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Comparison of the mating systems and breeding behavior of a resident and a migratory tropical flycatcher

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ABSTRACT. Little is known about the genetic mating systems of tropical passerines and how they vary among species. We studied the Lesser Elaenia (*Elaenia chiriquensis*) and the Yellow-bellied Elaenia (*E. flavogaster*) near Gamboa, Panama. These species breed in the same habitat, but Lesser Elaenias are intratropical migrants with seasonal territories and Yellow-bellied Elaenias are permanent residents that remain paired and defend territories throughout the year. Lesser Elaenias exhibited greater breeding synchrony (15–18 %) than Yellow-bellied Elaenias (9–10%). For Lesser Elaenias, 10 of 15 (67%) nests contained extra-pair young and 14 of 38 (37%) young resulted from extra-pair fertilizations (EPFs). In contrast, only one extra-pair nestling (4%, $N = 24$ nestlings) was found in 13 Yellow-bellied Elaenia nests. Neither species exhibited strong mate guarding. The higher rate of EPFs in Lesser Elaenias is consistent with the hypothesis that year-round territorial tropical passerines with low breeding synchrony have little or no extra-pair behavior compared with species that breed seasonally. Although the low singing rates of Lesser Elaenias (7 songs/h) suggest that this not an important cue for female extra-pair mate choice, the role of conspicuous male dawn song remains to be investigated. Further studies of tropical passerines are needed to help disentangle the effects of synchrony, density, and other ecological and behavioral factors that have influenced the evolution of extra-pair mating systems in passerines.

SINOPSIS. **Comparación del sistema de apariamiento y la conducta reproductiva de un papamoscas residente y uno migratorio del trópico**

Se conoce muy poco sobre la genética y el sistema de apariamiento de paserinos tropicales y como esta varía entre especies. Estudiamos al Papamoscas Menor (*Elaenia chiriquensis*) y al de Pecho Amarillo (*E. flavogaster*), cerca de Gamboa, Panamá. Estas especies se reproducen en el mismo habitat, pero el Papamoscas Menor es un migratorio dentro del trópico con territorios estacionales y el de Pecho Amarillo es un residente permanente que se mantiene apareado y defiende un territorio durante todo el año.El Papamoscas Menor mostró mayor sincronización reproductiva (15–18%) que el de Pecho Amarillo (9–10%). En 10 de los 15 (67%) nidos del Papamoscas Menor encontramos pichones de otros individuos y 14 de 38 (37%) pichones fueron el resultado de copular con otros individuos que no fueran su pareja. En contraste, en 13 nidos estudiados del Pecho Amarillo, tan sólo encontramos un pichón (4%, $N = 24$ pichones) resultante de copulación con otro individuo que no fuera la pareja. Ninguna de las dos especies exhibio estricta vigilancia de su pareja. La tasa mayor de copulaciones con otros miembros fuera de la pareja encontrados en el Papamoscas Mayor, es consistente con la hipótesis que este patrón es poco común en especies que defienden un territorio durante todo el año y muestran poca sincronización en la reproducción comparado con especies que se reproducen estacionalmente. Aunque la tasa baja de cantos en el Papamoscas Menor (7 cantos/hora) sugiere que el canto no es una pista de importancia para seleccionar a otros machos fuera de la pareja, el rol del canto llamativo y conspicuo por parte de machos al amanecer permanence sin ser investigado. Se necesitan otros estudios de paserinos tropicales para ayudar a entender los efectos de la sincronización, densidad y otros factores ecológicos y de conducta que puedan haber influido en la evolución de sistemas de producir progenie con otro miembro fuera de la pareja.

Key words: breeding synchrony, *Elaenia chiriquensis*, *Elaenia flavogaster*, extra-pair fertilizations, Lesser Elaenia, nesting behavior, song rates, Yellow-bellied Elaenia

Although extra-pair mating systems are often considered typical for socially monogamous passerines (Birkhead and Møller 1996, Griffith et al. 2002), most paternity studies have involved North American and European species. Rates of extra-pair fertilizations (EPFs) often exceed 20% of the young in Neotropical migrant songbirds (Stutchbury et al. 2005). Tropical passerines differ from their north-temperate counterparts in many aspects of their nesting ecology and behavior (Stutchbury and Morton 2001), and there is growing evidence that many tropical passerines do not have extra-pair mating systems. Stutchbury and Morton (1995) found that testes mass of resident passerines in the

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Neotropics was lower than that of Nearctic-Neotropical migrants, suggesting they also have fewer EPFs. Testis mass, relative to body size, is strongly and positively correlated with levels of extra-pair paternity, reflecting the high levels of sperm competition that occur when males are competing to inseminate females (Møller and Briskie 1995, Dunn et al. 2001).

Stutchbury and Morton (1995) argued that the long breeding seasons of tropical residents did not favor extra-pair mating systems because few females are fertile simultaneously in a population. Interspecific comparisons show that breeding synchrony is an important correlate of the frequency of EPFs (Stutchbury 1998, Møller and Ninni 1998, Stutchbury et al. 2005). Comparative tests of the breeding synchrony hypothesis have been hampered by the small number of studies available, particularly for tropical birds, and hence the difficulty of separating the effects of synchrony from confounding factors like migration and breeding density (Westneat and Sherman 1997, Stutchbury 1998, Griffith et al. 2002).

The results of paternity analyses of tropical passerines provide support for the association between breeding synchrony and extra-pair mating systems. For example, Dusky Antbirds (*Cecromacra tyrannina*) in Panama maintain permanent pairs and territories and had no extra-pair young (EPY) (Fleischer et al. 1997). Buff-breasted Wrens (*Thryothorus leucotis*) have a similar territorial system and only 3% of broods contained EPY (Gill et al. 2005). Both of these species have long breeding seasons (April–October) and low breeding synchrony (8–10%). Moore et al. (1999) compared the tropical Mangrove Swallow (*Tachycineta albilinea*) with its migratory congener, the Tree Swallow (*Tachycineta bicolor*), that breeds in North America and found that 54% and 15% of Tree Swallow and Mangrove Swallow nestlings, respectively, were extra-pair. Mangrove Swallows have a 5-month-long breeding season in Panama (8% synchrony), whereas Tree Swallows have a 2-month breeding season (47% synchrony). Tropical species that breed seasonally are expected to have extra-pair mating systems. Clay-colored Robins (*Turdus grayi*) breed relatively synchronously at the onset of the dry season in Panama and have many EPFs (38% of young and 53% of broods; Stutchbury et al. 1998). Similarly, Blue-black Grassquits (*Volatinia jacarina*) breed seasonally at the end of the rainy season in Brazil when grass seeds are abundant, and have a high frequency of EPFs (50% of young and 63% of broods; Carvalho et al. 2006). Although tropical species have tremendous potential for testing hypotheses concerning the ecological and behavioral factors favoring the evolution of extra-pair mating systems, too few species have been studied to permit comparative analyses.

Here we report on the mating systems and behavior of two tropical congeners, one migratory and the other resident. We compare two flycatchers in the genus *Elaenia* that breed in the same habitat in Panama during the dry season (January–May). Yellow-bellied Elaenias (*Elaenia flavogaster*) are nonmigratory and have year-round pair bonds and territory defense, so are expected to have few or no EPFs. Females sing and duet with males, and males assist with nest building and feeding young, but not with incubation. Lesser Elaenias (*E. chiriquensis*) are intratropical migrants so are present only during the dry season and are expected to have an extra-pair mating system. Females usually do not sing, and males feed the young, but do not build nests or incubate (Skutch 1960, Stutchbury and Morton 2001). The objective of our study was to compare the mating systems of these closely related, but ecologically different species.

METHODS

Our study was conducted near Gamboa, Panama, from January to March in 1998 and 1999. Both Lesser and Yellow-bellied Elaenias inhabit open country, dry scrub, and grassy areas with scattered trees and bushes (Hosner 2004). We observed no interspecific interactions despite much territorial overlap. Our study areas encompassed a number of abandoned houses and a golf course that was no longer used, but was mowed and contained many clumps of native trees and shrubs. All that remained of the housing area were cement streets and foundations interspersed with weedy former-lawn areas and exotic trees and native shrubs and trees such as *Cochlospermum vitifolium* and *Cecropia* sp. Territories were mapped by noting the locations of singing, color-banded males, and border disputes. Males were considered unpaired if there was no female nesting in their territory, and, in the case of Yellow-bellied Elaenias, if no female was observed duetting with the male.

We found nests by observing birds carrying nesting material or food to nestlings. During the two breeding seasons, we located 40 Yellow-bellied Elaenia nests and 44 Lesser Elaenia nests and were able to obtain first egg dates for 25 and 31 of those nests, respectively, to calculate breeding synchrony (Kempenaers 1993). To capture adult Yellow-bellied Elaenias, we played their characteristic male and female duets from a speaker located near nests and captured them in mist nets. For Lesser Elaenias, males were captured in mist nets using playback of dawn song. Female Lesser Elaenias did not respond to playback of male song, so incubating or brooding females were captured on nests using a single-cell Potter Trap modified by removing its hardware-cloth bottom (Fig. 1). The bottomless trap was secured in position covering nests after temporarily removing eggs and replacing them with dummy eggs. We then positioned a section of mist net under the nest and attached it to the bottom of

Fig. 1. To capture female Lesser Elaenias, we tied a 30 × 30 × 30 cm bottomless potter trap to branches near the nest with string. We first removed the eggs and substituted wooden dummy eggs to avoid damaging the real eggs. A piece of mist net tied to the bottom and under the nest kept females from escaping through the bottom of the trap.

the sides of the trap with clips. Females usually entered the trap within 20 min. No nests were abandoned due to use of this capture method.

Sample collection. Captured birds were banded with colored plastic bands for individual identification. In addition, we obtained a blood sample (25–100 μl) via brachial puncture and measured (tarsus and wing chord) and weighed each bird. Birds were sexed by noting the presence of either a cloacal protuberance (males) or brood patch (females), and sex was verified during nest watches when behavioral differences (e.g., incubation by females) were evident. When nestlings were at least 5 days old, we obtained a blood sample (25 μl) from each nestling.

Genetic analysis. All blood samples were stored at 4°C in 500 μl of Queen's Lysis buffer (Seutin et al. 1991). DNA extractions involved cell lysis and the use of either ammonium acetate and isopropanol to precipitate DNA (L. De Sousa, unpubl. protocol) or a Qiagen DNeasy Kit (Qiagen Corporation, Hilden, Germany). We genotyped each individual at hypervariable microsatellite loci isolated from the Least Flycatcher (*Empidonax minimus*; EMIZ 01, 23, 27, and 46; Tarof et al. 2001), Eastern Phoebe (*Sayornis phoebe*; SAP32 and 53, Watson et al. 2002), and Yellow-bellied Elaenia (ELN 22 and 27; Gregory et al. 2004) and compared the genotype of young with those of the putative parents to identify nestlings resulting from extrapair matings. Details of the genetic protocols and microsatellites used for each species are summarized in Tables 1 and 2.

Allelic variation at each locus was quantified either by visualizing radioactively labeled DNA fragments on autoradiographs or by quantifying the size of fluorescently labeled DNA fragments using the CEQ 8000 Genetic Analysis System (Beckman Coulter, Inc., Fullerton, CA). Only one method was used for each locus, that is, all individuals were genotyped at a specific locus using only autoradiographs or automated sequencing, thereby eliminating the need to compare genotypes generated using two different methods.

For radioactively labeled fragments, we amplified genomic DNA from each individual in a 10-μl PCR reaction mixture containing 3.73 μl water, 1 μl PCR reaction buffer (Biobasic Inc., Markham, Ontario, Canada), 3.0 μl 20 mM $MgSO_4$, 1 μl BSA (Amersham-Pharmacia, Piscataway, NJ), 0.3 μl 10 μM dNTPs, 0.03 μl of 10 μM forward primer, 0.06 μl of 10 μM reverse primer, 0.05 μl 5 U/μl TSG (BioBasic, Inc.), 0.277 μl radioactively labeled forward primer cocktail, and 50 ng of DNA. The primer cocktail (per 30 samples) included 1.0 μl 10-μM forward primer, 1.0 μl 10X Polynucleotide Kinase (PNK) reaction buffer (New England Biolabs, Ipswich, MA)/water mix in a 6:4 ratio, 3.0 μl T4 PNK, and 1.0 μl [γ^{33}P]-ATP (Amersham-Pharmacia). The reaction protocol involved incubation at 37°C for 30 min, and 68°C for 10 min. PCR reactions were performed in one of several different thermocyclers using the following protocol: an initial 2-min 94°C denaturing step followed by 35 cycles of: 20 s at 96°C, 20 s at the primer-specific annealing temperature (see Table 1), 30 s extension at 72°C, followed by a final extension step of 72°C for 5 min. To visualize PCR products, each sample was run on a 6% denaturing polyacrylamide gel. Several positive (samples of known size) and negative controls were always included on each gel. After electrophoresis, gels were dried and exposed to autoradiograph film for 24–48 h. We scored the size of each PCR fragment by comparing bands to the reference samples run on each gel.

For fluorescently labeled fragments, we amplified genomic DNA in 10 μl volumes comprised of the following reaction cocktail: 50 ng genomic DNA, 0.2 μl (10 μM) forward fluorescently labeled primer (Well-Red; Beckman-Coulter, Fullerton, CA), 0.2 μl (10 μM) reverse primer, 0.2 μl of 10 μM dNTPs, 0.08 μl X 5 U/μl TSG, 1.0 μl 10X reaction buffer, and 3.0 μl (20 mM) $MgCl_2$. PCR amplification was performed using a touchdown method (Don et al. 1991), with an initial denaturing step at 94°C for 3 min, followed by a total of 35 cycles of 94°C at 30 s, 30 s at Tm (Table 1), and 30 s at 72°C for DNA extension, followed by an additional 5 min at 72°C to complete DNA extension. The resulting fragment sizes were determined using the fragment analysis application module of the CEQ 8000 Genetic Analysis System.

For both species, we calculated the frequency of each allele from the total population of adults genotyped and calculated the average probability of parental exclusion for each locus (Tables 1 and 2). This is the probability, averaged over all alleles at that locus, that a randomly chosen nonparental male will not possess the paternal allele belonging to a given offspring, given that the

Table 1. Microsatellite loci used to analyze parentage in Lesser Elaenias.[a]

Locus	Primer sequence in 5′–3′ direction	T_a (°C)	No. alleles	*N*	P(E)
EAPH32[b,c]	F-TGCTTTTCCAACTGCAACAG R-GGACCCAATGTCTCTTAAGGG	48	4	25	0.60
EAPH53[b,d]	F-CCAAGAACAGCTTTTGCTCC R-CCCGTGTGTTCAAATAGGCT	48–50	6	24	0.50
EMIZ01[c,e]	F-AGGTGAGTGGGACAAGTTAGC R-GAGGAACAATAGCCTGCCAGT	48	3	28	0.10
EMIZ23[c,e]	F-ACTTGCTGTTCTGCAAGGGTTG R-ATACCCTAAGGCAAGCCACAGC	48	4	28	0.38
EMIZ27[c,e]	F-CGTGTCAGAGCAAGGCAGTG R-ACTGATCTGCACGTGAGCACC	48	2	28	0.18
EMIZ46[c,e]	F-CAAGTGGGTGATGTGCTAGAGATG R-TTGTCTGCATCTGAGACCTCCTG	48	5	26	0.38
ELN22[d,f]	F-CCCGGGAAAGGCTTCGTCTTC R-GGAGATTTTATATCGGTGGC	50–53	5	26	0.42
ELN27[d,f]	F-GTGTCAGAGCAAGGCAGT R-TGATCTGCACGTGAGCAC	50–53	4	22	0.24

[a]Annealing temperatures (T_a), number of alleles per locus, number of adults (*N*), and average probability of parental exclusion [P(E)]. Total probability of exclusion was 0.96.
[b] From Watson et al. (2002).
[c]Samples genotyped using radioactively labeled primers on autoradiographs.
[d]Samples genotyped with fluorescently labeled primers on an automated sequencer.
[e]From Tarof et al. (2001).
[f]From Gregory (2004).

genetic mother is known (Jamieson 1994). We then calculated the total probability of exclusion P(E) for all loci combined, defined as the probability that a randomly chosen male will not possess the paternal allele of an offspring at one or more of the loci (Chakraborty et al. 1988). We used the computer program Micro-Checker (Van Oosterhout et al. 2004) to assist in the identification of genotyping errors and null alleles.

For Lesser Elaenias, each of the eight loci used were moderately variable (2–6 alleles), there was

Table 2. Microsatellite loci used to analyze parentage in Yellow-bellied Elaenias.[a]

Locus	Primer sequence in 5′–3′ direction	Ta (°C)	No. alleles	*N*	P(E)
EAPH53[b,c]	F-CCAAGAACAGCTTTTGCTCC R-CCCGTGTGTTCAAATAGGCT	48–50	5	18	0.41
EMIZ01[d,e]	F-AGGTGAGTGGGACAAGTTAGC R-GAGGAACAATAGCCTGCCAGT	48	3	17	0.22
EMIZ27[d,f]	F-CGTGTCAGAGCAAGGCAGTG R-ACTGATCTGCACGTGAGCACC	48	3	20	0.24
ELN22[c,g]	F-CCCGGGAAAGGCTTCGTCTTC R-GGAGATTTTATATCGGTGGC	50–55	6	20	0.61
ELN27[c,g]	F-GTGTCAGAGCAAGGCAGT R-TGATCTGCACGTGAGCAC	50–55	3	21	0.23

[a]Annealing temperatures (T_a), number of alleles per locus, number of adults (*N*), and average probability of parental exclusion [P(E)]. Total probability of exclusion was 0.91.
[b]From Watson et al. (2002).
[c]Samples genotyped with fluorescently labeled primers on an automated sequencer.
[d]From Gregory (2004).
[e]Samples genotyped using radioactively labeled primers on autoradiographs.
[f]From Tarof et al. (2001).

no evidence of null alleles at any locus, and probabilities of exclusion ranged from 0.10 to 0.53 (Table 1). The total exclusion probability of the eight loci was P(E) = 0.96, indicating that we could exclude socially paired males as genetic parents with a high degree of certainty. We genotyped 19 adult males, 11 adult females, and 38 nestlings.

For Yellow-bellied Elaenias, each of the five loci was moderately variable (2–5 alleles) and there was evidence of null alleles at the loci LEFL 01 and ELN 22 (Table 2). The probabilities of exclusion ranged from 0.09 to 0.40 and the P(E) of the five loci was 0.91, indicating that we could exclude socially mated males as genetic parents with a reasonably high degree of certainty. We genotyped 14 adult males: 7 adult females and 24 nestlings.

Genetic parentage analysis. Adults were excluded as putative genetic parents if the adult and nestling allele(s) mismatched by two or more base pairs at two or more loci (Thusius et al. 2001, Tarof et al. 2005) . Given the likelihood of null alleles at two of the loci used to genotype the Yellow-bellied Elaenia, several mismatches between offspring and father where at least one individual was designated as a homozygote were not considered to indicate an extra-pair mating (e.g., Webster et al. 2001).

Because there was no indication of intraspecific brood parasitism in either species, we assumed that the social female was also the genetic female parent in all cases. This assumption was also supported by the genetic data. For Lesser Elaenias, we sampled and genotyped the social female parent in 13 of 15 (86%) family groups. Four chicks (10%) mismatched the social mother, but only by two base pairs at a single locus ($N = 3$) or at a locus where the female was heterozygous. In all four cases, we attributed mismatches to either laboratory artifacts (e.g., PCR amplification error or template-primer mismatch) or mutations. For Yellow-bellied Elaenias, we sampled and genotyped the social female parent for 84% of the nests ($N = 13$). In all but one instance ($N = 22$), the social female matched the offspring in her nest. In that case, the female was homozygous at the mismatched locus and we attributed the mismatch to PCR artifacts.

Behavioral observations. We monitored nests during 1-h observation periods during the period from 07:30 to 10:30 during the nest-building, egg-laying, and incubation stages. We observed nine pairs of Lesser Elaenias and eight pairs of Yellow-bellied Elaenias for 38 h and 20 h, respectively. Because of the open habitat, observations were made about 25–30 m from the nest tree. During observations, we noted nest-building trips by male and female and two measures of mate guarding. First, we noted the percentage of trips where the female left the nest territory and was followed immediately by her mate. Second, we determined the percentage of time when the female was on territory that the male was also present. We also collected data on mate-following behavior when females were nest building and egg laying (and potentially fertile) for five pairs of Yellow-bellied Elaenias (10 h) and nine pairs of Lesser Elaenias (23 h). Finally, we recorded the rate of vocalizations (solo or pair duets for Yellow-bellied Elaenias, and *beer*, *wee*, and double calls for Lesser Elaenias) and any extra-pair copulation attempts and intrusions.

RESULTS

Frequency of extra-pair paternity. We determined paternity for 15 family groups ($N = 38$ nestlings) of Lesser Elaenias and 13 family groups ($N = 24$ nestlings) of Yellow-bellied Elaenias. For Lesser Elaenias, the mean brood size was 2.5 ± 1.1 (SD) (range 1–4; $N = 15$) nestlings. Ten of the 15 nests (67%) had at least 1 EPY, and 14 (37%) of 38 nestlings were sired by a male other than the social male parent. The proportion of EPY in nests ranged from 0 to 1.0; seven nests had one EPY, two had two, and one had three. We observed male Lesser Elaenias intruding and chasing females, but did not witness any extra-pair copulations.

The mean brood size for Yellow-bellied Elaenias was 1.7 ± 0.5 (range 1–2; $N = 13$) nestlings. Only 1 of the 13 nests (8%) had EPY, and only 1 of 24 nestlings (4%) was sired by a male other than the social male parent. Yellow-bellied Elaenias had significantly fewer EPY than Lesser Elaenias (Fisher's Exact test, two-tailed, $P = 0.005$), and significantly fewer broods with EPY ($P = 0.002$).

Breeding synchrony and density. Breeding synchrony (percentage of females simultaneously fertile) was calculated based on first egg dates for nesting females in the population (Kempenaers 1993). The average

breeding synchrony index was 15–18% for the Lesser Elaenia (14.6 ± 4.5% in 1998, and 17.5 ± 7.8% in 1999), and peaked with about 40% of females fertile simultaneously during both early February (first nests) and early March (renests; Fig. 2). The average breeding synchrony index for Yellow-bellied Elaenias was 9–10% (9.7 ± 6.8% in 1998, and 9.6 ± 2.4% in 1999) and the percentage of females simultaneously fertile never exceeded 25% (Fig. 2). Differences in mean breeding synchrony between species were significant in both 1998 ($t_{27} = 2.34$, $P = 0.027$) and 1999 ($t_{34} = 3.67$, $P = 0.001$). Many Yellow-bellied Elaenia pairs began building nests at one location then either changed to another location or interrupted nest building for many days before resuming. Several pairs sporadically built their nests for a month before egg laying actually began and 4 of 12 pairs in 1999 had not completed nests when we ended data collection in early April (Fig. 2) and could not be used when calculating the synchrony index. Prolonged nest-building activity is common among year-round tropical residents (Stutchbury and Morton 2001). In contrast, female Lesser Elaenias built nests and laid clutches with little delay, resulting in a higher degree of nesting synchrony. All Lesser Elaenia females completed their first clutches by mid-March (Fig. 2).

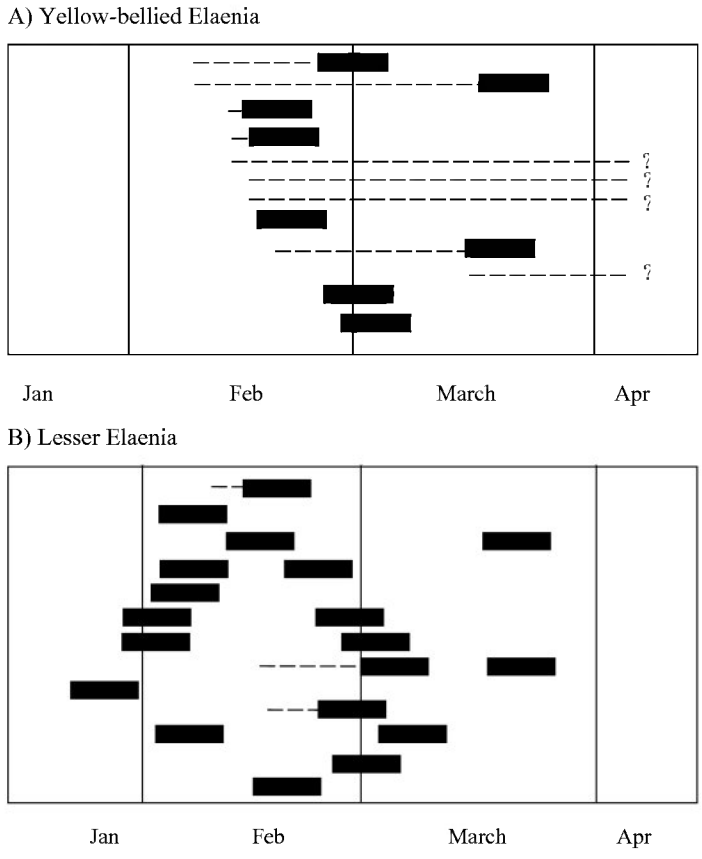

Fig. 2. Nesting chronologies of Yellow-bellied Elaenias (A) and Lesser Elaenias (B) in Gamboa, Panama, in 1999. The black boxes indicate fertile periods for individual females, with fertile periods defined as the 5-day period before, and the day of, the laying of the penultimate egg in a clutch. This was usually the day the first egg was laid because clutch size was typically two eggs. The dashed line indicates the period of nest site searching and nest building. The question marks (?) in A indicated that we ended the study before the first egg for that pair had been laid.

In 1999, the mean nearest neighbor distance was 76 ± 22 m ($N = 24$ territories) for Lesser Elaenias and 103 ± 23 m ($N = 22$) for Yellow-bellied Elaenias (Mann–Whitney U-test, $Z = 3.71$, $P < 0.001$). Results were similar in 1998 because many territories were used in both years.

Behavioral observations. All male Yellow-bellied Elaenias were monogamously paired (37/37) and participated in nest building, making 17.2% of all trips to nests with nest material. Only 79% of male Lesser Elaenias were paired (34 of 43 males observed) and none participated in nest building.

Both species have territorial systems called "fruit influenced," with off-territory fruit sources used extensively and pairs leaving their territories frequently (Stutchbury and Morton 2001). Rates of territorial intrusion were low for both species (none for Yellow-bellied Elaenia, and a mean rate of 0.31 ± 0.15 for Lesser Elaenias; $Z = 1.67$, $P = 0.19$). Female Yellow-bellied Elaenias who left their territory were followed by mates only 6.8 ± 3.1% of the time ($N = 5$ females, average of 21 trips per female), and female Lesser Elaenias were followed by mates only 1.7 ± 0.7% of the time ($N = 9$ females, average of 45 trips per female). There was no significant difference between species in mate-following behavior (Mann–Whitney U-test, $Z = 1.18$, $P = 0.24$). However, when their mates were on territory, male Yellow-bellied Elaenias were with them 28.6 ± 7.9% of the time, significantly more than male Lesser Elaenias (4.8 ± 2.6%; $Z = 2.66$, $P = 0.008$).

Singing rates of paired and unpaired male Lesser Elaenias differed, and we did not see any known female (e.g., color banded and sexed in the hand) sing. The mean singing rate of mated males ($N = 8$) during the nest-building and egg-laying stages was 6.8 ± 9.8 songs/h , but unpaired males ($N = 3$) sang at a rate of 168 ± 94.8 songs/h. Yellow-bellied Elaenias differed in that all males were paired, females sang frequently, and pair members often sang in duets. During the nest-building or egg-laying stages, male Yellow-bellied Elaenias ($N = 8$) sang alone 0.8 ± 0.6 time/h, pairs duetted at a rate of 9.1 ± 4.4 times/h, and females sang alone 5.6 ± 4.6 times/h. Males of both species sang distinctive dawn songs for 10–20 min before sunrise.

DISCUSSION

Although both species in our study were socially monogamous, migratory Lesser Elaenias exhibited a higher level of EPFs (67% of broods and 37% of nestlings) than resident Yellow-bellied Elaenias (8% of broods and 4% of nestlings). Our behavioral observations were consistent with these genetic results. Despite the open habitat, we never observed male Yellow-bellied Elaenias intruding onto a neighbor's territory or male–female chases. Although infrequent, male intrusions and male–female chases were more common in Lesser Elaenias.

The low level of extra-pair paternity in Yellow-bellied Elaenias is not simply a result of mate guarding. During nest building and egg laying, female Yellow-bellied Elaenias spent about 75% of their time on territory without the male present, and males rarely followed females off territory. Although Lesser Elaenias had a significantly higher breeding density, these flycatchers routinely commute hundreds of meters off-territory to obtain fruit. Thus, it is unlikely that the difference between species in mean nearest neighbor distance alone accounts for the difference in frequency of extra-pair matings. In addition, breeding density generally does not correlate well with frequency of EPFs in comparative studies (Griffith et al. 2002).

The near absence of extra-pair matings by Yellow-bellied Elaenias is consistent with the two other socially monogamous, permanently paired, and resident passerines studied to date in the Neotropics (Fleischer et al. 1997, Gill et al. 2005). This contrasts sharply with the high levels of extra-pair paternity typical of Neotropical migratory passerines. Neotropical migrants average a remarkable 46% of broods with EPY with 32% of the nestlings resulting from EPFs (Stutchbury et al. 2005) and off-territory forays in search of extra-pair copulations are often common (Tarof et al. 2005, Woolfenden et al. 2005). Our results build on the existing evidence from paternity studies and comparisons of testes size (Stutchbury and Morton 1995) that many tropical passerines are both socially and genetically monogamous.

Tropical species that breed relatively synchronously are expected to have extra-pair mating systems (Stutchbury et al. 1998, Carvalho et al. 2006). One way to determine if breeding synchrony influences genetic mating systems is

to compare closely related species that breed in similar habitat, but differ in breeding synchrony (Morton et al. 1998). Although a series of pair-wise congeneric tests involving a number of taxonomic groups would be ideal, even a single pair-wise comparison can be informative. For instance, Morton et al. (1998) compared the mating systems of two migratory species that breed in the same temperate zone forest, and found that Red-eyed Vireos (*Vireo olivaceus*) had both greater breeding synchrony and higher levels of extra-pair paternity than Blue-headed Vireos (*V. solitarius*). For the tropical *Elaenias* in our study, the intratropical migrant had significantly greater breeding synchrony and more EPFs.

Although occupying seasonal territories, male Lesser Elaenias did not have an unusually high singing rate for a tropical songbird (7 songs/h), and actually sang less than pairs of Yellow-belled Elaenias (16 song/h). This implies that singing rates may not be important for extra-pair mate choice in Lesser Elaenias. The possible role of dawn song in the extra-pair mating success of males needs additional study (Otter et al. 1997, Double and Cockburn 2000), particularly because male eleanias sing a distinctive song during the predawn hours. Unpaired male Lesser Elaenias sang at a high rate (168 songs/h), suggesting that song is important for social pairing.

Although intriguing, our results do not establish a causal link between breeding synchrony and extra-pair mating. The two species of elaenias we studied also differ in other characteristics that could affect extra-pair behavior, including migratory behavior, duration of the pair bond, breeding density, and the percentage of unpaired males. In Blue-black Grassquits, for instance, high levels of EPFs may be due to both territory aggregations and seasonal breeding (Carvalho et al. 2006). Further research is needed to determine the ecological and behavioral factors that contribute to differences in mating systems. Some populations of Lesser Elaenia in South America are thought to be resident (Hosner 2004), so might have lower breeding synchrony and fewer EPFs. Some populations of Yellow-bellied Elaenia near Veracruz, Mexico, are thought to be migratory (Hosner 2004), so may have higher levels of breeding synchrony and extra-pair behavior than found in our study. One way to separate the effects of migration (e.g., short-term pair bonds and territory defense, and constraints on female assessment of males) from the effects of breeding synchrony would be to compare resident populations in the same geographic area that differ in the timing of breeding due to differences in rainfall patterns (Stutchbury and Morton 2001). Although within- and between-population comparisons have not revealed consistent relationships between levels of extra-pair paternity and either breeding synchrony or breeding density in temperate areas (Griffith et al. 2002), it is not clear whether this will also prove true for tropical birds where breeding synchrony is driven by factors other than climate.

Experimental manipulation of female fertile periods is difficult to achieve in field studies, so comparative studies may be the only way to test the breeding synchrony hypothesis. One advantage of studying tropical birds is the variation in territory systems, parental care, and timing of breeding among species, even close relatives. Tropical birds also present challenges, with long breeding seasons and pairs that build nests but delay egg laying for weeks or months. High levels of nest predation and small clutch sizes result in relatively modest sample sizes for paternity analysis. Nevertheless, paternity studies in central Panama conducted to date have revealed an impressive diversity of genetic mating systems, suggesting that additional studies of tropical passerines may provide important insight into the evolution of avian mating systems.

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