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# Local male breeding density affects extra-pair paternity in a south temperate population of grass wrens *Cistothorus platensis*

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Demographic factors can affect the frequency of extra-pair paternity (EPP) in birds, as the distribution and availability of potential mates in both space and time influence the rate of encounters between females and males. Over three breeding seasons, we intensively studied the breeding system of a south temperate population of grass wrens *Cistothorus platensis* by genotyping 73 broods (319 nestlings) and estimating EPP rates for those broods. Using five different radii (80, 160, 240, 320 and 400 m) around each nest with assigned paternity, we examined the effects of local breeding synchrony, male breeding density and adult sex ratio (ASR) on the EPP rate. The majority of extra-pair offspring (~80%) were sired by neighboring males. Neither local breeding synchrony nor ASR consistently explained the EPP rate variation as their effects were only statistically significant within 320 and 400 m. However, the EPP rate increased as the local male breeding density increased within every radius category, strongly suggesting that neighboring male abundance might play an important role in the extra-pair mating behavior in this species. Our study also highlights the relevance of using a local scale approach when studying mating behavior.

Keywords: demography, male abundance, parentage, sex ratio

## Introduction

The discovery of extra-pair paternity (EPP) – the siring of offspring by a male other than the female's social partner – radically changed our understanding of mating systems (Gowaty 1985, 1996, Bennett and Owens 2002). Although monogamy is the predominant social mating system in birds (Lack 1968), the prevalence of genetic polygamy can be an important source of variance in male reproductive success, increasing the arena in which sexual selection can act (Hill et al. 1994, Møller and Ninni 1998). Why some individuals engage more frequently in extra-pair copulations than others remains a central question for understanding the importance of sexual selection in socially monogamous species (Griffith et al. 2002, Westneat and Stewart 2003,

Macedo et al. 2008). Demographic variables are thought to play a central role in shaping genetic mating systems, as the frequency of EPP is expected to be constrained by the distribution and availability of potential mates in both space and time (Westneat and Sherman 1997, Griffith et al. 2002, Shuster and Wade 2003). However, there is still no consensus on the general role of demographic variation in driving variation in EPP among individuals and across species (Brouwer and Griffith 2019, Beck et al. 2020).

A high encounter rate between males and females might increase the frequency of EPP (Westneat et al. 1990). Thus, it is expected that higher breeding densities increase EPP rates by facilitating mating opportunities (Westneat and Sherman 1997, Mayer and Pasinelli 2013, Brouwer et al. 2017). Some studies have investigated the relationship between breeding density and EPP with mixed results (Hasselquist et al. 1995, Chuang et al. 1999, Thusius 2001, Stewart 2006, Mayer and Pasinelli 2013, Schlicht et al. 2015). For instance, in the great reed warbler *Acrocephalus arundinaceus*, nests with extra-pair offspring are located in territories with more male neighbors within a 100 m radius than nests with no extra-pair offspring (Hasselquist et al. 1995). Similarly, in the reed bunting *Emberiza schoeniclus*, the EPP rate increases with breeding density within a radius of 170 m (Mayer and Pasinelli 2013). In contrast, no effects of breeding density on EPP have been reported in black-throated blue warblers *Setophaga caerulescens* (Chuang et al. 1999). Several experimental studies have also shown that breeding density affects EPP rates. For example, in cavity nesters, manipulating the nest-box availability to create high-density breeding areas increases the likelihood of EPP in the brood (Gowaty and Bridges 1991, Charmantier and Perret 2004, Stewart et al. 2010).

Another factor proposed to influence EPP variation is breeding synchrony: the proportion of females that are simultaneously fertile (Stutchbury and Morton 1995). Breeding synchrony includes both the spatial and temporal distribution of individuals, which plays an important role in extra-pair mating behavior (Stutchbury and Morton 1995, Weatherhead 1997). From the male perspective, breeding synchrony might promote EPP if mate guarding is not important in assuring paternity, which allows males to invest time pursuing extra-pair copulations (Chuang et al. 1999). On the contrary, breeding synchrony may constrain EPP if males face a tradeoff between mate guarding and seeking extra-pair copulations (Birkhead and Biggins 1987, Birkhead and Møller 1992). From the female perspective, breeding synchrony may increase EPP rates if the simultaneous display of males allows females to more accurately select the best-quality extra-pair males (Stutchbury 1998a, b, Stutchbury and Morton 2001). The empirical evidence for a relationship between breeding synchrony and EPP is mixed. Intra-population studies have reported a positive relationship between breeding synchrony and EPP rates when assessing either population synchrony (within-seasonal variation) or local synchrony (within clusters of neighboring territories). In the hooded warbler *Wilsonia citrina* the frequency of EPP is higher early in the breeding season when more females are simultaneously fertile (Stutchbury et al. 1997).

Similarly, in the black-throated blue warbler *Setophaga caerulescens*, higher EPP rates are associated with higher synchrony among female neighbors (Chuang et al. 1999). In contrast, several studies have reported the opposite result. In common yellowthroats *Geothlypis trichas*, EPP is higher when population breeding synchrony is low (Thusius et al. 2001), while in Australian golden whistlers *Pachycephala pectoralis* EPP is higher when synchrony within a radius of three territories is low (van Dongen and Mulder 2009). Finally, some studies have failed to find an association between synchrony and EPP. For example, the EPP rate is unrelated to local synchrony in both a north temperate and a south temperate population of house wrens *Troglodytes aedon* (LaBarbera et al. 2010). Similarly, in great reed warblers *Acrocephalus arundinaceus* there is no relationship between EPP rates and breeding synchrony when considering either population or local synchrony (Arlt et al. 2004).

Adult sex ratio (ASR; defined as the proportion of adults that are males in a population) is a fundamental demographic variable that influences mating competition (Kokko and Jennions 2008, Liker et al. 2014). A male-biased ASR (higher number of males in relation to females) is typical of many bird populations (Donald 2007). However, there is still no consensus on how ASR affects EPP. For example, EPP is thought to increase with a male-biased ASR either because there are more potential extra-pair males to choose from (Liker et al. 2014, Székely et al. 2014) or because male coercion drives extra-pair fertilizations through forced copulations (Adler 2010). In contrast, it has been proposed that EPP should decrease with a male-biased ASR because males may intensify mate-guarding behavior to avoid losing paternity (Harts and Kokko 2013). Liker et al. (2014) found that EPP is more frequent in socially monogamous species with male-biased than in those with female-biased ASR. However, Grant and Grant (2019) reported that extra-pair mating decreases in male-biased populations of the medium ground finch *Geospiza fortis* and cactus finch *G. scandens*. Although the ASR should strongly affect mate availability, the role of local ASR in determining EPP rates in clusters of territories within the same population has rarely been investigated.

Several studies have found that extra-pair offspring are most frequently fathered by the nearest neighbor (Gibbs et al. 1990, Westneat 1993, Dunn et al. 1994, Stutchbury et al. 1994, Chuang et al. 1999, LaBarbera et al. 2010, Grant and Grant 2019). Thus, a local demographic approach that explicitly considers the spatial clustering of territories and proximity among neighbors may best reflect the interactions between males and females that determine patterns of EPPs (Chuang et al. 1999, Canal et al. 2012).

In this study, we describe the genetic mating system of a south temperate grassland specialist, the grass wren *Cistothorus platensis*. The grass wren is a socially monogamous passerine distributed from Cape Horn to Mesoamerica (Zarco and Llambías 2018, Llambías et al. 2019a, Fujikawa and Tubelis 2020, Remsen et al. 2021). Until recently it was considered conspecific with the largely migratory sedge wren *Cistothorus stellaris* of North America (Chesser et al. 2021),

making the grass wren an excellent model for future comparisons between tropical and temperate populations. Using data from three breeding seasons and parentage analyses of 73 broods, we estimate the population EPP rate (proportion of extra-pair offspring in the study population) per season and then explore the influence of breeding synchrony, male breeding density and ASR on the EPP rate (proportion of extra-pair offspring in each brood) from a local scale perspective.

## Material and methods

### Study species

The grass wren is a small (10 g), insectivorous, passerine (Zarco and Llambías 2018, Remsen et al. 2021). Males develop complex songs which are used to defend their territory and attract females (Kroodsma et al. 1999). Both sexes collaborate in building the nest and in feeding the nestlings, but only females incubate the eggs and brood the young (Llambías et al. 2019a, b). At our study site, grass wrens are year-round residents and frequently lay two successive clutches of 4–6 eggs per breeding season (Llambías et al. 2019a).

### Study site

Fieldwork was conducted in the floodplain of the Uspallata Stream and Mendoza River (32°38′10″S, 69°22′16″W, 1800 m a.s.l.), Mendoza Province, Argentina. Our study site spanned approximately 120 ha and consisted of small swamps and pockets of riparian grasslands dominated by pampa grass *Cortaderia selloana* (Martínez Carretero 2000). Seasonality is pronounced, with temperatures below freezing and occasional snowfall during the austral winter (mean temperature = 6.88°C) and higher temperatures in the austral summer (mean temperature = 13.78°C).

### General field procedures

We carried out intensive fieldwork over three breeding seasons (from October to February, 2015–2017). At the beginning of each season, we captured males with mist-nets by stimulating aggressive behavior with song play-back. Females were caught by herding them to the mist-net or by setting the mist-net close to the nest while they were feeding nestlings (11–12 days old). Adults were marked with a numbered aluminum ring and a unique combination of colored leg bands. Blood samples (20–50 µl) were collected for paternity analysis. We monitored territories daily to determine social pairs and located nests by using parental behavioral cues (i.e. observing individuals with nesting material or with food to feed nestlings) and by systematic searching. Thus, we were able to identify both social female and male from every nest.

Nests were monitored until all nestlings fledged or the nesting attempt failed. We marked nestlings aged 7–10 days with numbered aluminum rings and collected blood samples. All blood samples were collected from the brachial vein and stored in lysis buffer until DNA extraction. Since some breeding pairs raised two broods during the same year, we avoided pseudoreplication by randomly choosing one brood of each breeding pair to measure EPP rate.

### ddRAD sequencing and SNP data analysis

DNA was extracted from blood samples following a standard protocol of dehydration and precipitation with ethanol and NaCl (Miller et al. 1988). We performed double-digest restriction site-associated DNA sequencing (ddRAD) for single nucleotide polymorphism (SNP) discovery by following the protocol of Peterson et al. (2012) with the modifications described in Thrasher et al. (2018). The ddRAD sequencing protocol together with the SNP data analysis were described in Arrieta et al. (2020). This procedure resulted in 762 SNPs for the 2015 season, 906 SNPs for 2016 and 855 SNPs for 2017.

### Parentage analysis

We used CERVUS ver. 3.0.7 (Kalinowski et al. 2007) to infer parentage and calculate the EPP rate for each season. CERVUS assigns paternity using the natural logarithm of the likelihood ratio (LOD score), which provides the likelihood of paternity of each candidate male relative to a random male in the population. Positive LOD scores indicate that a candidate male is more likely to be the genetic father than a randomly chosen male, while a zero or negative number indicates that the candidate male is as likely or less likely to be the genetic father than a randomly chosen male.

Before paternity analyses, we ran simulations for 10 000 offspring based on population allele frequencies, the number of candidate fathers, the proportion of candidate fathers sampled, the proportion of loci typed and the overall genotyping error rate. Only adults were included to estimate allele frequencies (Flanagan and Jones 2019). We set the number of candidate fathers to a value of 8 based on the typical number of nearest neighbors in this population (Lemons et al. 2015). The remaining parameters followed those in the CERVUS user manual. Within these parameters, critical LOD scores are calculated to assign paternity at either 80% or 95% confidence.

CERVUS calculates pair LOD scores by comparing the genotypes of the candidate father and offspring. Trio LOD scores involve the same comparison, while also considering the genotype of the known mother. Field data allowed us to include the putative mother in the maternity analyses. CERVUS ranks males based on both pair and trio LOD scores values. The highest-ranked male was assigned as the genetic father. We also set an allowable number of offspring-male loci mismatches related to the observed maximum number of loci mismatches between a known father (i.e. putative father confirmed by CERVUS) and his offspring. Thus, the genetic father was assigned when the highest-ranked male showed a positive (or slightly negative) pair LOD score and the offspring had 8 or fewer mismatches (~1% of total loci). This criterion applied for either the social or sampled

extra-pair males. When an assignment was ambiguous, we accepted it if the highest-ranking male also showed a positive trio LOD score. Whenever an extra-pair male was assigned (i.e. the genetic father was not the male attending the nest in the field), we confirmed it by verifying that the extra-pair and social males occupied neighboring territories. When the highest-ranked male was not the putative father, showed more than 8 loci mismatches with the offspring, and had a high negative value for pair LOD score, we considered those nestlings to have been fathered by an unsampled extra-pair male. Only 1 out of 73 broods in our dataset contained an unsampled putative father because we were not able to collect a blood sample. However, as we sampled the putative mother, the offspring and the neighboring males, CERVUS output allowed us to discriminate between within-pair and extra-pair offspring.

Based on the results of paternity analyses we calculated different indexes of EPP. We reported the population EPP rate (proportion of extra-pair offspring in the study population) and the percentage of broods with extra-pair offspring to describe the genetic mating system. To analyze the influence of local demographic factors on EPP, we estimated the EPP rate (proportion of extra-pair offspring in the brood).

### Local demographic variables

We defined different radii (up to 80, 160, 240, 320 and 400 m) around each nest (hereafter, focal nest) with assigned paternity (n = 73) with the program Garmin BaseCamp ver. 4.7.3. Including neighboring breeding territories encompasses scales at which social interactions among individuals are more frequent, which are critical to compare and select extra-pair partners (Chuang et al. 1999). In addition, using different radii allowed us to evaluate whether results change according to radius size.

We counted the number of paired males (both socially monogamous and polygynous males) within the radius of the focal nest as a measure of local male breeding density. In our study site, grass wrens were predominately socially monogamous over nine years (2010–2018). However, almost 60% of males established polygynous associations (i.e. one male mates with multiple females) during the breeding season of 2015. This unusual behavior might be explained by a female-biased ASR observed during that season (Arrieta et al. unpubl.).

We also counted the number of males and females to calculate the local ASR (number of adult males/number of adult females). While male breeding density considered only paired males, ASR also included single males defending a territory. Field observations suggest that 'floaters' (i.e. non-territorial individuals) are rare in our population: 1) most males and females of known age (banded as nestlings) started breeding during their first year, 2) all adult birds that were captured in mist nets and banded were observed breeding or defending territories the year when they were captured and 3) several territories remain vacant every

year. When calculating both variables, we only considered those individuals present during the breeding attempt at the focal nest.

We calculated the local synchrony index for each focal nest following Kempenaers (1993). We counted the number of neighboring females that were fertile during each focal female's fertile period. We assumed that a female's fertile period started five days before the first egg was laid through the day the penultimate egg was laid (Yezerinac and Weatherhead 1997, Johnson et al. 2002). A local synchrony index of 0 indicates a completely asynchronous group of neighboring females with no overlap of fertile periods, whereas a synchrony index of 1 indicates a completely synchronous group of neighboring females. All nesting attempts that resulted in at least one egg were included in our calculations.

### Spatial distribution of extra-pair paternity

We determined the most frequent distances that grass wrens moved to obtain extra-pair fertilizations. After assigning paternity to extra-pair offspring, we calculated the distance from the nest with paternity loss to the nest attended by the extra-pair father with the program Garmin BaseCamp ver. 4.7.3. We then plotted the number of nests with mixed paternity within each radius category.

We also determined the availability of nests attended by fertile females within each radius. To evaluate whether the distances that grass wrens moved to obtain extra-pair fertilizations were affected by the number of potential mates, we first counted the number of available nests (i.e. nests attended by females whose fertile periods overlapped the focal female's by at least one day) within each radius of focal nests. We then plotted the number of available nests within each radius category.

### Statistical analyses

We used generalized linear models with a negative binomial distribution and log link function to examine how the number of extra-pair nestlings in the brood varied with fixed effects, with the log of the number of nestlings in the brood as an offset. We included the male breeding density, synchrony and ASR as explanatory variables. We found that male breeding density and ASR were correlated in four out of five radii (80 m, rho = 0.21, p = 0.10; 160 m, rho = 0.31, p = 0.01; 240 m, rho = 0.43, p < 0.01; 320 m, rho = 0.60, p < 0.01; 400 m, rho = 0.63, p < 0.01). Thus, we ran separate models to avoid problems related to collinearity. While 'Model A' included both breeding synchrony and male breeding density, 'Model B' included ASR and breeding synchrony. Additionally, we standardized the explanatory variables to evaluate their relative biological importance.

Analyses were performed in the R language and environment (ver. 4.0.3; <www.r-project.org>). Model simplification was performed by backward stepwise elimination of non-significant terms from the full model by using a $\chi^2$ likelihood ratio test (Crawley 2012).

## Results

### Genetic mating system and extra-pair paternity

Over three breeding seasons, we genotyped 105 adults and 319 offspring from 73 broods. CERVUS assigned paternity to 96.8% of the nestlings and identified extra-pair males from 30 out of 33 (90.1%) nests with mixed-paternity. Every sampled extra-pair male was paired with a female. CERVUS was not able to assign paternity to seven nestlings from three nests. However, CERVUS identified the attending female as the genetic mother and assigned some of their siblings to the putative father. Thus, we concluded that they had been fathered by unsampled extra-pair males.

Three nestlings (from three different nests) were not genetically related to any sampled adult. We considered them cases of conspecific brood parasitism by an unsampled female who had mated with an unsampled male because CERVUS assigned their nestmates to both putative parents in those three nests (Arrieta et al. 2020).

Twenty-seven nests with mixed paternity (~80%) occurred within 160 m of the extra-pair father's nest location (Fig. 1a). On average, individuals moved up to 130 ± 16 m (mean ± SE; n = 34) to obtain extra-pair fertilizations. However, the number of available nests for potential extra-pair fertilizations was similar between radii (Fig. 1b).

Paternity analysis revealed that the population EPP rate ranged between 8.5 and 27.0% among seasons (Table 1). The percentage of broods with extra-pair offspring ranged between 28.0 and 57.1% among seasons (Table 1). The number of extra-pair offspring in the nest varied, 40% had one extra-pair offspring, 33.3% had two and 27.3% had three.

### Influence of local demographic factors on paternity

In our study population, male breeding density ranged between 1 and 22, breeding synchrony ranged between 0 and 0.89 and ASR ranged between 0.33 and 1.50. The EPP rate increased with increasing male breeding density within every radius (model A, Table 2). We also found a positive association between the EPP rate and ASR within 320 and 400 m (model B, Table 2). However, the EPP rate was positively related to breeding synchrony only within 320 m (model A, Table 2).

## Discussion

South temperate grass wrens at our study site had moderate rates of EPP; however, we observed a high percentage of broods with extra-pair offspring (Table 1). Most of the nests with mixed paternity were located within 160 m of the extra-pair father's nest (Fig. 1a) despite that the availability of fertile females was similar between radii (Fig. 1b), suggesting that individuals preferred to seek extra-pair fertilizations close to their breeding territory. Our analyses revealed that the EPP rate increased as local male breeding density increased within all radii, strongly suggesting that male abundance may play an important role in determining the extra-pair mating behavior of grass wrens. Neither local breeding synchrony nor ASR consistently explained the EPP variation in the studied population.

The population EPP rates of grass wrens (8.5–27%) were similar to those reported in other south-temperate socially monogamous passerines (house wren, *Troglodytes aedon* = 15.7%, LaBarbera et al. 2010; blue-black grassquit *Volatinia jacarina* = 21.1%, Manica et al. 2016; ringed
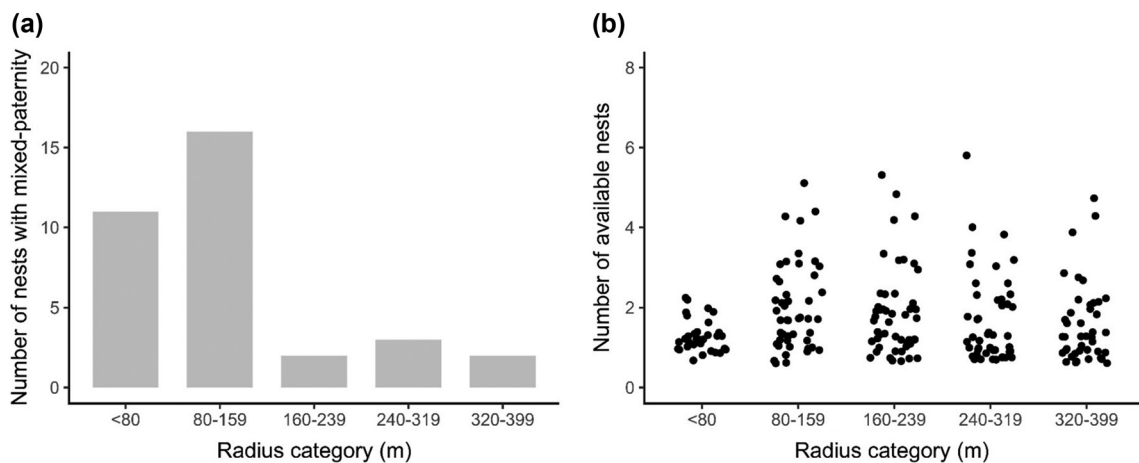


Figure 1. South temperate grass wrens obtained most of their extra-pair fertilization from neighboring mates, even though nests were available within the five radii considered. (a) Number of nests with mixed-paternity in relation to the distance between the focal nest (mixed-paternity) and the nest attended by the extra-pair father. (b) Number of available nests for potential extra-pair fertilizations within each radius. Available nests include only those nests belonging to females whose fertile periods overlapped the focal female's (i.e. female attending the nest with paternity data) by at least one day. Jitter was added to the x-axis to increase the visibility of data points.

Table 1. Results of paternity analyses in grass wrens for three breeding seasons. We report the number of nestlings and broods sampled, the number of extra-pair nestlings, the population EPP rate (percentage of extra-pair offspring in the population) and the percentage of broods with extra-pair offspring.

| Season | No. of nestlings | No. of extra-pair nestlings | No. of broods | Population EPP rate (%) | Broods with extra-pair offspring (%) |
|--------|------------------|-----------------------------|---------------|-------------------------|--------------------------------------|
| 2015 | 118 | 10 | 25 | 8.5 | 28.0 |
| 2016 | 89 | 24 | 21 | 27.0 | 57.1 |
| 2017 | 112 | 28 | 27 | 25.0 | 51.8 |

warbling-finch, *Poospiza torquata* = 20.6%, Sánchez et al. 2018; thorn-tailed rayadito, *Aphrastura spinicauda* = 5.8–14%, Botero-Delgadillo et al. 2020). Although a generally small proportion of nestlings within a brood were sired by an extra-pair male, more than half of the broods had at least one extra-pair offspring in 2016 and 2017 (Table 1). In contrast, a smaller proportion of broods with extra-pair offspring have been reported in other south-temperate socially monogamous passerines (*T. aedon* = 32.5%; LaBarbera et al. 2010, *V. jacarina* = 30.5%; Manica et al. 2016; *P. torquata* = 42.8%; Sánchez et al. 2018, *A. spinicauda* = 14.3–20.7%; Botero-Delgadillo et al. 2020). Our results suggest that genetic polygamy is a frequent mating strategy in south temperate grass wrens. Future comparisons between mating strategies of tropical and temperate populations of grass wrens and other species of the genus *Cistothorus* may allow the identification of life history and environmental variables that affect the genetic mating system.

Intra-population studies comparing the rate of EPP among birds with different local densities have reported variable results (reviewed by Griffith et al. 2002). The lack of a general relationship between density and EPP might be related to the spatial scale of measurement that does not always reflect mating opportunities accurately (Chuang et al. 1999, Wang and Lu 2014). In our study, the majority of extra-pair nestlings were sired by one or two neighboring males, suggesting that our local approach most likely reflected extra-pair mating opportunities. Our results also suggest that high male abundance in relative proximity affects the extra-pair mating behavior of grass wrens and provide further evidence for the general pattern that increasing local breeding density influences EPP (Richardson and Burke 2001, Charmantier and Perret 2004, Mayer and Pasinelli 2013, Schlicht et al. 2015, Brouwer et al. 2017, Landgraf et al. 2017). From the female perspective, a high male density might increase the probability to engage in extra-pair fertilizations and facilitate better assessment of potential extra-pair mates (Westneat et al. 1990, Birkhead and Møller 1992). In passerines, there is convincing evidence that females frequently foray off territory to seek extra-pair fertilizations (e.g. superb fairy-wren *Malurus cyaneus*, Double and Cockburn 2000; hooded warbler *Setophaga citrina*, Chiver et al. 2008). Females might engage in extra-pair copulations with neighboring males to maximize genetic diversity among their offspring (Westneat et al. 1990) or to obtain good genes for their offspring (Westneat et al. 1990, Birkhead and Møller 1992). However, a positive association between male breeding density and EPP may also result from a higher proportion of neighboring males forcing females to engage in extra-pair copulations to avoid further male harassment (Westneat and Stewart 2003).

In birds, ASR plays an important role in regulating social interactions and mating systems (Liker et al. 2013, 2014, Székely et al. 2014). The effect of ASR on mating behavior is difficult to study in wild populations as ASR does not show enough intra-population variation in most bird species (Liker et al. 2013). Accordingly, few studies have evaluated how the ASR affects EPP in birds. We found that the EPP rate increased as more males (higher local ASR) were available within 320 and 400 m. However, this pattern might not be biologically relevant. We expected that local ASR also influences the EPP rate within 80 and 160 m because most extra-pair offspring were sired by males defending a territory less than 160 m away. A bigger sample would be necessary to evaluate this association properly.

Table 2. Results of generalized linear models evaluating the influence of local demographic factors on the extra-pair paternity rate in grass wrens for different radii.

| Radius (m) | n | Model A | | | | Model B | | | |
|------------|---|---------|---|---|---|---------|---|---|---|
| | | Term | Estimate (SE) | z | p | Term | Estimate (SE) | z | p |
| 80 | 59 | Density | 0.50 (0.13) | 3.73 | **< 0.01** | ASR | 0.20 (0.18) | 1.13 | 0.27 |
| | | Synchrony | −0.14 (0.18) | −0.77 | 0.44 | Synchrony | −0.13 (0.18) | −0.74 | 0.49 |
| 160 | 69 | Density | 0.41 (0.16) | 2.56 | **0.02** | ASR | 0.21 (0.17) | 1.27 | 0.26 |
| | | Synchrony | 0.20 (0.17) | 1.16 | 0.26 | Synchrony | 0.10 (0.17) | 0.62 | 0.56 |
| 240 | 72 | Density | 0.40 (0.15) | 2.61 | **0.02** | ASR | 0.22 (0.17) | 1.34 | 0.14 |
| | | Synchrony | 0.25 (0.16) | 1.57 | 0.11 | Synchrony | 0.16 (0.16) | 1 | 0.33 |
| 320 | 72 | Density | 0.42 (0.14) | 3.00 | **< 0.01** | ASR | 0.40 (0.16) | 2.57 | **0.01** |
| | | Synchrony | 0.31 (0.14) | 2.18 | **0.03** | Synchrony | 0.28 (0.14) | 1.95 | 0.06 |
| 400 | 72 | Density | 0.45 (0.15) | 3.10 | **< 0.01** | ASR | 0.45 (0.16) | 2.80 | **< 0.01** |
| | | Synchrony | 0.29 (0.15) | 1.88 | 0.06 | Synchrony | 0.24 (0.15) | 1.63 | 0.11 |

Sample size (n), parameter estimates with standard errors (in parentheses), z-values and p-values of likelihood-ratio tests are given.
Terms were standardized to facilitate the comparison between regression coefficients.
Significant p-values are indicated in bold (significance level considered: $p < 0.05$).

In line with previous studies (Yezerinac and Weatherhead 1997, Arlt et al. 2004, LaBarbera et al. 2010, García-Navas et al. 2014, Wang and Lu 2014, Schlicht et al. 2015), we did not find convincing evidence that local breeding synchrony affects the occurrence of extra-pair fertilizations in grass wrens. Local breeding synchrony explained the EPP variation within 320 m in model A, but we considered it a false positive. Additionally, this result might not be biologically relevant for the same reasons discussed above. Stutchbury and Morton (1995) state that higher breeding synchrony in migratory than in resident passerines is expected. In migratory passerines, males arrive earlier than females to the breeding grounds and establish a territory from where they sing to attract a female (Kokko et al. 2006). Hence, when females arrive at the breeding grounds, they encounter males displaying synchronously. However, breeding synchrony might not influence the extra-pair behavior in our study population as grass wrens are year-round residents, allowing females to assess social and extra-pair mates throughout the year rather than primarily during courtship (Petrie and Kempenaers 1998). Further studies focused on intraspecific tests are needed to determine the relationship between migratory behavior, breeding synchrony and EPP.

In conclusion, our results suggest that neighboring male abundance influences the extra-pair mating behavior in grass wrens and highlight the importance of demographic variables in shaping extant genetic mating systems. However, an experimental approach where male density is manipulated will be necessary to confirm this hypothesis. In grassland birds, overgrazing by cattle often reduces the abundance of individuals (Sliwinski and Koper 2015, Elliott and Johnson 2017), with the potential of affecting the genetic mating system and disrupting sexual selection processes. This study also highlights the importance of using a local demographic approach that effectively reflects the interactions among individuals in future studies.

## Author contributions

**Ramiro S. Arrieta**: Conceptualization (lead); Data curation (lead); Formal analysis (lead); Funding acquisition (equal); Investigation (lead); Methodology (lead); Project administration (lead); Resources (supporting); Software (equal); Visualization (lead); Writing – original draft (lead); Writing – review and editing (equal). **Leonardo Campagna**: Formal analysis (equal); Funding acquisition (supporting); Methodology (equal); Resources (equal); Software (equal); Supervision (supporting); Validation (supporting); Writing – review and editing (equal). **Bettina Mahler**: Conceptualization (supporting); Formal analysis (supporting); Funding acquisition (lead); Methodology (equal); Resources (equal); Supervision (equal); Writing – review and editing (equal). **Irby Lovette**: Funding acquisition (lead); Resources (equal); Supervision (supporting); Writing – review and editing (equal). **Paulo E. Llambías**: Conceptualization (equal); Data curation (equal); Formal analysis (supporting); Funding acquisition (lead); Investigation (equal); Methodology (equal); Project administration (supporting); Resources (lead); Supervision (equal); Validation (equal); Writing – original draft (supporting); Writing – review and editing (equal).

## Transparent Peer Review

## Data availability statement

## References

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