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High rates of extra-pair paternity in two equatorial populations of rufous-collared sparrow, *Zonotrichia capensis*

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The latitudinal increase in extra-pair paternity (EPP) rates in birds suggests broad selective benefits to low EPP rates in the tropics. However, we have few EPP data from tropical birds, particularly from species with close relatives at high latitudes. Here, we report EPP rates in two resident equatorial populations of rufous-collared sparrow *Zonotrichia capensis*, a genus well-represented at high latitudes. We found 64% and 60% of broods contained extra-pair offspring, and 42% and 52% of all young were extra-pair. EPP rates were similar in these populations, despite clear differences in elevation, temperature, rainfall, and breeding season length. These findings provide evidence that EPP rates in tropical birds can be as high as those observed in temperate birds, and suggest that the selective pressures acting on EPP rates vary markedly across tropical birds.

The discovery that the occurrence of extra-pair paternity (EPP) in socially monogamous birds is the rule rather than the exception is perhaps one of the major advances in the field of behavioural ecology in the last decades. Whereas males clearly benefit from engaging in extra-pair fertilizations, the reasons for females to do so remain poorly understood. Possibly extra-pair fertilizations gain them genetic advantages for their offspring (Westneat et al. 1990) or insure against the infertility of their social mate (Wetton and Parkin 1991). Despite uncertainties about the ultimate causes of EPP, it is apparent that the occurrence and rate of EPP among birds vary dramatically, not just between species, but also between populations of the same species and among individuals of the same population (Griffith et al. 2002). The inter-specific variation in EPP rates has a large phylogenetic component, with more than 55% of the variation occurring at or above the level of family (Arnold and Owens 2002). An extensive comparative study, correcting for phylogenetic non-independence of data, indicated that latitude of breeding may be a good predictor of the prevalence of EPP, with rates increasing with latitude (Spottiswoode and Møller 2004). The proximate mechanisms behind this pattern, and variation in EPP rates in general, are currently not well understood and are a matter of ongoing debate. A multitude of intra- and inter-specific studies have linked variation in EPP to social and environmental factors, such as breeding synchrony, breeding density, migration distance, adult mortality rate, etc. (reviewed by Griffith et al. 2002, Spottiswoode and Møller 2004).

Despite years of research, a pressing issue in the study of avian mating systems in general, and EPP in particular, remains: nearly all EPP studies have been conducted on northern temperate species. To our best knowledge, EPP rates have been estimated for only 16 socially monogamous tropical bird species (reviewed by Douglas et al. 2012), representing approx. one tenth of all bird species for which EPP rates are known. As the majority of bird species live in the tropics, more research on low latitude species is needed before generalized statements regarding the relationship between EPP and latitude can be made (Macedo et al. 2008). In the current study, we therefore estimated rates of EPP in two equatorial populations of the rufous-collared sparrow *Zonotrichia capensis*. Interestingly, despite the fact that our study populations are situated only ~ 25 km apart, they differ markedly in many characteristics, including elevation, temperature, rainfall, and the length of the breeding season (in one population breeding is restricted to 3–4 months annually, whereas in the other population breeding occurs year-round; Moore et al. 2005, Class and Moore 2011, Class et al. 2011).

Methods

Field sites and field methods

We studied two non-migratory populations of rufous-collared sparrow in the eastern Andes of Ecuador (Napo Province): one located near the town of Papallacta (0°21′S,

78°9′W, ~3300 m elevation) and one at the Yanayacu Biological Research Station near Cosanga (0°37′S, 77°55′W, ~2100 m elevation). The Papallacta population was studied from October 2006 through January 2007, and the Yanayacu population from December 2009 through January 2010.

Rufous-collared sparrows in our study populations inhabit rural farmlands and other human-disturbed habitat, and density can reach 10 pairs ha^{-1} in both sites (FB, CE, PRM, ITM, unpubl.). Typical clutch size is 2–3 eggs, and both parents provision the young (Miller and Miller 1968; FB, CE, PRM, ITM, unpubl.). At Papallacta, most breeding is restricted to the months of September through December, whereas at Yanayacu, active nests can be found throughout the year, with seasonal variation in the proportion of individuals that are breeding (Moore et al. 2005, Class and Moore 2011, Class et al. 2011). The causes of the breeding phenology differences between these populations are unknown. Seasonal rainfall and temperature patterns are similar for the two populations, but the amounts of precipitation and air temperatures differ dramatically (Class et al. 2011). The higher elevation Papallacta population experiences lower temperatures and less rainfall year-round, which could limit resources required for breeding. However, whether limitation of resources explains the timing and duration of the breeding seasons in our study populations is questionable as experimental food supplementation in Yanayacu stimulated moult rather than breeding (Class and Moore in press).

In both populations, we located active nests through behavioral observations of adults. Once an active nest (i.e. containing eggs or young) was located, we captured adults using mist nets placed close to the nest. We then collected a blood sample (10–225 µl), and marked each bird with a unique combination of one metal and three plastic color bands to allow subsequent individual identification. After hatching, we observed nests by a telescope or binoculars until the identities of the adults provisioning the young were verified. In this species, only the social pair provision offspring, thus adults provisioning young in a given nest were defined as the social parents attending that nest. We collected a small blood sample (10–50 µl) from all nestlings a few days after hatching. All blood samples were collected into microcapillary tubes via brachial venipuncture and stored in 100% ethanol at room temperature or without preservative but frozen at −20°C until DNA extraction. Parentage analysis was done only with families for which we obtained a blood sample from both social parents and all offspring. With the exception of one pair that completed two nesting bouts during the sampling period, we estimated EPP rates from one brood per social pair.

Genotyping and parentage analysis

We extracted DNA from blood samples for all individuals using the Qiagen DNeasy kit, following the manufacturer's instructions for nucleated blood cells. We then genotyped all individuals at a minimum of 7 of 8 variable microsatellite loci (Table 1, mean number of alleles per locus 8.13 at Papallacta, 6.13 at Yanayacu) using polymerase chain reaction (PCR) to amplify DNA followed by separation and detection using capillary gel electrophoresis.

Table 1. The eight microsatellite loci used in parentage analysis for *Zonotrichia capensis*.

Primer name	Source
Dpµ_01	Dawson et al. 1997
Dpµ_16	Dawson et al. 1997
Pdoµ_5	Griffith et al. 1999
Zole_B01	Poesel et al. 2009
Zole_C06	Poesel et al. 2009
Zole_C11	Poesel et al. 2009
Zole_E02	Poesel et al. 2009
Zole_E11	Poesel et al. 2009

We used the program Cervus (ver. 3.0.3) to detect incidents of EPP (Kalinowski et al. 2007), with the female attending the nest assigned as a known parent and all males sampled within the population designated as candidate sires. Our probability of detecting extra-pair sired offspring (i.e. sire-exclusion probability) was $>98\%$ in each population. In one case, a sampled offspring did not share alleles with either the attending female or male at 4 of 8 loci, likely representing a case of conspecific brood parasitism (previously undescribed in this species). This individual was not included in the analyses, and is not included in the sample sizes reported. Forty-four of the remaining 47 offspring shared at least one allele in common with the female attending the nest at all loci, and three shared alleles at all but one of the loci.

Results

In the Papallacta population, 64% of broods contained at least one extra-pair young (EPY) ($n=11$), 42% of young were extra-pair ($n=24$), and the mean percentage of EPY per brood was 45% ($n=11$). In the Yanayacu population, these estimates were 60% ($n=10$), 52% ($n=23$), and 50% ($n=10$), respectively. These EPP rates were similar (Fisher's exact test, all $p>0.5$) for both equatorial populations of rufous-collared sparrow. Of the 21 males for which EPP rates were determined across both populations, seven lost all young to extra-pair sires, whereas eight males were not cuckolded. The remaining six males had broods of mixed paternity.

Discussion

Our study revealed high EPP rates in rufous-collared sparrows breeding on the equator. Given the combined sample size of 21 broods sampled in both populations, the 95% confidence interval around our estimate of 48% EPY per brood was ± 20%, or 28% to 68%. Even with this wide confidence interval, the lower limit of 28% EPP still is higher than that of the majority of high latitude species (Griffith et al. 2002, Spottiswoode and Møller 2004). The EPP rates in our equatorial resident *Zonotrichia* populations are also comparable to those observed in high latitude populations of the congeners *Zonotrichia leucophrys* and *Zonotrichia albicollis* (McDougall-Shackleton et al. 2002, Tuttle 2003, Bonier et al. 2007). Other studies of resident tropical birds have reported percentage of EPY varying from 0–51%, with a mean of 17% (reviewed by Macedo et al. 2008, Douglas et al. 2012). From these few studies, we see that EPP rates in

birds breeding in the tropics are variable, and include values similar to those observed in temperate birds.

In their extensive comparative study, Spottiswoode and Møller (2004) showed that in birds EPP rates were positively correlated with latitude. A possible explanation for this relationship is that more synchronous breeding at higher latitudes may both facilitate female extra-pair mate choice as well as increase a male's likelihood of encountering fertile extra-pair females (Stutchbury and Morton 1995, Stutchbury 1998, Stutchbury et al. 1998). Despite the fact that both our study populations lie on the equator, they differ markedly in the timing and length of the breeding season (Moore et al. 2005, Class and Moore 2011, Class et al. 2011). Exactly why they differ phenologically is currently unclear (Methods), but the difference in breeding season length very likely affects the breeding synchrony in these populations; when females produce a single clutch annually, but breed throughout the year, the breeding synchrony in the population must be lower than when breeding is restricted to a few months a year. Indeed, in a previous study (including six months of nest searching to determine lay dates in the year-round breeding population) the breeding synchrony index, calculated using the formula by Kempenaers (1993), was 13.8% at the year-round breeding population, and 19.2% at the population where breeding is restricted to 3–4 months annually (Class et al. 2011). It must be noted that, as a consequence of the formula's inherent assumption that the population is studied throughout the entire breeding season, the 13.8% breeding synchrony at the year-round breeding population is an overestimate (Class et al. 2011). Despite differences between our two study populations in the length of the breeding season, EPP rates were very similar. However, our results can not reject the breeding synchrony hypothesis because we do not know the proportion of the populations that were breeding during our sampling. Future work on these populations may allow a test of the breeding synchrony hypothesis, in addition to tests of the selective pressures that favour such high EPP rates relative to many other tropical birds.

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