

The function of seasonal song in a tropical resident species, the Red-throated Ant-tanager (*Habia fuscicauda*)

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Abstract Recent studies of tropical birds with year-round territoriality indicate that song rate is relatively low year-round in association with lower seasonality and relatively stable social environment over the year. We examined seasonal variation in the song rate of Red-throated Ant-tanagers (*Habia fuscicauda*), a socially monogamous species in which males predominantly sing during the breeding season, to determine to what extent increased song rate is driven by (1) increased territory defense, (2) pair coordination of nest initiation, and (3) competition for extra-pair mating opportunities. Song was infrequent during the non-breeding season, increased dramatically prior to nesting (17 % time spent singing), and increased even more so during nesting (32 % time spent singing). Male song output was inversely related to frequency of interactions between pairs at territorial borders; however, interactions may also serve in a mating context. Extra-pair matings were common, and 17 of 41 nestlings in ten of 19 broods were extra-pair young, suggesting that high male song output may be sexually selected via extra-pair mate choice. Although males spent much less time singing prior to nesting, our results also indicate that male initiation of singing was

associated with female egg-laying date, indicating also a role in within-pair coordination of reproduction. Our results suggest that high song output during the breeding season may be more common in omnivorous species that experience increased seasonality and that it may be indicative of an extra-pair mating system. More studies of species with diverse life histories are needed to examine the ecological conditions that explain interspecific variation in song output.

Keywords Extra-pair mating · Neotropics · Pair coordination · Seasonality · Song output · Year-round territoriality

Zusammenfassung

Die Funktion saisonalen Gesangs bei der tropischen Rotkehl-Ameisentangare (*Habia fuscicauda*)

Aktuelle Untersuchungen von tropischen Vögeln mit ganzjähriger Territorialität deuten darauf hin, daß die Gesangsrate das ganze Jahr über relativ niedrig ist, was mit einer geringeren Saisonalität und einer relativ stabilen sozialen Umwelt über das Jahr hinweg zusammenhängt. Wir untersuchten saisonale Unterschiede in der Gesangsrate der Rotkehl-Ameisentangare (*Habia fuscicauda*), einer sozial monogamen Art, bei der die Männchen vor allem während der Brutsaison singen, um festzustellen, in welchem Maße eine Zunahme der Gesangsrate angeregt ist durch (1) Revierverteidigung, (2) Paarkoordination bezüglich des Nistbeginns und (3) Konkurrenz um Gelegenheiten für „extra-pair mating“. Der Gesang war selten außerhalb der Brutzeit, nahm dramatisch zu vor der Nistzeit (Gesang 17 % der gesamten Zeit), und umso mehr während der Nistzeit (Gesang 32 % der Gesamtzeit). Der Umfang des

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Gesangs der Männchen hing umgekehrt zusammen mit der Häufigkeit von Interaktionen von Paaren an den Territoriumsgrößen; allerdings können Interaktionen auch eine Rolle spielen im Zusammenhang mit der Paarung. „Extra-pair mating“ war häufig und 17 von 41 Nestlingen in 10 von 19 Brutpaaren waren paarfremde Junge, was darauf hindeutet, dass hohe Gesangsaktivität möglicherweise über die Wahl paarfremder Partner selektiert wird. Obwohl die Männchen vor dem Nistbeginn eine viel geringere Zeit mit Singen zubrachten, deuten unsere Ergebnisse auch darauf hin, dass der Beginn des Gesangs assoziiert war mit dem Datum der Eiablage, was wiederum darauf hindeutet, dass der Gesang eine Rolle spielt bei der Koordination des Brutpaares beim Brutgeschäft. Unsere Ergebnisse legen nahe, dass hohe Gesangsaktivität während der Brutsaison häufiger vorkommt bei omnivoren Arten, die eine erhöhte Saisonalität erfahren und dass sie ein Zeichen sein könnten für ein „extra-pair mating“-System. Mehr Studien an Arten mit unterschiedlicher Lebensgeschichte sind notwendig, um die ökologischen Bedingungen zu untersuchen, die Unterschiede in der Gesangsaktivität zwischen verschiedenen Arten erklären.

Introduction

Song rate has been an important focus of studies on the behavioral ecology of birds as it provides important insight into the context and function of song and the ecological drivers of singing behavior (Morton 1996). To date, studies of singing effort in birds have focused mostly on temperate-zone species, which live in highly seasonal environments and for which song is largely restricted to the breeding season. In these species, song rate is high in both a territorial context, when males first arrive on the breeding grounds and re-establish territories, and a mating context, when males attract social mates and compete for extra-pair matings (e.g., Forstmeier and Balsby 2002; Byers and Kroodsma 2009). However, a high proportion of bird species are residents of tropical environments, which often exert different selective pressures on singing behavior (Stutchbury and Morton 2001). The tropics are characterized by lower seasonality, and thus a large proportion of species are residents year-round, often defending a territory and maintaining their pair bond throughout the year. Recent studies examining singing behavior in tropical residents found that song rate is relatively low outside of the brief dawn song period and is fairly constant year-round, suggesting that song plays an important role outside of the breeding context and particularly in territory maintenance (Fedy and Stutchbury 2005; Topp and Mennill 2008; Tobias et al. 2011).

Nevertheless, even in tropical environments, there often is variation in the extent of seasonality depending on habitat type, forest stratum, or foraging guild (Levey and Stiles 1992). Species living in secondary forests, edges, or canopy experience larger variations in climatic conditions and food supply than those in understory mature forest habitats (Levey 1988; Smythe 1982). Similarly, fruit or nectar resources are more responsive to fluctuations in climatic conditions than insects, thus frugivores often experience increased seasonality in food resources (Janzen 1967; Faaborg and Terborgh 1980; Terborgh 1980). Although little is known about singing behavior among tropical species that experience increased seasonality, several studies have indicated increased song output predominantly during the breeding season in several species living in secondary or more open woodland habitats or which feed on fruit (e.g. Clay-colored Thrushes, *Turdus grayi*, Stutchbury et al. 1998; Dusky Bush-tanagers, *Chlorospingus semifuscus*, Bohorquez and Stiles 2002; Rufous-collared Sparrows, *Zonotrichia capensis*, Addis et al. 2010). Increased seasonality is expected to result in re-establishment of territorial boundaries and increased competition for mating opportunities if breeding synchrony increases, activities which are associated with high song output (Stutchbury et al. 1998; Stutchbury and Morton 1995, 2008).

In the study reported here, we examined seasonal variation in male song output in a tropical resident species with year-round territoriality, the Red-throated Ant-tanager (*Habia fuscicauda*, Willis 1972). Red-throated Ant-tanagers are typically found in secondary growth and edge habitat and are omnivorous, eating a mixed diet of insects and fruit. The species is characterized by sexual dimorphism, males are larger and brighter, and only males sing. Previous studies indicate that song is more frequent during the breeding season (Willis 1960, 1961).

We examined the extent of seasonal variation in song output in light of three non-mutually exclusive hypotheses: “Territorial Defense,” “Mate Stimulation,” and Extra-pair Mate Competition.” According to the “Territory Defense” hypothesis, a seasonal increase in song output could be a result of increased frequency and risk of territorial challenges (Levin 1996). If male song increases primarily as a result of territorial defense, seasonal patterns in song output should be positively correlated with the frequency and threat of territory loss. The “Mate Stimulation” hypothesis states that in a breeding context, song functions in stimulation of the social mate and within-pair synchronization of nesting, both of which have been proposed to be particularly important among species living in tropical environments because of the lower predictability of seasonal changes (Dilger 1953; Kunkel 1974). In captive studies, male song can advance the date of nest-building and egg-

laying of their mates, suggesting that song may play an important role in mate stimulation and synchronization (reviewed in Mota and Depraz 2004). If song is important for within-pair coordination of nesting, then male song output is expected to peak before females begin nest-building, and female nesting date is predicted to be correlated with the social mate's timing of song initiation and song output. The "Extra-Pair Mate Competition" hypothesis states that high song output can benefit males both via defense against intrusions by extra-pair males and in attracting neighboring females (Morton 1996; Stutchbury 1998). To date, the extent of extra-pair paternity expected in species with year-round territoriality and pair bonds remains under debate (Stutchbury and Morton 2001, 2008; Macedo et al. 2008). Highly seasonal or temporal increases in food resources and/or mating partners may drive increased sexual selection pressures on males (Stutchbury and Morton 1995; Griffith et al. 2002). If male song functions in an extra-pair mating context, it would be expected to peak during the nesting season when females become fertile (Greig-Smith 1982; Mace 1987; Forstmeier and Balsby 2002), and DNA paternity testing should reveal high rates of extra-pair paternity. A high rate of extra-pair paternity would suggest that male song output could influence male mating success either through preventing paternity loss in their own nest or through gaining extra-pair fertilizations (Chiver et al. 2008).

Materials and methods

Methods

We studied a population consisting of 22 families of Red-throated Ant-tanagers, from January 11–May 13 2010, in Soberania National Park near Gamboa, Republic of Panama (9°N, 79°W). Red-throated Ant-tanagers live in pairs or, on about one-third of the territories, are accompanied by one to three males or females, presumably young from the previous season, that may contribute to feeding of the young (Willis 1961). Birds were banded with a numbered metal band and a unique combination of two to three color bands as part of a previous study (Chiver 2011), except for two territorial males, which were captured during the present study using mist-nets and playback of conspecific calls. During banding, we collected a small amount of blood (10–30 μ l) by puncturing the brachial vein with a 46-gauge needle, and this sample was stored in Queen's lysis buffer at 4 °C for paternity analysis (Seutin et al. 1991). We conducted behavioral observations from February to mid-May, which encompasses the period of non-breeding and the initiation of breeding.

Song output and territorial interactions

Territories, mapped as the area where pairs were observed singing or calling, range in size from 3 to 5 ha, and pairs or groups have one to three neighboring pairs (Chiver 2011). Starting 11 January, we visited each territory every 2–3 days to confirm the presence and identity of individuals, and we observed song output and territorial interactions for pairs on all territories. However, because some pairs were difficult to observe continuously due to difficult terrain ($n = 6$) and for other pairs we were unable to locate first nests ($n = 3$, see section "First egg dates"), our analysis of singing behavior was limited to 13 pairs. For the analysis we included song observations and territorial interactions starting on 1 February, when we monitored the same numbers of pairs throughout. Observations took place from 0600 to 1300 hours. Each observation period involved finding the focal pair and following them for an average of 44.2 ± 14.3 min (during 336 total observation periods, average of 25.5 ± 4.8 observation periods per male). We rotated the first territory observed each morning to ensure that each pair was observed at a range of times during the day. When not singing, pairs were located using pair contact calls, which are given throughout the day (Willis 1960). Males sing at a rate of 10–15 songs min^{-1} in bouts of 1–15 min where the same song is repeated separated by silent intervals of approximately 5–7 s. To measure song output, we defined a song bout as over when the male was silent for >60 s to avoid long pauses. We used a timer to record the duration of each song bout, and we calculated the proportion of time spent singing by dividing singing time by the total amount of observation time. To estimate variation in territorial intrusions and defense, we noted the occurrence of territorial interactions, where individuals from different groups met at a territorial border or when one or more individuals trespassed onto a neighboring territory. Territorial interactions involve prolonged vocal contests during which males sing or, together with females, call repeatedly with loud harsh "ahrr ahrr ahrr" sounds (Willis 1960). Chases may ensue between individuals from different groups.

First egg dates

To determine first egg date, we monitored nesting behavior of the pairs. Females build nests alone over the course of 4–7 days (Willis 1960). Males rarely help with nest construction, but the pair remains in close contact during this period. Prior to egg-laying, females frequently solicit copulations. When females are egg-laying or incubating, they are at the nest for ≥ 20 min at one time and males sing or forage alone (Willis 1960). We used observations of (1) nest-building activity, (2) copulations, and (3) female

absence from group or pair interactions for >20 min to determine when pairs initiated their first nest. During observations of singing behavior, we monitored for any indications of nest initiation, and first nests were located for 13 females. To determine first egg date, we monitored completed nests on a daily basis until we recorded the presence of an egg. Using the first clutches we encountered, we calculated the breeding synchrony index (Kempnaers 1993), which estimates the proportion of females that are fertile simultaneously in the population. This measure can provide an estimate of the extent of breeding seasonality and a measure of extra-pair mating opportunities.

Paternity analysis

To determine genetic parentage, we used microsatellite analysis at three loci, two isolated from Cherrie's Tanager (loci: Rcaat11 and Rcaaag406, *Ramphocelus costaricensis*; Krueger and Williams 2006) and one from Grasshopper Sparrow (locus Asu18, *Ammodramus savannarum*; Bulgin et al. 2003).

Samples from complete families are difficult to obtain because of high nest predation. In 2010, only 20 nestlings representing nine clutches from eight family groups were obtained. We included samples collected during three previous field seasons (2007–2009), i.e., an additional 21 nestlings representing ten clutches from nine families. In addition to the breeding pairs (14 males and their mates, 3 of which were replaced over the course of the 4 years for a total of 31 adults), we included in the genotyping analysis 28 additional adults from the population, including group members and neighboring males (12 adult males, 16 females and juvenile group members). Thus, we included samples from 59 adults and 41 nestlings representing 19 clutches and 17 family groups (3 of the 17 families represent pairs where males re-mated with a second female after they lost their mate).

DNA extraction was conducted using Qiagen DNA extraction kit for tissue or blood samples (Qiagen, Hilden, Germany). DNA was amplified by PCR in a 10- μ l reaction volume containing 2.5 μ l DNA solution (at 20 ng μ l⁻¹), 1.5 μ l water, 5.0 μ l PCR buffer (Promega, Madison, WI), 0.5 μ l of 10 μ M fluorescently labeled forward primer, and 0.5 μ l of 10 μ M reverse primer. The PCR cycling assay consisted of a 2-min denaturing step at 94 °C, followed by 35 cycles of 20 s at 72 °C, 20 s at the annealing temperature (45–50 °C for Rcaat406, 50–55 °C for Asu18, and 55–60 °C for Rcaat11), and 30 s at 72 °C. Forward primers were labeled with fluorescent dyes, and the PCR product at the three loci was multiplexed and sized using GeneScan™ 500 LIZ 500 size marker (Life Technologies, Carlsbad, CA). Fragment size was analyzed using the ABI 3730XL

Genetic Analyzer and the software Genemapper 3.0 (Applied Biosystems, Foster City, CA).

To determine genetic parentage of the nestlings, we used Cervus 3.0 (Kalinowski et al. 2007), which uses a maximum likelihood approach to assign paternity. Given the allele frequency of adults in the population, Cervus provides confidence intervals based on a simulation of possible nestling genotypes and their correct assignment to the parents. We first checked for Hardy–Weinberg equilibrium and the presence of null alleles at each locus (Dakin and Avise 2004) and then calculated the probability of exclusion per locus and overall (Table 1). We tested the assignment of the social mother to nestlings, and at all nests the social mother matched the nestlings except at one clutch, where the putative breeding female mismatched with the nestlings and a secondary female in the group matched all three nestlings. We included this secondary female as the mother of the nestlings. To simulate critical differences between likelihood ratios of nestling–father pairs, we assumed a genotyping error rate of 0.06 (determined from the number of mismatches between nestling and mother pairs) and that 90 % of candidate parents were sampled (we banded all territorial males heard in the area except for those on two territories that were inaccessible due to Panama Canal operations; 26 of 28 known territorial males). Floater males may also be present; however, we assumed that they were rarely successful at obtaining copulations (Woolfenden et al. 2005). First, we used each assigned mother as a known candidate and then assigned or excluded her social mate as the sire of the nestlings. We then performed an open analysis including all sampled males as candidate sires of the nestlings.

Statistical analyses

To determine if song output changes with breeding behavior and with nesting stage we divided observations into the following stages: (1) non-breeding ($n = 13$ pairs, 144 observations), 20–40 days before nest construction; (2) pre-breeding ($n = 13$ pairs, 107 observations), 20 days

Table 1 Genetic diversity at 3 microsatellite loci in 59 Red-throated Ant-tanager (*Habia fuscicauda*) adults

Locus	Number of alleles	H_O	H_E	P_{EX}
RcAAAG406	27	0.89	0.89	0.78
RcAAT11	10	0.63	0.70	0.47
Asu18	9	0.82	0.84	0.65
Average	15.3	0.78	0.81	–
Overall	–	–	–	0.99

H_O , Observed heterozygosity; H_E , expected heterozygosity; P_{EX} the probability of exclusion given that one parent is known

before and until initiation of nest construction; (3) breeding ($n = 13$ pairs, 85 observations), including nest construction, egg-laying, and incubation stage. We used a mixed-model analysis of variance (ANOVA) to evaluate the effect of breeding stage on male song output as we observed the same pairs repeatedly over time and our data therefore lacked independence. We used a generalized least squares model for the fixed effects (breeding stage) and mean squares model for the random effects (male identification). The model was weighted to allow for different variance values for each stage, as this resulted in a better fit model and homogeneity of residuals.

To test whether the rate of territorial interactions changed with breeding behavior, we calculated average interaction rate per pair (as number of interactions per hours of observations) at each of the non-breeding, pre-breeding, and breeding stages, respectively, and, because interaction rate was non-normally distributed, we used a non-parametric ANOVA (Kruskal–Wallis test) to look at variation with breeding status. We used correlation tests to determine whether song output was negatively related to the frequency of territorial interactions an individual male experienced (i.e., if increased song output may prevent territorial challenges). We also used correlation tests to examine if male timing of song initiation and song output were associated with female first egg date. Males vary in the timing of song initiation within the year by 1 to >4 weeks (Chiver 2011). We defined “timing of first song” as the average Julian date between the date when males were first observed to spend $\geq 20\%$ of their time singing during two consecutive observation periods and the date of the previous observation period. Our analysis did not change if instead we used a 15% song output threshold. All statistical analyses were conducted in R 2.14.0 (The R Foundation for Statistical Computing, Vienna, Austria). Means with 1 standard deviation are reported unless otherwise indicated.

Results

Seasonal variation in male song output

Male song increased from almost no song during the non-breeding season ($3 \pm 3\%$ of time spent singing) to an average of 17% ($\pm 10\%$) of time spent singing during the pre-breeding season, to 32% ($\pm 10\%$) once females initiated nests (Fig. 1). Song output varied significantly with breeding stage (ANOVA whole model $F_{1,336} = 35.1$, $P < 0.001$; breeding stage effect $F_{2,336} = 82.4$, $P < 0.001$). Male song increased during the pre-breeding period and more so once females started nest building.

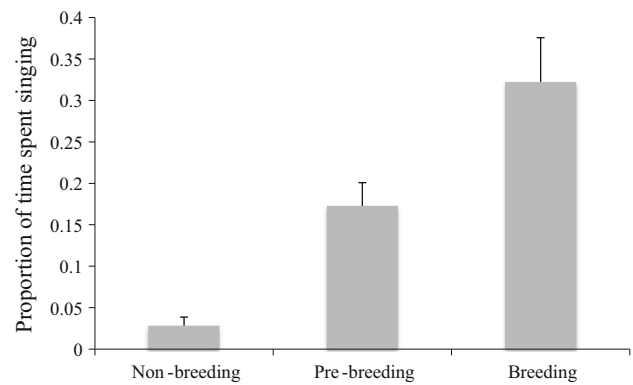


Fig. 1 Proportion of time male Red-throated Ant-tanager (*Habia fuscicauda*) adults spent singing (mean + standard error) during the non-breeding, pre-breeding, and breeding stages, respectively. Mean at each stage was calculated using the average song output of each male ($n = 13$). Song output increased significantly from the non-breeding to the pre-breeding stage (≤ 20 days before nest building) and from the pre-breeding stage to the breeding stage (the latter includes nest-building, egg-laying, and incubation periods)

Territorial interactions

In total, we observed 54 territorial interactions between birds on neighboring territories: nine during the non-breeding period, 23 during the pre-breeding period, and 22 during the breeding period. Interactions took place at territorial borders between neighboring territory owners, and the majority occurred between pairs (44/54, 81%), while a smaller proportion occurred with unpaired females that had lost their mate and were defending territories alone (10/54, 19%). None of the territorial interactions between pairs resulted in changes in territory ownership. Following interactions, pairs often remained and foraged close to the territorial border, often attempting repeated challenges. For the 12 observed territorial interactions where the pairs were involved only in one interaction, the mean duration of the dispute was 13.5 (range 5–24) min. Twelve territorial interactions were between pairs and single individuals: ten were by two territorial females without mates (8 and 2 by each of the two females) and two by a male whose mate was incubating. One of the two females was observed interacting with neighboring pairs on seven occasions during the non-breeding and pre-nesting stages and once during the nest construction stage, and this female joined a neighboring pair as a helper when this pair had a complete clutch and the mated female started incubating. The second female was observed interacting with neighboring pairs on two occasions—once during the non-breeding period and a second time during the pre-nesting period; during subsequent observations, she was observed consorting with the breeding male, while the original female was not observed on the territory, indicating she had been evicted or had died.

Average rate per pair of territorial interactions did not vary significantly with breeding stage (Kruskal–Wallis test $\chi^2_{2,13} = 1.65$, $P = 0.44$). However, we found that overall song output (average of non-breeding, pre-breeding, and breeding song output) was inversely related to overall interaction rates experienced by the territorial pair (Fig. 3; Pearson correlation test $R = -0.59$, $P = 0.032$).

Male song output and female nesting date

First clutches of 13 pairs in the population were initiated from 24 March through to 26 April (mean Julian Date 101 ± 10.5). Considering these first nest attempts only, the calculated breeding synchrony index was 14 % (range 0–22 % among females).

We examined if male singing behavior influenced the timing of female nesting. “Timing of first song” varied by >40 days (mean Julian Date 79 ± 12.5), and female first egg date varied over a range of 33 days (mean Julian 101 ± 10.5). The timing of first song but not pre-breeding song output was significantly correlated with the date of female nest initiation (Fig. 2; timing of first song: Spearman correlation test $R_s = 0.66$, $P = 0.014$; pre-breeding song output: Pearson correlation test $R = 0.11$, $P = 0.72$).

Paternity analysis

Of the 41 nestlings in 19 broods, 17 (41 %) nestlings in ten (53 %) broods were sired by a male other than the social

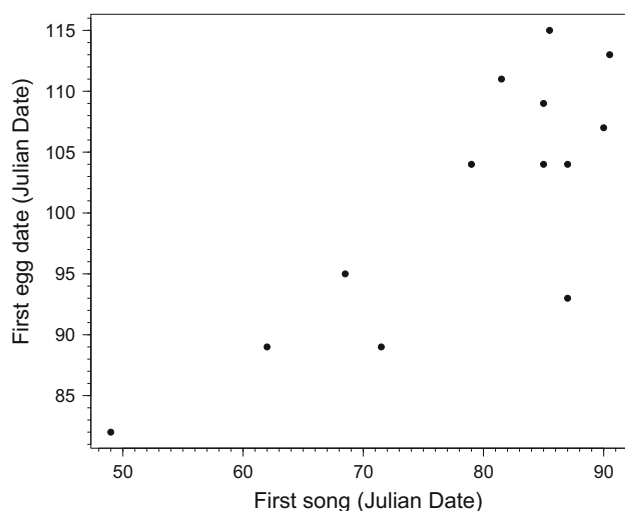


Fig. 2 Male song initiation measured by the timing of first song (the average between the date when a male was first observed to spend >20 % of the time singing during two consecutive observations and the previous date observed) was positively correlated with female nesting date ($n = 13$; Spearman correlation test $R = 0.66$, $P = 0.014$)

father. For the 16 broods with more than one young, five had mixed paternity, seven contained young fathered solely by the social father, and four contained young none of which were sired by the social father. For eight of the 17 extra-pair young, the identified father was a neighboring male; four males each sired one extra-pair young and two males sired 2 extra-pair young each. One male lost paternity for both young in the nest during 1 year and gained paternity in two of the neighbor’s nests the second year. For the other nine extra-pair young, the genetic father could not be identified with confidence.

Discussion

We studied singing behavior in Red-throated Ant-tanagers, a tropical species with year-round territoriality. In this species, males sing very little outside of the breeding season and significantly more with the onset of nesting (Fig. 1). We examined the importance of three non-mutually exclusive hypotheses, including competition for territories, pair reproductive coordination, and competition for extra-pair matings, in explaining the increase in male song during the breeding season.

The peak of song output during the nesting period suggests that male song may function primarily in a mating context. Previous studies indicate that territorial defense may be more pronounced prior to the breeding period because that is when young individuals establish territories on their own (Greenberg and Gradwohl 1983; Gill and Stutchbury 2006, 2010; Tarwater and Brawn 2010). During systematic observations of focal pairs, we did not observe territorial challenges or take-overs by non-territorial males. However, in 2007–2008, prior to this study, we observed four second-year males living as helpers in the neighborhood aggressively challenge adjacent territory owners during the pre-breeding period (Chiver 2011). Three of these males succeeded in replacing the owners, while one established in a territorial vacancy. During our study, the majority of territorial interactions were between neighboring pairs, and their frequency was not significantly associated with breeding stage. However, our results show that males with higher song output were involved in fewer territorial interactions (Fig. 3). It is possible that, as in temperate migratory species, territorial interactions may be important both in territory maintenance as well as in mate competition and thus male song output during the breeding season could function both in territoriality and paternity defense. Playback experiments combined with radiotracking of individual males (Fedy and Stutchbury 2004) could further test how song output influences the behavior of territorial intruders at different stages of breeding.

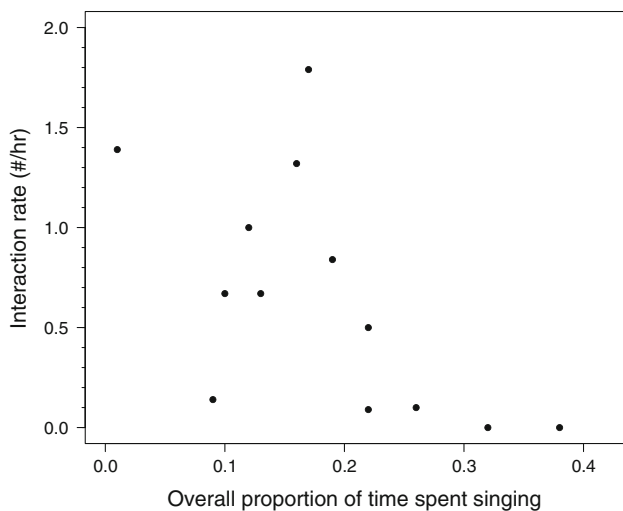


Fig. 3 Higher male song output (average proportion of time spent singing across the non-breeding, pre-breeding, and breeding periods) was associated with lower territorial interaction rates experienced by the pair ($n = 13$; Pearson correlation test $R = -0.59$, $P = 0.032$)

The presence of extra-pair matings (41 % of nestlings were extra-pair young) suggests that song could be important in the extra-pair mating system (reviewed in Forstmeier and Balsby 2002). Sustained song output during the breeding season suggests that song may function in attracting assessing extra-pair mates. Previous studies in other species indicate that female off-territory foray rate and resulting frequency of extra-pair young in the nest are related to the social mate's song characteristics (e.g., Forstmeier et al. 2002; Chiver et al. 2008). High song output may also function in mate guarding to deter extra-pair copulation attempts by neighboring males (Evans et al. 2008; Douglas et al. 2012). Radio-tracking of fertile females is necessary to establish how females respond behaviorally to song output by the social mate and whether neighbors with high song output are more likely to be preferred by females (Chiver et al. 2008). We cannot address the relationship between song output and pair variation in frequency of extra-pair matings statistically because we had few pairs where both behavioral and paternity data are available. However, the presence of an extra-pair mating system and the resulting increased sexual selection pressures on males is expected to be an important predictor of interspecific variation in male song output during the breeding season (Morton 1996). Song output varies greatly among year-round territorial passerines, ranging from low (5–17 songs h^{-1}) in White-bellied Antbirds (*Myrmeciza longipes*) and Buff-breasted Wrens (*Cantorchilus leucotis*; Fedy and Stutchbury 2005; Gill et al. 2005), to high (approx. 150 songs h^{-1}) in Red-throated Ant-tanagers. Paternity analysis has been performed on few such species, making it difficult to formally

test the prediction that song output correlates with frequency of extra-pair fertilizations. Buff-breasted Wrens having low song rates also have few extra-pair young (<5 % nestlings; Gill et al. 2005). Our results indicate that Red-throated Ant-tanagers feature high song rate and a high rate of extra-pair matings.

Our findings show that song output outside of the breeding period is significantly lower but that male initiation of singing is correlated with their mate's egg-laying date (Fig. 2). It is possible that male song functions secondarily in within-pair mate stimulation, which has been proposed to be important in tropical species (Dilger 1953). In Red-throated Ant-tanagers, nesting starts with the first rains of the wet season, and pairs that have a higher proportion of insects in their diet start nesting earlier (Chiver 2011). Early song may be a reliable indicator for females of suitable breeding conditions. An alternative is that females assess changes in environmental conditions independently of their mate and that both pair members respond to the same environmental cues. We cannot differentiate between these two possibilities, however given that female egg-laying follows male song initiation by about 3 weeks, it would be beneficial if females responded to male song to fine-tune egg-laying, particularly because this is a relatively costly energetic investment. For several species, including in the wild, it has been shown that female egg-laying may be finely tuned to male singing behavior, likely because this has important fitness consequences (Mota and Depraz 2004).

Seasonality in resources is generally lower in tropical environments, nevertheless the extent of seasonality may vary even among closely related species of similar foraging guild sharing the same habitat (Ahumada 2001). The ecological factors influencing the extent of sexual selection pressures on individuals in socially monogamous species have been under debate, but breeding synchrony, which is likely related to resource seasonality, is proposed to be important because it represents the extent of mating opportunities (Stutchbury and Morton 1995, 2008; Griffith et al. 2002; Stutchbury et al. 2007; Macedo et al. 2008). Our results indicate that Red-throated Ant-tanagers, while showing moderate levels of breeding synchrony, have a high frequency of extra-pair matings and that male song in this species likely serves in a mating context. Our estimate of breeding synchrony excludes breeding attempts following nest predation, as we were unable to follow these; however, as both predation and clutch replacement occur with high frequency (Willis 1961), our calculation of breeding synchrony is most likely an underestimate. More studies on species with diverse life histories are needed to examine how different ecological conditions have shaped breeding seasonality and to explain interspecific differences in male singing behavior.

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References

- Addis EA, Busch DS, Clark AD, Wingfield JC (2010) Seasonal and social modulation of testosterone in Costa Rican rufous-collared sparrows (*Zonotrichia capensis costaricensis*). *Gen Comp Endocrinol* 166:581–589
- Ahumada JA (2001) Comparison of the reproductive biology of two neotropical wrens in an unpredictable environment in northeastern Colombia. *Auk* 118:191–210
- Bohórquez CI, Stiles FG (2002) The paradoxical social system of the Dusky Bush-Tanager (*Chlorospingus semifuscus*): lekking in a nine-primaried oscine? *J Field Ornithol* 73:281–291
- Bulgin NL, Gibbs HL, Vickery P, Baker AJ (2003) Ancestral polymorphisms in genetic markers obscure detection of evolutionarily distinct populations in the endangered Florida grasshopper sparrow (*Ammodramus savannarum floridanus*). *Mol Ecol* 12:831–844
- Byers BE, Kroodsma DE (2009) Female mate choice and songbird song repertoires. *Anim Behav* 77:13–22
- Chiver I (2011) Timing of nesting and breeding behaviour of Red-throated Ant-tanagers (*Habia fuscicauda*) a resident species of lowland Panama. PhD dissertation, York University, Toronto
- Chiver I, Stutchbury BJM, Morton ES (2008) Do male plumage and song characteristics influence female off-territory forays and paternity in the hooded warbler? *Behav Ecol Sociobiol* 62:1981–1990
- Dakin EE, Avise JC (2004) Microsatellite null alleles in parentage analysis. *Heredity* 93:504–509
- Dilger WC (1953) Duetting in the crimson-breasted barbet. *Condor* 55:220–221
- Douglas SB, Heath DD, Mennill DJ (2012) Low levels of extra-pair paternity in neotropical duetting songbird, the Rufous-and-white Wren (*Thryothorus rufalbus*). *Condor* 114:393–400
- Evans ML, Stutchbury BJM, Woolfenden BE (2008) Off-territory forays and genetic mating system of the wood thrush (*Hylocichla mustelina*). *Auk* 125:67–75
- Faaborg JR, Terborgh JW (1980) Patterns of migration in the West Indies. In: Keast A, Morton ES (eds) *Migrant birds in the neotropics: ecology, behavior, distribution, and conservation*. Smithsonian Institution Press, Washington, DC, pp 157–171
- Fedy BC, Stutchbury BJM (2004) Territory switching and floating in white-bellied antbird (*Myrmeciza longipes*), a resident tropical passerine in Panama. *Auk* 121:486–496
- Fedy BC, Stutchbury BJM (2005) Territory defence in tropical birds: are females as aggressive as males? *Behav Ecol Sociobiol* 58:414–422
- Forstmeier W, Balsby TJS (2002) Why mated dusky warblers sing so much: territory guarding and male quality announcement. *Behaviour* 139:89–111
- Forstmeier W, Kempenaers B, Meyer A, Leisler B (2002) A novel song parameter correlates with extra-pair paternity and reflects male longevity. *Proc R Soc Lond Ser B Biol Sci* 269:1479–1485
- Gill SA, Stutchbury BJM (2006) Long-term mate and territory fidelity in neotropical Buff-breasted Wrens (*Thryothorus leucotis*). *Behav Ecol Sociobiol* 61:245–253
- Gill SA, Stutchbury BJM (2010) Delayed dispersal and territory acquisition in neotropical Buff-breasted Wrens (*Thryothorus leucotis*). *Auk* 127:372–378
- Gill SA, Vohnhof MJ, Stutchbury BJM, Morton ES, Quinn JS (2005) No evidence for acoustic mate-guarding in duetting Buff-breasted Wrens (*Thryothorus leucotis*). *Behav Ecol Sociobiol* 57:557–565
- Greenberg R, Gradwohl J (1983) Sexual roles in the Dot-winged Antwren (*Microphias quixensis*), a tropical forest passerine. *Auk* 100:920–925
- Greig-Smith PW (1982) Seasonal patterns of song production by male Stonechats *Saxicola torquata*. *Ornis Scand* 13:225–231
- Griffith SC, Owens IPF, Thuman KA (2002) Extra pair paternity in birds: a review of interspecific variation and adaptive function. *Mol Ecol* 11:2195–2212
- Janzen DH (1967) Synchronization of sexual reproduction of trees within dry season in Central America. *Evolution* 21:620
- Kalinowski ST, Taper ML, Marshall TC (2007) Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Mol Ecol* 16:1099–1106
- Kempenaers B (1993) The use of a breeding synchrony index. *Ornis Scand* 24:84–84
- Krueger TR, Williams DA (2006) Microsatellite loci for Cherrie's tanager (*Ramphocelus costaricensis*). *Mol Ecol Notes* 6:853–855
- Kunkel P (1974) Mating systems of tropical birds: the effects of weakness or absence of external reproduction-timing factors, with special reference to prolonged pairbonds. *Z Tierpsychol* 34:265–307
- Levey DJ (1988) Spatial and temporal variation in Costa Rican fruit and fruit-eating bird abundance. *Ecol Monogr* 58:251–269
- Levey DJ, Stiles FG (1992) Evolutionary precursors of long-distance migration—resource availability and movement patterns in neotropical landbirds. *Am Nat* 140:447–476
- Levin RN (1996) Song behaviour and reproductive strategies in a duetting wren, *Thryothorus nigricapillus* 2. Playback experiments. *Anim Behav* 52:1107–1117
- Mace R (1987) The dawn chorus in the great tit *Parus major* is directly related to female fertility. *Nature* 330:745–746
- Macedo RH, Karubian J, Webster MS (2008) Extrapair paternity and sexual selection in socially monogamous birds: are tropical birds different? *Auk* 125:769–777
- Morton ES (1996) A comparison of vocal behavior among tropical and temperate passerine birds. In: Kroodsma DK, Miller TE (eds) *Ecology and evolution of acoustic communication in birds*. Cornell University Press, Ithaca, pp 258–268
- Mota PG, Depraz V (2004) A test of the effect of male song on female nesting behaviour in the serin (*Serinus serinus*): a field playback experiment. *Ethology* 110:841–850
- Seutin G, White BN, Boag PT (1991) Preservation of avian blood and tissue samples for DNA analyses. *Can J Zool* 69:82–90
- Smythe NM (1982) The seasonal abundance of night-flying insects in a Neotropical rain forest. In: Leigh EG Jr, Rand AS, Windsor DM (eds) *The ecology of a tropical rainforest: seasonal rhythms and long-term changes*. Smithsonian Institution Press, Washington, DC, pp 309–318
- Stutchbury BJM (1998) Extra-pair mating effort of male hooded warblers, *Wilsonia citrina*. *Anim Behav* 55:553–561

- Stutchbury BJM, Morton ES (1995) The effect of breeding synchrony on extra-pair mating systems in songbirds. *Behavior* 132:675–690
- Stutchbury BJM, Morton ES (2001) Behavioral ecology of tropical birds. Academic Press, London
- Stutchbury BJM, Morton ES (2008) Recent advances in the behavioral ecology of tropical birds—the 2005 Margaret Morse Nice lecture. *Wilson J Ornithol* 120:26–37
- Stutchbury BJM, Morton ES, Piper WH (1998) Extra-pair mating system of a synchronously breeding tropical songbird. *J Avian Biol* 29:72–78
- Stutchbury BJM, Morton ES, Woolfenden B (2007) Comparison of the mating systems and breeding behavior of a resident and a migratory tropical flycatcher. *J Field Ornithol* 78:40–49
- Tarwater CE, Brawn JD (2010) Family living in a Neotropical bird: variation in timing of dispersal and higher survival for delayed dispersers. *Anim Behav* 80:535–542
- Terborgh JW (1980) The conservation status of Neotropical migrants: present and future. In: Keast A, Morton ES (eds) *Migrant birds in the neotropics: ecology, behavior, distribution, and conservation*. Smithsonian Institution Press, Washington, DC, pp 21–30
- Tobias JA, Gamarra-Toledo V, Garcia-Olachea D, Pulgarin PC, Seddon N (2011) Year-round resource defence and the evolution of male and female song in suboscine birds: social armaments are mutual ornaments. *J Evol Biol* 24:2118–2138
- Topp SM, Mennill DJ (2008) Seasonal variation in the duetting behaviour of rufous-and-white wrens (*Thryothorus rufalbus*). *Behav Ecol Sociobiol* 62:1107–1117
- Willis E (1960) Voice, courtship, and territorial behavior of ant-tanagers in British Honduras. *Condor* 62:73–87
- Willis E (1961) A study of nesting ant-tanagers in British Honduras. *Condor* 63:479–503
- Willis E (1972) Taxonomy, ecology, and behavior of the Sooty Ant-Tanager (*Habia gutturalis*) and other Ant-Tanagers (Class: Aves). *Am Mus Novit* 2480:1–38
- Woolfenden BE, Stutchbury BJM, Morton ES (2005) Male Acadian Flycatchers, *Empidonax virescens*, obtain extrapair fertilizations with distant females. *Anim Behav* 69:921–929