8$; Fig. 2, Supporting information).

Within-pair relatedness of social mates was not significantly associated with paternity loss (Table 4c). Relatedness values were similar between the female and male of social pairs whose broods included EPO (0.14 ± 0.04 , $n=7$ pairs) and those whose broods lacked EPO (0.20 ± 0.05 , $n=13$ pairs).

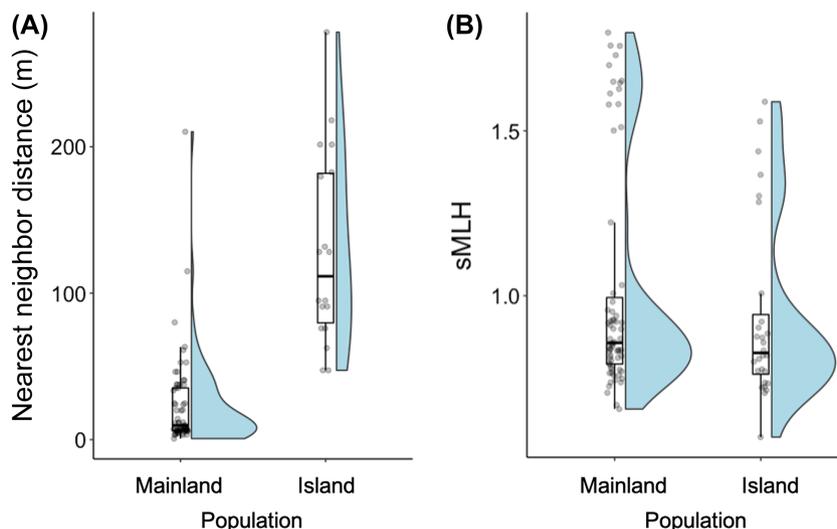


Figure 1. Rain cloud plots comparing (a) nearest neighbor distance (NND) and (b) genetic diversity of two populations for the two black catbird populations in our study. (a) NND (linear distance to the nearest nest) is inversely related to breeding density: short distances correspond to high density and long distances to low density. (b) We used standardized multi-locus heterozygosities (sMLH) as a metric of genetic diversity based on 414 single-nucleotide polymorphisms (SNPs) typed for mainland ($n=67$ individuals) and island ($n=28$ individuals) birds. Blue shapes graph the density function for each population. Boxes contain first and third quartiles while whiskers extend to the largest value not further than $1.5 \times$ the inter-quartile range above or below the box, and points represent raw data.

Table 3. Model results of breeding density represented by nearest-neighbor distance (NND). Model (a) was a linear model of the natural log transformed NND and population (mainland or island) as a predictor. Model (b) was a generalized linear model with a binomial fit and used CERVUS parentage results. The binary response was whether any extra-pair offspring were detected in a social father's nest (1) or not (0), and predictors included population and NND z-scores.

	Estimate \pm SE	t or Z value	p
(a) Breeding density (populations)			
Intercept	4.736 \pm 0.233	20.33	< 0.001
Population (mainland)	-2.150 \pm 0.265	-8.11	< 0.001
(b) Breeding density and parentage (nests)			
Intercept	-0.384 \pm 0.874	-0.440	0.660
Nearest neighbor distance	0.470 \pm 0.738	0.637	0.524
Population (mainland)	-0.905 \pm 1.483	-0.610	0.542

Discussion

We confirmed that the black catbird exhibits social monogamy with bi-parental care, and documented moderate rates of EPP (21% of offspring were sired by extra-pair males and 30% of nests contained EPO). Contrary to the predictions of the breeding density hypothesis, the incidence of EPP did not differ between the island and mainland, despite far higher breeding densities in the mainland population. Indeed, our results suggested a trend toward higher rates of EPP in the island population, where NNDs were more than five times greater than on the mainland. Evidence for the genetic diversity hypothesis was mixed: at the individual level, neither the social father's heterozygosity (sMLH) nor within-pair relatedness predicted paternity loss. However, extra-pair nestlings were more heterozygous than were within-pair nestlings (across all nestlings, as well as within nests of mixed parentage), suggesting a possible role for genetic diversity in mating decisions. At the population level, our results were consistent with the genetic diversity hypothesis because the two populations did not differ in overall heterozygosity nor in the overall incidence of EPP. However, the stronger test of this hypothesis would have been to compare two populations differing in underlying heterozygosity. While our results are consistent with the hypothesis, they are inconclusive as to whether

genetic diversity drives population differences in EPP for the black catbird.

However, we caution that these results, especially at the population level, were based on incomplete sampling and relatively small sample sizes, which could have obscured differences in mating patterns between the two populations. As in most studies that require genetic information from nestlings, our samples were biased towards successful nests, since nests that were depredated before the nestlings reached 7–10 days of age could not be sampled. Importantly, nest predation rates were significantly higher on the mainland than on the island (Roldán-Clarà et al. 2013), such that a lower proportion of known nests was sampled on the mainland than on the island (12% versus 57%, respectively). It is possible that depredated and non-depredated nests (and pairs) differ in traits that could influence mating decisions, and this could have affected our estimates of EPP. For example, if cuckolded males invest less in parental care (Dixon et al. 1994) and if paternal care reduces nest failure rate (Lifjeld et al. 1998), then nests containing EPO could theoretically be more vulnerable to predation and be under-sampled in our data set. This would result in an erroneously low estimate of EPP for the mainland population. Male black catbirds do invest heavily in nestling care (in one instance, a male fledged two of three young after his mate disappeared several days before fledging; LaPergola

Table 4. Genetic diversity model results. Model (a) was a generalized linear model (GLM) fit with a binomial distribution and the response variable was whether any extra-pair offspring (EPO) were detected in a social father's nest (1) or not (0). Population (mainland or island) and the social father's z-transformed standardized multi-locus heterozygosity (sMLH) were included as a predictors. Model (b) was a linear mixed-effects model with sMLH of individual offspring as the response variable, parentage type (extra-pair, EPO or within-pair offspring, WPO) and population as categorical fixed effects, and nest ID as a random effect. All offspring with paternity information were included (n=38). Model (c) was a GLM fit with a binomial distribution and the binary paternity loss result as the response (1=pairs had \geq 1 EPO, 0=pairs had no EPO). The predictor variables include the Wang relatedness estimator for social pairs and population.

	Estimate \pm SE	df	t or Z value	p
(a) Parentage for social fathers				
Intercept	-0.491 \pm 0.968		-0.507	0.612
sMLH	-2.326 \pm 1.432		-1.624	0.104
Population (mainland)	-2.157 \pm 1.194		-1.807	0.071
(b) sMLH of all offspring				
Intercept	1.556 \pm 0.138	30.224	11.238	< 0.001
Parentage (WPO)	-0.554 \pm 0.128	18.471	-4.333	< 0.001
Population (mainland)	-0.092 \pm 0.154	14.987	-0.597	0.559
(c) Within-pair relatedness and parentage loss				
Intercept	0.093 \pm 0.662		0.141	0.888
Relatedness	-0.975 \pm 0.696		-1.401	0.161
Population (mainland)	-2.020 \pm 1.364		-1.481	0.139

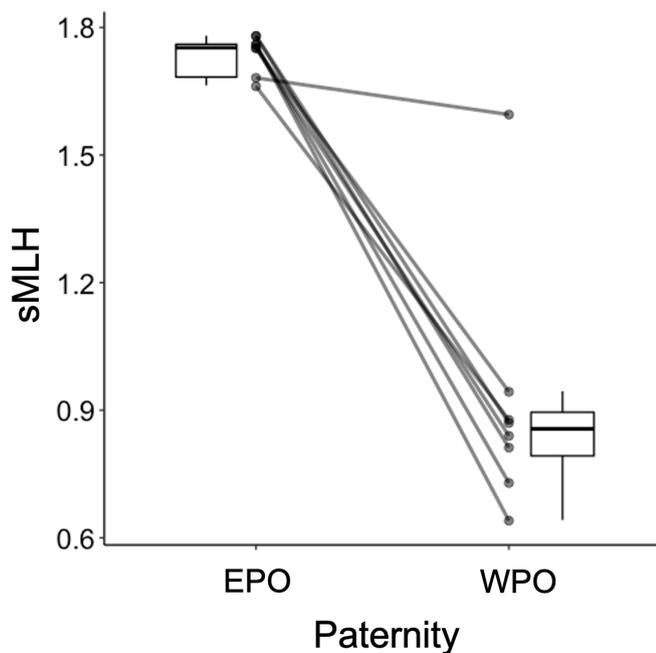


Figure 2. Boxplot and slope graph of standardized multi-locus heterozygosity (sMLH) of black catbird nestlings from mixed parentage broods. Individual points represent extra-pair (EPO, $n=5$) and within-pair offspring (WPO, $n=8$), and each line connects offspring from the same nest ($n=5$ nests).

2012), but we lack data on the effect of paternal care on nest predation. To adequately test this hypothesis, one would need to sample all nests, perhaps using incubators and replacement eggs to increase the percentage of broods sampled before nest failure (Tori et al. 2006) and to compare EPP levels of successful and unsuccessful nests. Increased sampling by using incubators would also address our concerns regarding sample size. Griffith et al. (2002) recommended 200 offspring as a sample size that optimized the trade-off between accuracy and costs of additional sampling. Our sample sizes for both populations (mainland = 21 offspring from 12 broods; island = 17 offspring from 11 broods) were thus below the target recommended by Griffith et al. (2002). With an average black catbird clutch size of 2.3 (LaPergola 2012), we would need to completely sample 87 nests in each population to reach the recommended 200 offspring.

The finding that extra-pair young were more heterozygous than within-pair young is intriguing and deserves future study. The difference in heterozygosity was particularly striking when comparing offspring within broods, suggesting that female black catbirds may pursue extra-pair copulations to increase the genetic diversity of their broods. Support for this hypothesis is, however, qualified by the finding that social fathers that lost paternity were no less heterozygous than social fathers that did not, and breeders within pairs with EPO were not more closely related to each other than pairs without EPO. It is possible that on an individual basis, genetic compatibility might drive extra-pair mate choice (Blomqvist et al. 2002) but we did not test this hypothesis;

doing so would require more extensive sampling of nests and genotyping fitness-relevant genes, such as those in the major histocompatibility complex (Richardson et al. 2005).

It is also possible that female catbirds engage in EPP with males that are more heterozygous than their social mates, regardless of within-pair relatedness. Unfortunately, we lack information on the identity of extra-pair sires in our populations, so direct comparison of social mates and extra-pair mates was not possible. Future work with more intensive sampling of adult catbirds could potentially provide a test of this prediction. Evidence that heterozygosity is linked to offspring fitness is similarly lacking for catbird nestlings (and for most species in which this hypothesis has been tested). In some studies, heterozygosity correlates with recruitment success of fledglings (Hansson et al. 2001) and metrics of adult fitness (Seddon et al. 2004, Velando et al. 2015). Whether EPP actually leads to indirect benefits for female catbirds thus remains an intriguing but unresolved possibility.

In an earlier comparative study, Griffith (2000) found that insular populations of passerines generally exhibited lower rates of EPP than mainland populations, supporting the hypothesis that reduced genetic variation on islands leads to a corresponding reduction in variation in quality among males and lower benefits for EPP. Our study of black catbirds failed to find such a difference in EPP, possibly because overall levels of genetic variation were not higher on the mainland. The Cozumel catbird population is quite large and located relatively close to the mainland, and immigration from the mainland may have prevented genetic bottlenecks that typically characterize island populations. Numerous other insular black catbird populations exist in Mexico (e.g. Isla Mujeres and Isla Contoy) and Belize (e.g. Caye Caulker (Miller and Miller 1991) and Ambergris Caye (Miller and Miller 1998, Johnson and Haight 2011)), affording other opportunities to investigate the effects of insularity on mating system in this species.

Finally, these results underscore the need for more studies of the genetic mating systems of tropical birds to provide a more inclusive and complete picture of avian behavioral ecology. Although earlier syntheses predicted that tropical birds might be more monogamous than their northern counterparts (Stutchbury and Morton 2001), more recent studies have found that EPP rates in the tropics can be just as high as those in the temperate zone (reviewed by Riehl 2020). The levels of EPP that we report for black catbirds at both the level of individual offspring (21%) and nests (30%) were close to the global averages for socially monogamous species with bi-parental care: 19% and 33%, respectively (Brouwer and Griffith 2019). These results are consistent with an emerging consensus that patterns of EPP across birds are more strongly influenced by phylogeny than by proximate constraints, and that not all tropical songbirds exhibit low rates of EPP (Macedo 2008, Brouwer and Griffith 2019). More genetic parentage studies on tropical birds are still needed to elucidate the role of latitude in EPP across species, and to test adaptive hypotheses for extra-pair mate choice.

Acknowledgements – We gratefully acknowledge Jesús Gustavo Marina Hipolito, Ligia Torres Abán, Jan F. Gogarten, Adèle Michon and other volunteers for assistance with data collection; Felipe Juárez, Rafael Chacón, Omar Ortiz and CONANP, the Fundación de Parques y Museos de Cozumel, Fundación Plan Estratégico de Cozumel, Dirección de Ecología Municipal, Island Endemics Foundation, Amigos de Sian Ka'an A. C., Global Vision International and Boca Paila Fishing Lodge for generous logistical support; and the subject editor, two anonymous reviewers, Daniel R. Ardia, Vikram K. Iyengar and Walter D. Koenig, for their valuable comments on earlier versions of the manuscript and constructive input at various stages of the project. We also thank members of Cornell's Dept. of Neurobiology and Behavior Lunch Bunch and Princeton's Integrated Behavioral Research Group for helpful feedback and suggestions. We also thank Dhriti Tandon for splitting the MiSeq library with us. Finally, we thank Wei Wang and the staff of the Princeton Univ. Genomics Core Facility for assistance with library preparation and sequencing.

Funding – Villanova Univ. and a Florida Ornithological Society William B. and Mary J. Robertson Fellowship awarded to JBL provided funding. JBL was also supported by a grant from the National Science Foundation (grant no. 1755279 award to CR). JEM-G was a postdoctoral fellow at the Inst. de Ecología, A.C. while we conducted this research.

Permits – We completed all fieldwork in accordance with SEMARNAT permits SGPA/DGVS/02595/08 and SGPA/DGVS/03358/09, RBSK004/2008, and Villanova Univ. IACUC protocol no. AS 08-06.

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Transparent peer review

The peer review history for this article is available at <<https://publons.com/publon/10.1111/jav.03034>>.

Data availability statement

Data are available from the Dryad Digital Repository: <<https://doi.org/10.5061/dryad.2bvq83btg>> (LaPergola et al. 2022).

Supporting information

The Supporting information associated with this article is available with the online version.

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