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EVALUATING THE INFLUENCE OF ENVIRONMENTAL FACTORS ON THE RATE OF EXTRA-PAIR

MATINGS IN TROPICAL AND TEMPERATE POPULATIONS OF THE HOUSE WREN (*TROGLODYTES*

AEDON)

by

Kaitlin C. McKenney

A Thesis Submitted in

Partial Fulfillment of the

Requirements for the Degree of

Master of Science

in Biological Sciences

at

The University of Wisconsin-Milwaukee

December 2013

ABSTRACT

EVALUATING THE INFLUENCE OF ENVIRONMENTAL FACTORS ON THE RATE OF EXTRA-PAIR MATINGS IN TROPICAL AND TEMPERATE POPULATIONS OF THE HOUSE WREN (TROGLODYTES AEDON)

by

Kaitlin C. McKenney

The University of Wisconsin-Milwaukee, 2013
Under the Supervision of Drs. Emily K. Latch and Linda A. Whittingham

Considerable variation exists in the rate of extra-pair matings (EPMs) in birds. Environmental variability likely influences EPM rates within species, but the effects of local environmental factors on EPM rates are largely unpredictable. To determine whether broad-scale environmental factors might be better predictors of EPM rates within species, we quantified levels of extra-pair paternity in the house wren (*Troglodytes aedon*) in four populations spanning a range of latitude, elevation, and primary productivity (measured by actual evapotranspiration rates). Our results indicated an intermediate and variable level of EPM among populations (6 -31% extra-pair young) that was not significantly affected by 3 broad-scale environmental factors. We found no correlation between EPM rate and parental relatedness, sire genetic diversity, or offspring male bias. Overall, we suggest that broad-scale environmental factors likely have weak effects on EPM, and that local-scale factors may be more important drivers of intraspecific EPM rates.

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INTRODUCTION

Extra-pair matings (EPMs), or copulations outside of the social bond, occur in socially

monogamous species from a wide array of taxonomic groups, from mammals (Goossens et al.

1998; Munshi-South 2007) and primates (Fietz et al. 2000), to fish (Dierkes et al. 2008) and

amphibians (Liebgold et al. 2006). In birds, EPMs have been documented in over 75% of species,

and in over 86% of passerines (reviewed by Griffith et al. 2002), for which genetic data have

been used to infer paternity.

There exists considerable variation in the rate of EPMs across populations and species

(Westneat and Sherman 1997; Petrie & Kempenaers 1998); EPMs can be absent in some species

and widespread in others, and can vary temporally within populations of the same species

(Petrie and Kempenaers 1998). While the passerine group has been especially well studied in

the EPM literature (see Hasselquist and Sherman 2001), the direct causes of such large variation

in EPM rates are still poorly understood. However, both males and females are expected to

benefit from EPMs under certain conditions.   A polygynous male may successfully increase his

reproductive success by engaging in EPMs and successfully defending paternity in multiple

broods through effective mate guarding. However, polygynous males incurring costs associated

with cuckoldry may have a lower realized reproductive success than that of monogamous, multi-

brooded males (i.e., sequential monogamy; Poirier et al. 2004).

Females may gain either direct or indirect benefits by engaging in EPMs. Although

evidence for direct female benefits of EPMs is limited in birds, the most familiar suggested direct

benefit for females is that of fertility insurance (Birkhead 1998). Females may also directly

benefit from engaging in EPMs by gaining foraging access or predator defense in her EP male's

territory (e.g., Gray 1997). For example, female red-winged blackbirds mated to an extra-pair

male were allowed to forage in his territory, whereas females that did not engage in an extra-pair mating with the male were actively prevented from doing so (Gray 1997). Indirect female benefits of EPMs have also been proposed regarding increased genetic diversity and quality of extra-pair young (EPY; Birkhead 1998); for example, several studies have shown that increased offspring heterozygosity results from mating with less related males, leading to enhanced fitness in EPY (Brown 1997; Masters et al. 2003; Foerster et al. 2003; Suter et al. 2007).

Variation in the occurrence of EPMs is likely influenced by a variety of local scale environmental factors. EPM rates are expected to be higher in a population where it greatly benefits a female to modify the paternity of her clutch and where the energy costs of pursuing EPMs are low (Petrie and Kempenaers 1998). The level of EPMs is expected to increase with improved territory or nest site quality, which is commonly associated with greater food availability, though the effect is not always realized in empirical studies (Hoi-Leitner et al. 1999, Vaclav et al. 2003). Additionally, females mated to poor quality males may seek copulations with EP males (Moller 1992), leading to an elevated rate of EPMs in environments where there is a large amount of variation in the genetic quality of males and where females can readily assess male quality (Robertson et al. 2001). Increased local predation risk might also result in a higher occurrence of EPMs if EP males may provide assistance to females through added parental care and nest defense beyond that of the social male (Gray 1997). EPMs are more likely to occur if the level of parental care by the social male is not negatively affected by female engagement in EPMs (Gowaty 1996) or females are able to compensate for the loss (e.g., Mulder et al. 1994).

EPM rates are also apt to vary with broad scale environmental factors that vary across larger geographic areas. For example, the longer breeding seasons typical of tropical, and

similarly low elevation species, may limit the occurrence of extra-pair mating due to a lack of simultaneously fertile females (Stutchbury and Morton 1995; Badyaev 1997; Badyaev and Ghalambor 2001). While breeding synchrony is a commonly studied factor expected to yield differences in EPM rates at a broad scale, the relative effects of breeding synchrony have proven controversial in the literature and vary widely between studies and species (see also Birkhead and Biggins 1987; Neudorf 2004).

Additional broad-scale factors of environmental variation may help to explain variation in EPM rates across different environments. Consistent with the hypothesis that females seek to increase genetic variation of their offspring in more variable environments (Westneat et al. 1990), Botero and Rubenstein (2012) demonstrated that environmental variability is positively associated with promiscuity in analyses including data from 277 avian species. Abiotic environmental conditions (e.g., climate, Johnsen and Lifjeld 2003; Bouwman and Komdeur 2006) have been found to influence EPM levels, and biotic aspects of the environment (e.g., terrestrial primary productivity measures; Dunn et al. 2000) have been found to influence other features associated with avian mating strategies (e.g., clutch size variation) in certain environments. Therefore, factors representative of environmental variation, and therefore potential resource levels in an area, are important components in understanding how broad-scale environmental factors might affect avian extra-pair mating strategies.

Differences in environmental variability are perhaps most pronounced when comparing temperate and tropical environments, which experience a range of environmental variability throughout the year. Whereas temperature is largely stable in the tropics, it serves as a dominant driver of environmental variation in the temperate zone; conversely, precipitation is largely stable in temperate environments, but fluctuates to a greater degree during wet and dry

seasons in the tropics. While growing seasons are shorter in the temperate zone, dry seasons in the tropics may encourage dramatic fluctuations in insect abundances, suggesting variability in primary productivity levels in both environments. Therefore, comparisons between tropical and temperate environments provide a useful dichotomy to assess the importance of broad-scale environmental variability on EPM rates.

Despite the interesting contrast provided by comparing EPM rates in temperate and tropical environments, over 90% of parentage studies in socially monogamous birds examine species breeding in the temperate zone (Stutchbury and Morton 2001). Studies in the temperate zone have provided important insights into the role of environmental variation on rates of EPM but alone cannot offer an unambiguous answer. It remains unclear whether the same environmental factors that influence EPM rates in temperate systems are applicable to tropical systems, or if broad-scale features of the environment might be important drivers of EPM rates in species that occur across a broad geographic range. Few studies have directly investigated mechanisms of EPM variation in tropical bird species (Rabenold et al. 1990; Stutchbury et al. 2007; Stutchbury et al. 1998; Cramer et al. 2011); recent work has called attention to the paucity of data from tropical environments and highlighted the distinctive opportunities provided by EPM studies in tropical systems to investigate mechanisms of EPM variation within and among species (Moore et al. 1999, Stutchbury and Morton 2008, Macedo et al. 2008). In particular, intraspecific variation in EPM rates between temperate and tropical populations would be helpful in determining whether broad-scale environmental factors, rather than local factors, are driving EPMs rates.

In addition to environmental variation, genetic variation of males and biased sex ratios of offspring may also be indicative of EPM rate variation in birds. A female may seek EPMs in

order to increase the genetic variation and fitness potential of her offspring. Therefore, she may be more likely to mate with an EP male who is less related (Blomqvist et al. 2002), or more genetically variable (Foerster et al. 2003) than her social mate. Biased sex ratios of young may also be indicative of EPM in birds, especially in polygynous systems, where there is often large variation in male reproductive success (Whittingham and Dunn 2004). In these systems, the high fitness potential for male offspring may encourage females to bias their offspring sex ratios toward males, especially for EPY sired by high quality males (sex allocation theory; Charnov 1982).

In this study, we compared EPM rates between temperate and tropical populations of the house wren (*Troglodytes aedon*). The house wren has the largest latitudinal range of any native passerine in the New World and exhibits a moderate level of EPMs that vary across environments (Whittingham and Dunn 2004, Johnson et al. 2009, LaBarbera et al. 2010, Masters et al. 2003). Since few systems permit such broad comparisons of EPM, intraspecific data from both tropical and temperate populations provides a unique opportunity to investigate how the rate of EPMs vary across environments as a consequence of broad-scale ecological factors. If increased environmental variability causes females to increase the variation of their offspring, then we predict that EPMs will be less frequent in tropical or low elevation environments where environmental variation may be less extreme. Since females may seek to engage in EPMs with less related and more genetically variable males, we expect that EP males will exhibit lower relatedness to the social female and higher genetic variability than social males. Finally, since offspring with the greatest fitness potential in polygynous systems are typically male, we expect offspring sex ratios to be more heavily male-biased, especially offspring sired by EP males.

METHODS

STUDY SPECIES

The house wren *(Troglodytes aedon)* is a sexually monomorphic, insectivorous, cavity nesting passerine distributed throughout a wide geographic and altitudinal range, with breeding populations reaching from southern Canada to southernmost South America, and from sea level up to approximately 2700 meters in elevation in Costa Rica (Skutch 1953).

The mating system of the house wren is complex, with males showing different types of social mating behavior, including single-brooded monogamy, sequential monogamy, or polygyny (Poirier et al. 2004). In the temperate zone, breeding seasons of house wrens usually span 2-3 months, from May to approximately July (Skutch 1953; Belles-Isles and Picman 1986). Female house wrens in the temperate zone typically raise two broods annually, laying one egg per day until the average clutch of 5-7 eggs is produced (Kendeigh 1941). In the tropics, house wren breeding seasons typically begin in February or March and continue through August or September (Skutch 1953; Young 1994). During these longer seasons, females may raise up to three or four broods annually, though clutch sizes are generally smaller with 3-4 eggs, on average (Skutch 1953). In both temperate and tropical environments, the female alone incubates the clutch for approximately 13 days; during the incubation period, males may invest time attracting additional mates and acquiring EPMs via intrusion into nearby territories (Johnson and Kermott 1989). Once the eggs have hatched, males return to assist females in provisioning young (Kendeigh 1941).

FIELD METHODS

Blood samples were collected from populations of house wrens breeding in nest boxes in both temperate and tropical environments. Two populations with two different elevations were sampled at each site. In the temperate zone, house wrens were sampled at the Sierra Nevada Aquatic Research Laboratory (SNARL) in Mammoth Lakes, California, USA (37.6°N 118.8°W, elevation 2,150m), and at Marshall Canyon, just east of Bonita, California, USA (34.1°N 117.7°W, elevation 550m). Tropical populations sampled included La Selva Biological Station near Puerto Viejo, Heredia, Costa Rica (10.4°N 84.0°W, elevation 50m) and the San Luis Research Station (now the University of Georgia Costa Rica campus), near Santa Elena, Guanacaste, Costa Rica (10.3°N 84.8°W, elevation 1,050m). Data from La Selva and San Luis we collected in two different years (La Selva: 2002 and 2004; San Luis: 2001 and 2004), while data from SNARL and Marshall Canyon reflect collection from one sampling year (SNARL: 2003; Marshall Canyon: 2005). The dataset included samples from 108 nests: 56 nests from La Selva (n=282 birds), 21 from San Luis (n=99), 16 from SNARL (n= 127), and 15 from Marshall Canyon (n=113).

Species in both tropical and temperate latitudes are affected by environmental variation at different scales. Generally, temperature is a dominant source of variation in the temperate zone, evidenced by greater annual temperature fluctuations than in the tropics. Since diurnal average temperatures in our tropical populations rarely fluctuate by more than 3°C over the course of the year (Matlock and Hartshorn 1999), large differences in average monthly rainfall between wet and dry seasons is the primary source of environmental variation in these areas. In terms of the biotic environment, variation in temperature and precipitation are manifested in the availability of water and solar energy in the environment. Actual evapotranspiration (AE) quantifies this resource availability by measuring the amount of water delivered to the

atmosphere by evaporation and transpiration. Thus, AE represents a valid predictor of primary production (and therefore resource availability during the growing season) in the environment (Rosenzweig 1968). Increased yearly productivity, as well as increased plant species diversity and complexity in the tropics, lends to greater insect species diversity (Janzen and Schoener 1968), suggesting that AE may also be a useful indicator of food availability and therefore EPM incentive for insectivorous birds. We calculated average monthly temperature and precipitation for each site using the University of Delaware's *Web*WIMP modeling program (http://climate.geog.udel.edu/~wimp/; Table 1). AE rates for known house wren breeding months (May through July in the temperate zone, Skutch 1953; Belles-Isles and Picman 1986, and March through August in the tropics, Skutch 1953; Young 1994) were also calculated for each site using the same program.

GENOTYPING

DNA was extracted from 621 house wren blood samples using a BioSprint 96 DNA Workstation. Genotypes were collected from 621 individuals at seven microsatellite loci developed in house wrens and related species (TA-B4-2, TA-A5-15, TA-C3B-2, TA-A5-2, TA-C6-7,Cabe and Marshall 2001; ThPl-14, Brar et al. 2007; Mcyu4, Double et al. 1997).Ten microliter amplification reactions consisted of 10ng genomic DNA, 5 pmol each primer, 1.0mM (TA-A5-15 and ThPl14) or 1.5mM (all other loci) $MgCl_2$, 0.2 mM each dNTP, and 0.75 U of Taq DNA polymerase in 1 x reaction buffer. Thermocycler conditions for loci TA-B4-2, TA-A5-15, and TA-C3B-2 were: a 2-min initial denaturation step at 95°C; 30 cycles of 30s at 95°C, 30s at 55°C, and 30s at 72°C; followed by a soak at 60°C for 45 min. TA-A6-7 and TA-A5-2 differed only in the annealing temperature (56°C), and for TA-A5-2 the extension step was additionally increased to 45s. ThPl14 and Mcyu4 were amplified using touchdown protocols. Following the initial

denaturation step, cycles decreased by 0.5°C/cycle from 56°C (ThPl14) or 51°C (Mcyu4). Once

the annealing temperature reached 45°C, additional cycles were carried out at 45°C to reach a

total of 35 cycles. Amplification for both loci included a final soak at 60°C for 45 min.

PCR products were electrophoresed on an ABI 3130 and scored in GeneMapper (version

4.0). Any trio (chick and both social parents) with mismatching genotypes was re-amplified and

re-genotyped an additional two times at the mismatched loci to ensure accuracy of all

multilocus genotypes in the dataset. Additionally, 48 samples were randomly selected for

repeated genotyping at all 7 loci for quality control. In total, we obtained 599 duplicate

genotypes out of 4,347 total genotypes (13.7%). We identified 5 instances in which repeated

genotyping yielded inconsistencies (5/599=0.8% error rate); in all instances the error resulted

from allelic dropout and was corrected. Expected and observed heterozygosities, deviations

from Hardy-Weinberg equilibrium (calculated using GENEPOP, Raymond and Rousset 1995), and

tests for the presence and frequencies of null alleles (calculated in CERVUS, Version 3.0,

Marshall et al. 1998, Kalinowski et al. 2007) were calculated from the genotypes of all unique

adults in each population (La Selva: n=85, San Luis: n=34, SNARL: n=30, Marshall Canyon: n=28).

All loci were retained for subsequent analyses, since the seven microsatellite loci showed no

evidence for departures from HWE in any population (α=0.002 after FDR correction for multiple

comparisons [Benjamini and Hochberg 1995] and P>0.002 in all cases) and no chicks were found

to mismatch the social female, indicating that any observed heterozygote deficiencies were

unlikely to be caused by null alleles. Gender was determined for all samples by amplifying a

diagnostic region of the CHD gene via PCR, using primers 2550F and 2718R (Fridolfsson and

Ellegren 1999). Gender determination reactions were repeated for 96 samples (15.5%) to assess

data quality, and corrected in one instance where a female was incorrectly scored as a male

(1.0% error rate).

PATERNITY ASSIGNMENT

Measures of genetic diversity were calculated using CERVUS (Table 2). The seven microsatellite loci constituted a powerful marker set for parentage analysis in house wrens, with an average of 15 alleles per locus (range 6-26). The probability of paternal exclusion at each locus ranged from 0.33 to 0.86 and the combined probability of paternal exclusion for all loci (Jamieson and Taylor 1997), given known mother, was 0.999 across all populations.

The genotypes of all chicks were compared against the genotypes of all sampled males present in the study site that year, using an open analysis in CERVUS with the mother as the known parent since all chicks matched at least one of their social mothers' alleles at all loci. Statistical confidence for parentage assignment in CERVUS, determined by critical LOD (likelihood) score values, was calculated using the following simulation parameters: 10,000 offspring, 99.8% individuals typed, 1% error rate, and 80% proportion of the population sampled. Simulations were initially run representing 20, 50, 80, and 90% of the population sampled. After detecting no quantifiable change in the accuracy of parentage assignment following CERVUS simulations and parentage analyses, 80% was considered the standard parameter for future runs as a conservative estimate of the proportion of the population sampled. Paternity was initially assigned using a combination of manual match assessment and CERVUS LOD scores. All extra-pair assignments were verified by manually comparing the maternal and paternal alleles at each locus.

A sire was assigned if he was the only male in the population to match the chick and female at all 7 loci (lacking trio-wise mismatches, WP=260; EP=7). If multiple males (2 males: n=15; 3 males: n=6) completely lacked trio-wise mismatches, the male with the most positive trio-wise likelihood (LOD) score was considered mostly likely and assigned as the sire (WP=14,

EP=7). If LOD score differences were indistinguishable between the social male and the most likely candidate male, a conservative assignment of the social sire was made if he was the only male to sire other chicks in the nest without mismatch (n=6).

For offspring that mismatched the social male at only 1 locus (n=58), we calculated the probability of chance inclusion (Jeffreys et al. 1992) for the six matching loci. For 57 of these 58 offspring, probabilities of chance inclusion were low (<0.05; mean=0.0010; range= <0.0001-0.0297) and were thus considered within-pair young (Johnsen et al. 2000); the remaining 1 chick exhibited high probabilities of chance inclusion for the 6 matching loci (>0.05; 0.0642) and was considered extra-pair. Offspring were assigned as EP status but not assigned a sire if all males in the population exhibited multiple trio-wise mismatches (n=52) or if multiple males completely lacked trio-wise mismatches and LOD score differences were indistinguishable (n=1).

STATISTICAL ANALYSIS

We calculated the proportion of EPY per nest and the proportion of broods containing EPY for each population, as well as the average number of sires for each population. In order to estimate the number of sires for nests containing EPY but where an EP sire could not be assigned (n=19 nests), we constructed theoretical sire genotype(s) for unassigned EPY (n=49) to determine the minimum number of sires required to account for the EPY genotypes. We analyzed the average proportion of EPY per nest among populations against 3 environmental predictors (elevation, latitude, and AE) using a generalized linear model (GLM) with a binomial distribution and logit link function in JMP, Version 10 (SAS Institute Inc 2012). The number of EPY in each nest was the dependent variable, with clutch size (i.e., the number of sampled young) as the binomial denominator, and elevation, latitude, and AE as predictors. A power analysis was conducted in JMP to determine the sample size (i.e., number of nests) necessary to

obtain sufficient power. Effect sizes for GLM (eta squared, or η^2; Cohen 1988) were then calculated to supplement significance tests and determine the magnitude of the effect of the predictors on the proportion of EPY per nest.

If females seek EPMs to increase genetic variation in their offspring, females may be more likely to mate with extra-pair males less related to themselves than their social mate (Blomqvist et al. 2002). To determine if the proportion of EPY in the nest increased as a function of relatedness between the social pair, we used a GLM with a binomial distribution and logit link function. The number of EPY in each nest was the dependent variable, with clutch size as the binomial denominator, and relatedness of the social pair (calculated in SPAGeDi [Hardy and Vekemans 2002] using the Queller and Goodnight [1999] estimator) as a predictor. Effect sizes for GLM (η^2) were calculated to determine the magnitude of the effect of relatedness on the proportion of EPY per nest. A small number of assigned EP males prohibited an analysis to determine if relatedness between females and social males in mixed nests was greater than between females and assigned EP males. However, females may also seek EPMs with males who are more genetically variable than their social mate (Foerster et al. 2003). In order to determine if extra-pair sires exhibited higher levels of genetic variation than within-pair sires, we used a Wilcoxon test in JMP to test for significant differences between assigned extra-pair sires and within-pair sires whose nests contained at least one EPY in two measures of genetic similarity, mean d^2 and mean heterozygosity (Coulson et al. 1998).

Data from an additional 5 house wren populations (Masters et al. 2003; Whittingham and Dunn 2004; Johnson et al. 2009; LaBarbera et al. 2010; Table 1) were incorporated into an analysis to determine whether differences in the site-wide proportion of EPY were attributable to environmental variables. To do this, we used GLMs with binomial distributions and logit link

function in JMP. The total number of EPY in each population was the dependent variable, with the total number of young from each population as the binomial denominator, and elevation, latitude, and AE as predictors. A power analysis was conducted in JMP to determine the sample size (i.e., number of house wren populations) necessary to obtain sufficient power. Effect sizes for GLM (η^2) were calculated to supplement significance tests and determine the magnitude of the effects of each predictor on the proportion of EPY per population in 9 house wren populations.

Sex Ratio of Young

In polygynous mating systems, where there is often large variation in male reproductive success (Whittingham and Dunn 2004), females may alter the sex ratio of their brood in order to obtain the greatest return on parental investment via the production of high quality male offspring. If females are biasing offspring sex ratios in relation to paternity from high quality sires, offspring sired by EP males may be more often male than those sired by WP males (Johnson et al. 2009). Chi-square (χ^2) tests in JMP were used to determine if the observed sex ratio of offspring was significantly different from expected (1:1) in each of four nest types: all nests, WPY only nests, nests with at least 1 EP chick (including EP only nests), and mixed nests containing both EPY and WPY. Wilcoxon tests were used to determine if a) EPY were more male-biased than WPY overall and b) if EPY were more male-biased than WPY in mixed nests.

<u>RESULTS</u>

As found in previous studies of this species, house wrens exhibited intermediate levels of extra-pair mating. Across all populations, 28% of nests contained EPY (30 of 108 nests) and

17% of young were sired by extra-pair males (68 of 405 young). The overall occurrence of EPY was intermediate in the tropical populations (13% and 21% EPY in La Selva and San Luis, respectively) and was more variable in the temperate zone populations (31% and 6% EPY in SNARL and Marshall Canyon, respectively; Table 1). Similarly, the percent of broods containing EPY was fairly consistent between our tropical populations (22% and 29% in La Selva and San Luis, respectively), and greatly variable between our temperate populations (63% and 13% in SNARL and Marshall Canyon, respectively). We were able to assign WP or EP status to all 405 nestlings, and confidently assigned (with zero mismatches) an EP sire to 14 of 68 EPY (21%). Average clutch sizes in our tropical populations were small (La Selva: 3.0±1.0, San Luis: 2.7±0.8), while clutch sizes in the temperate zone were larger, on average (SNARL: 5.9±1.2, Marshall Canyon: 5.5±1.8). Similarly, the average number of sires per nest in our tropical populations were consistently small (La Selva: 1.2±0.5, San Luis: 1.1±0.3), but varied more between our temperate populations (SNARL: 1.9±0.8, Marshall Canyon: 1.2±0.6).

EPY occurred within nests in each population and, where present, comprised an average of 65% of offspring within that nest. The proportion of extra-pair young per nest was significantly different across the 4 study populations (χ^2=9.5, df=3, P=0.024), with Marshall Canyon exhibiting a much lower proportion of EPY per nest than other populations (Figure 1). The proportion of EPY per nest was not correlated with elevation (χ^2=2.3, df=1, P=0.129; η^2=0.008), latitude (χ^2=1.0, df=1, P=0.317; η^2=0.003), or AE (χ^2=2.0, df=1, P=0.161; η^2=0.007). Power analyses indicated that our sample size was sufficient to detect a difference, should it exist; however, all effect sizes were small, suggesting only a weak relationship between the environmental factors and the proportion of EPY per nest.

Our data showed no evidence that females engaged in EPMs to increase genetic variation in their offspring. The relatedness of the social pair at each nest did not affect the proportion of EPY present in those nests (χ^2=1.0, df=1, P=0.321, η^2=0.003). Cuckolded within-pair males (n=26) and assigned EP sires (n=10) did not differ in genetic variation measures d^2 (χ^2=0.01, df=1, P=0.944) or heterozygosity (χ^2=0.5, df=1, P=0.501).

When all 9 house wren populations were considered, differences in the proportion of EPY between sites were observed (Table 1), but they were not significant (χ^2=8.0, df=8, P=0.434). The proportion of EPY among sites was correlated with elevation (χ^2=8.3, df=1, P=0.004) and AE (χ^2=5.5, df=1, P=0.019), but not latitude (χ^2=0.3, df=1, P=0.584). The average proportion of EPY in the 2 tropical sites (La Selva and San Luis; 17%±6%) was similar to the average proportion of EPY in the 7 total temperate house wren populations (16%±9%), while the 3 high elevation populations (San Luis, SNARL, and Wyoming) experienced a slightly higher average proportion of EPY (21%±9%) than the 6 low elevation populations (14%±6%). However, small effect sizes (η^2=0.07 for elevation, η^2=0.003 for latitude, η^2=0.05 for AE) indicate that data from nearly three times as many populations (n=27 populations to achieve 80% power) would be required to detect true effects of these environmental factors, when the unit of analysis is by site, rather than by nest. Given the non-significance of the broad-scale ecological factors in our nest-wise analyses, significant results from our site-wide analysis across 9 house wren populations should be viewed with caution, as they still exhibit relatively small effect sizes and may not represent true effects of these environmental variables, even when additional samples are obtained.

Sex Ratio of Young

The proportion of male offspring per nest varied between populations but was not significantly different (χ^2=2.3, df=3, P=0.507). However, when Marshall Canyon was excluded from the analysis (due to a small sample size of EPY), the remaining 3 populations differed in the proportion of male EPY per nest (χ^2=8.8, df=2, P=0.012), with SNARL experiencing more strongly biased EPY sex ratios (76% male) than the tropical populations (64% in La Selva, 50% in San Luis). All nest types analyzed exhibited an offspring sex ratio bias toward males. Male-biased sex ratios were observed in: all 108 nests (n=405 young; 59% male; χ^2=13.9, df=1, P<0.001), 78 nests containing only WPY (n=285 young; 58% male; χ^2=6.5, df=1, P=0.011), 30 nests containing at least one EP chick (n=120 young, including nests with only EPY [63% male; χ^2=8.5, df=1, P=0.004]), and 19 mixed nests containing both EPY and WPY (n=88 young, 66% male; χ^2=8.9, df=1, P=0.003). Extra-pair chicks were male biased over all nests (n=68 young; 68% male; χ^2=8.5, df=1, P=0.004), but not more so than within-pair chicks (n=337 young; 58% male; χ^2=3.8, df=1, P=0.051), although this was marginally significant. In mixed nests, extra-pair chicks (n=36 young; 78% male) were more male-biased than WPY (n=52 young; 58% male), though the difference was not significant (χ^2=0.7, df=1, P = 0.420).

DISCUSSION

Upon analyzing the effect of 3 environmental variables and 2 genetic measures hypothesized to influence EPM levels in the house wren, our results suggest that the proportion of EPY differs among our 4 sites but is not correlated with elevation, latitude, AE, relatedness between social mates, or genetic variation of males. Therefore, our results suggest that broad-scale environmental factors may not be playing a large role in determining EPM rates.

Elevation was not a significant environmental predictor of the proportion of EPY in our 4 study sites. We hypothesized that colder temperatures and greater seasonality common in high elevation environments may encourage higher EPM rates because it may benefit a female to increase the diversity of her clutch when environmental conditions are adverse or unpredictable. Consistent with our predictions, the high elevation populations we surveyed experienced higher EPM rates than their low elevation counterparts. However, the effect size for elevation was small, despite sufficient power to detect a difference at an individual nest scale. The small effect size of elevation indicates that the magnitude of the relationship between the proportion of EPY per nest and elevation is small in our populations, suggesting that additional sampling may still show elevation to be of limited biological importance to EPM rates.

The temperate, high elevation population we sampled (SNARL) approaches the upper altitudinal limit of where house wrens are found and exhibited a notably larger EPM rate than the other populations. Since house wrens have been known to inhabit environments at elevations approaching 3,000m, SNARL provides a baseline for future studies to determine whether or not the level of EPM observed in SNARL is typical of temperate, high elevation populations, or instead functions as an outlier in our analyses. Results from other studies have supported higher EPM rates in high elevation populations versus low elevation populations within the same species (e.g., 33% EPY in sub-alpine populations and 0% EPY in lowland populations of willow warblers, *Phylloscopus trochilus;* Bjornstad and Lifjeld 1997; Gyllensten et al. 1990). Although elevation itself was not an important predictor of EPM rates in our 4 populations, other environmental variables for which elevation may be a proxy might be influencing EPM rates and warrant further consideration. In the case of house wrens, further sampling of additional temperate, high elevation sites may be particularly interesting to directly compare to SNARL. If comparable high elevation sites exhibit EPM rates significantly lower than

SNARL, we may consider SNARL to be a true population outlier and future investigations could target alternative hypotheses for the high observed EPM rate. Conversely, similar EPM rates between SNARL and other temperate, high elevation sites might indicate that common environmental factors at such sites are important in determining EPM rates at high elevations.

In contrast to our prediction favoring lower EPM rates in the tropics, we found no evidence for a correlation between the proportion of EPY and either latitude or AE. A previous study investigating the effects of weather conditions on EPMs found that when EPY do occur, their frequency is more heavily influenced by air temperature, rather than precipitation, in a temperate population of bluethroats, *Luscinia svecica* (Johnsen and Lifjeld 2003). Since few comparable studies exist in tropical species, we expected that stable temperatures in the tropics, resulting in longer breeding seasons and the opportunity for multiple broods, would result in lower rates of EPM at low latitudes, despite the variable nature of precipitation during the wet season. Our expectations for the relationship between EPM rates and AE, however, were in contrast with those for latitude. We predicted elevated EPM rates in tropical environments, where high AE rates indicate greater primary productivity and therefore higher available resource levels; however, we observed no correlation between AE and EPM rate in our 4 populations.

It is possible that that latitude itself may not be as important a predictor of EPM rates as previously thought. Our data includes populations separated by over 23° latitude and provides sufficient power to detect an effect of latitude with adequate sample sizes from nest-level data in each population. While we only sampled two sites at each latitude, the variability in EPM rates that we did observe in the temperate zone suggests that some other environmental factor

(or combination of factors) that we did not measure could be influencing EPM rates between populations at the same latitude, especially for temperate populations.

It is also possible that the effects of latitude and AE offset one another. Since AE can be an indicator of food availability in the environment, and food availability may have a positive relationship with the likelihood of EPM (Hoi-Leitner et al. 1999), we expected that high AE rates in the tropics may be correlated with elevated EPM rates when compared to populations with lower AE rates. Our two low latitude populations both exhibited high AE rates, making it difficult to disentangle these potentially confounding effects.   It is important to note that the non-significance of AE in our analysis may also be largely due to the similarity of AE rates between sites at each latitude; therefore, AE in this analysis is more analogous to the factor of latitude and exhibits a similar, non-significant effect on the proportion of EPY per nest. Therefore, a larger variance in AE rates, and therefore a greater distinction from simply the factor of latitude, is required to better distinguish the relative effect of AE on the proportion of EPY per nest. Therefore, further sampling of populations not represented by our 4 study populations (e.g., temperate sites with high AE rates and tropical sites with low AE rates) may help clarify the relative importance of these variables.

When data from an additional five house wren populations were added into a population-based analysis with our 4 sites, we found an effect of elevation and AE on the proportion of EPY. This analysis enabled us to expand both the latitudinal and altitudinal range of our study by incorporating these additional populations, as well as account for greater variance in AE than our 4 populations provided alone, which allowed us to detect a significant effect of AE at this resolution. Previous studies have found relationships between reproductive success and AE (e.g., clutch size variation; Ricklefs 1980; Dunn et al. 2000), but studies showing a

specific correlation between AE and EPM rates in birds are lacking and require further investigation. However, it is important to note that while the analyses from our 4 populations included data per nest, the data from the analysis of 9 house wren populations included site-wide measures of the proportion of EPY, thereby lacking the statistical power of nest-level data. The additional house wren populations in this analysis were also derived from the temperate zone, however, further emphasizing the extreme lack of tropical studies to date. Since tropical species are affected by distinct ecological factors from species in the temperate zone, we encourage future research to specifically target tropical latitudes, in order to close the current gap in our understanding of how environmental factors affect the evolution of avian mating strategies in tropical systems.

We also found no correlation between the relatedness of the social pair at each nest and the proportion of EPY observed. We predicted that nests of highly related social pairs would contain higher proportions of EPY, although our results did not support this hypothesis. Genetic variation in assigned EP sires was also not significantly higher than cuckolded WP sires, contrary to our expectations. Some studies have shown that females seek EPMs when they share a higher degree of genetic similarity with their social mate (Blomqvist et al. 2002; Eimes et al. 2004; Tarvin et al. 2005) or to increase the genetic variation of their offspring, although our data did not support such findings. It has been often hypothesized that EP males may be more heterozygous than social males; however, there is little empirical evidence to date showing such a difference in genetic diversity between males, and our data similarly did not support this hypothesis. Our data suggest that EPM rates in these house wren populations may be more heavily influenced by environmental, rather than genetic variation, and perhaps on a more local-scale, as alluded to previously.

It has been hypothesized that female birds can theoretically adjust sex ratios of their young through a variety of mechanisms (Pike and Petrie 2003). Males typically experience greater variation in reproductive success than females in polygynous systems (Whittingham and Dunn 2004). Sex allocation theory predicts that females should bias offspring sex ratios toward male, especially when offspring are extra-pair, as they typically exhibit greater fitness potential than their within-pair counterparts (Charnov 1982). However, few empirical studies have noted a significant male bias in EPY. A previous study in blue tits provided evidence that in nests with mixed paternity, EPY were more often male than WPY (Kempenaers et al. 1997). Similarly, a study in house wrens showed that the proportion of male offspring increased by 8.5% when eggs were fertilized by extra-pair sperm (Johnson et al. 2009). Our results also show that although EPY were male-biased overall, they were only marginally more male-biased than WPY. In mixed nests, although the sex ratio of EPY was more biased than for WPY (78% versus 58%, respectively), the difference was not significant. The overall male biases in each of these nest types suggest that females may be able to partition resources to produce more males, in accordance with sex allocation theory. Although a small number of EPY in Marshall Canyon (n=5) prohibited statistical analysis of offspring sex ratios between all 4 populations, comparisons of the remaining 3 populations showed that sex ratios of EPY in SNARL were the most heavily skewed toward male, suggesting that sex ratios were more heavily biased in populations with a high proportion of EPY. However, it is unclear whether the skewed sex ratios we observed were directly associated with high EPM rates, or rather other factors known to affect offspring sex ratios that were not investigated in this study (i.e., territory quality, Dubois et al. 2006; maternal condition, Whittingham et al. 2002). If females are producing more male offspring as a consequence of being in better condition via greater access to food resources in high quality

territories, we may be able to more directly analyze how offspring sex ratios may be influenced by factors of broad-scale and local environmental variation.

Implications

Despite the house wren being a wide-ranging habitat generalist, the findings of this study suggest that broad-scale environmental factors may not be major drivers of EPM rates in house wrens. However, whether the observed variation in EPM rates across populations is driven by local scale environmental factors, or another factor entirely, is still unclear. The lack of correlation between the proportion of EPY and 3 broad-scale environmental predictors in our nest-based analysis, and coupled with small effect sizes, despite vastly different environments, suggests that sampling additional populations may increase statistical power, but perhaps not biological insight on how these environmental factors drive EPM rates in house wrens. The lack of large effect sizes for any of our broad-scale environmental variables across tropical and temperate lines or between elevation extremes suggests that local-scale environmental variation may instead play a more important role in determining EPM rates in a population. Given our findings, we suggest that future investigations into EPM rates across environments in the house wren or other wide-ranging species incorporate local environmental variation (e.g., local predation risk or insect abundance measures as a function of local temperature and precipitation fluctuation) to evaluate the relative importance of local and broad-scale environmental variation in determining EPM rates.

Our study adds to the limited but essential literature available characterizing mating systems in tropical bird species. Tropical populations continue to be integral to studies of extra-pair mating systems in birds since they encounter and are affected by a distinct suite of ecological factors from temperate species. There is consensus in the literature that tropical

populations are key to understanding how ecology affects the evolution of extra-pair mating systems (Stutchbury et al 2007; Moore et al 1999). The results of our study support this premise and suggest that, while not all systems allow for intraspecific comparisons across such broad geographical ranges, such studies provide fundamental data to support the development of testable hypotheses to better understand the ecological and evolutionary mechanisms that influence mating decisions both within and across species.
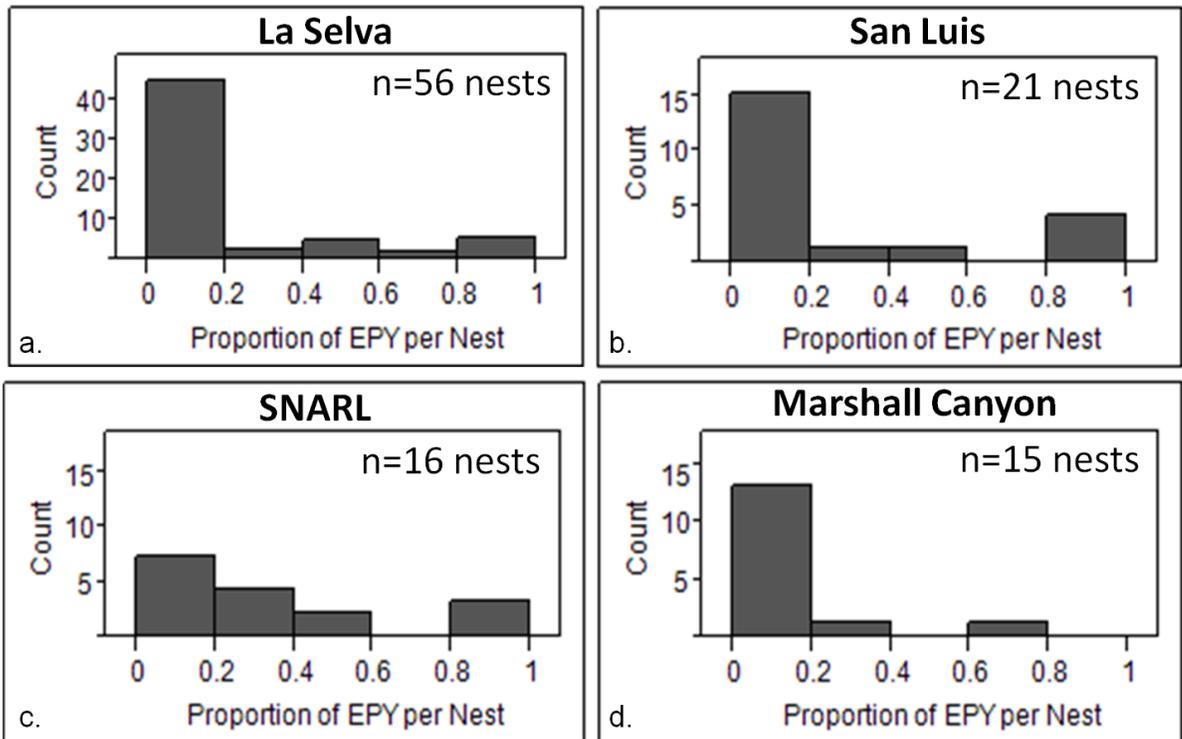
Figure 1. The proportion of EPY per nest and the frequencies at which they occur in each of four house wren populations by elevation and latitude class (a. La Selva [tropical, low elevation], b. San Luis [tropical, high elevation], c. SNARL [temperate, high elevation], and d. Marshall Canyon [temperate, low elevation]).

Table 1: Summary data from nine house wren populations, including proportions of EPY and EP broods, and environmental data from each of 9 total house wren populations.

Site	% EPY	Number of EPY/ Total Offspring	% EP Broods	Number of EP Broods/ Total Broods	Latitude/ Longitude	Elevation (m)	Av. Monthly Temp (°C)	Av. Monthly Precipitation (mm)	AE (mm)
La Selva, Costa Rica [1]	13	22/170	22	12/55	10.4°N 84.0°W	50	19.0±0.5	316.4±139.6	502
San Luis, Costa Rica [1]	21	12/57	29	6/21	10.3°N 84.8°W	1050	17.4±0.7	237.9±141.6	502
SNARL, California [1]	31	29/95	63	10/16	37.6°N 118.8°W	2150	9.3±6.9	31.6±23.3	165
Marshall Canyon, California [1]	6	5/83	13	2/15	34.1°N 117.7°W	550	15.1±5.7	43.0±44.6	84
Saukville, Wisconsin [2]	10	59/584	28	29/103	43.4°N 88.0°W	265	7.8±10.6	66.3±27.5	328
Mackinaw River, Central Illinois [3]	15	350/2345	37	176/476	40.7°N 88.9°W	215	10.5±10.4	76.8±23.8	370
Ithaca, New York [4]	25	94/377	54	44/82	42.5°N 76.5°W	330	7.1±9.7	81.4±9.3	329
Buenos Aires, Argentina [4]	16	26/166	33	13/40	36.4°S 56.9°W	1	14.8±4.7	87.3±16.8	450
Sheridan County, Wyoming [5]	12	60/500	37	29/79	44.7°N 105.9°W	1250	7.0±10.1	30.1±19.7	231

References: [1] This study; [2] Whittingham and Dunn 2004; [3] Johnson et al. 2009; [4] LaBarbera et al. 2010; [5] Masters et al. 2003

Table 2: Parameters of seven microsatellite loci used to assess paternity in house wrens: number of alleles per locus (k), average exclusion probability for one candidate parent (E-1P); average exclusion probability for one candidate parent, given the genotype of a known parent (E-2P); expected heterozygosity (h_e)/observed heterozygosity (h_o); χ^2 p values from Hardy-Weinberg Equilibrium Test (HWE); estimated null allele frequency (r).

Locus	Population	k	E-1P	E-2P	h_e/ h_o	HWE*	r
TA-B4-2 [1]	La Selva	8	0.379	0.560	0.694/0.751	0.047	+0.038
	San Luis	7	0.349	0.531	0.719/0.735	0.853	-0.022
	SNARL	10	0.456	0.631	0.814/0.900	0.962	-0.061
	Marshall Canyon	10	0.449	0.624	0.828/0.679	0.036	+0.083
TA-A5-15 [1]	La Selva	4	0.005	0.051	0.118/0.113	1.000	-0.021
	San Luis	4	0.059	0.182	0.288/0.294	0.474	<0.001
	SNARL	6	0.379	0.557	0.794/0.633	0.043	+0.104
	Marshall Canyon	5	0.331	0.514	0.774/0.536	0.042	+0.165
TA-C3B-2 [1]	La Selva	4	0.007	0.060	0.153/0.144	1.000	-0.031
	San Luis	3	0.124	0.245	0.516/0.412	0.216	+0.125
	SNARL	19	0.700	0.823	0.923/0.967	0.003	-0.032
	Marshall Canyon	20	0.731	0.845	0.944/0.893	0.061	+0.018
TA-A5-2 [1]	La Selva	6	0.172	0.349	0.471/0.506	0.092	+0.040
	San Luis	6	0.289	0.469	0.682/0.559	0.073	+0.102
	SNARL	14	0.549	0.711	0.844/0.567	0.007	+0.189
	Marshall Canyon	13	0.570	0.727	0.886/0.750	0.044	+0.078
TA-C6-7 [1]	La Selva	7	0.266	0.430	0.682/0.694	0.209	-0.002
	San Luis	4	0.195	0.336	0.622/0.500	0.412	+0.109
	SNARL	6	0.297	0.471	0.694/0.600	0.233	+0.072
	Marshall Canyon	5	0.262	0.431	0.723/0.607	0.334	+0.076
ThPl-14 [2]	La Selva	15	0.646	0.786	0.929/0.902	0.474	-0.019
	San Luis	15	0.640	0.781	0.911/0.853	0.255	+0.026
	SNARL	14	0.540	0.704	0.850/0.867	0.120	-0.024
	Marshall Canyon	15	0.663	0.798	0.921/0.786	0.030	+0.070
Mcyu4 [3]	La Selva	11	0.404	0.582	0.671/0.779	0.233	+0.075
	San Luis	8	0.370	0.548	0.782/0.882	0.250	-0.070
	SNARL	10	0.602	0.753	0.894/0.900	0.240	-0.012
	Marshall Canyon	12	0.586	0.740	0.885/0.821	0.063	+0.033

* α=0.002 after FDR correction for multiple comparisons

References: [1] Cabe and Marshall 2001; [2] Brar et al. 2007; [3] Double et al. 1997

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