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THE GENETIC MATING SYSTEM OF A TROPICAL TANAGER

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Abstract. We describe the genetic mating system of Cherrie's Tanager (*Ramphocelus costaricensis*) in southwestern Costa Rica. Our data represent the first analysis of the genetic mating system of any member of the tanager family, Thraupidae. For 59 nestlings sampled from 32 broods over two years, the social mother was the genetic mother for all but two nestlings. These two unmatched nestlings were in the only brood we observed of four young, which is double the modal brood size. We identified social fathers for 55 nestlings from 31 broods. Of these young, 49% of the nestlings in 55% of the broods resulted from extra-pair matings. Our data on Cherrie's Tanager do not support the prediction that asynchronously breeding, tropical species will have low levels of extra-pair fertilizations and highlight the need for more genetic studies of tropical species.

Key words: *Cherrie's tanager, mating system, parentage,* Ramphocelus costaricensis*, Thraupidae, tropical.*

Sistema de Apareamiento Genético de una Tángara Tropical

Resumen. Describimos el sistema de apareamiento genético de *Ramphocelus costaricensis* en el suroeste de Costa Rica. Nuestro estudio representa el primer análisis del sistema de apareamiento genético de un miembro de la familia de las tángaras (Thraupidae). De 59 polluelos pertenecientes a 32 nidadas, la madre social fue la madre genética de todos, a excepción de dos polluelos. Estos dos polluelos se encontraron en la única nidada con cuatro crías, lo cual equivale al doble de la moda del tamaño de nidada. Identificamos a los padres sociales para 55 polluelos de 31 nidadas. De estos, el 49% de los polluelos en 55% de las nidadas fueron el resultado de apareamientos extramaritales. Nuestro estudio sobre *R. costaricensis* no apoya la hipótesis de que, debido a su reproducción asincrónica, las especies neotropicales tienen niveles bajos de fertilización extramarital y resalta la necesidad de más estudios genéticos con especies tropicales.

Although monogamy is by far the most common social mating system among birds (Lack 1968), molecular analyses of parentage have revealed that strict genetic monogamy is relatively rare (Griffith et al. 2002, Westneat and Stewart 2003). Instead, the genetic mating system of most socially monogamous birds is promiscuity, with both males and females commonly mating and producing offspring with members of the opposite sex other than their social mates (Whittingham and Lifjeld 1995, Webster et al. 2001, Albrecht et al. 2007). Griffith et al. (2002) reviewed a large number of molecular genetic studies of birds and estimated that across socially monogamous species, 11% of young are extra-pair and that some extra-pair paternity occurs in 19% of broods.

Estimates of the frequency of extra-pair paternity may be geographically biased, as genetic mating systems have been studied mainly in temperate species, whereas most species of birds reside in the tropics. Stutchbury and Morton (1995) predicted that levels of extra-pair paternity would be relatively low in tropical species because breeding is relatively asynchronous in the tropics. Their logic is that greater breeding synchrony allows females to assess more males simultaneously, improving the ability of females to estimate male quality, and increasing the benefit they experience from extra-pair mating (Stutchbury and Morton 1995, Stutchbury 1998a, 1998b). Because breeding is spread over more of the year in many tropical birds, the benefits of extra-pair mating will be low for females in the tropics, and consequently so too will be the frequency of extra-pair mating. Others have criticized this logic (Weatherhead and Yezerinac 1998) and indeed have made the opposite prediction, that extra-pair mating should decrease with increasing breeding synchrony (Birkhead and Biggins 1987, Westneat et al. 1990). Empirical evidence has been mixed: some between-species comparative studies have found a positive association between breeding synchrony and extra-pair mating (Stutchbury 1998a, 1998b), whereas other comparative studies (Westneat and Sherman 1997) and many within-species studies (Griffith et al. 2002, Westneat and Mays 2005) have found no relationship. Early studies of the genetic mating systems of socially monogamous tropical birds found some support for the prediction of low levels of extra-pair paternity, at least in asynchronously breeding species (Fleischer et al. 1994, 1997, Moore et al. 1999).

Here we contribute to knowledge of extra-pair mating in socially monogamous tropical birds by using microsatellite markers to analyze the genetic mating system of Cherrie's Tanager (*Ramphocelus costaricensis*). Cherrie's Tanager is a year-round resident of the lowlands of the Pacific slope of Costa Rica and Panama. Previously, the species was subsumed in the Scarlet-rumped Tanager (*R. passerinii*) but was split from the Atlantic coast population and

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[4]Terry Krueger passed away, after a short battle with cancer, in August 2007.

The Condor, Vol. 110, Number 3, pages 559–562. ISSN 0010-5422, electronic ISSN 1938-5129. © 2008 by The Cooper Ornithological Society. All rights reserved. Please direct all requests for permission to photocopy or reproduce article content through the University of California Press's Rights and Permissions website, http://www.ucpressjournals.com/reprintInfo.asp. DOI: 10.1525/cond.2008.8546

designated as a separate species (AOU 1997) because of differences in mitochondrial genes and female plumage (Hackett 1996). The earlier name describes the appearance of adult males, whose brilliantly scarlet rumps contrast strongly with their otherwise black plumage. Females are drabber, with orange rumps, brown heads and wings, and olive backs. Skutch (1954) categorized the species as socially monogamous, based on his observation that most males attend a single female while she builds a nest and incubates, and then assist her with feeding the young. A small proportion of females feeds its young with little or no help from males, however, and Skutch (1954) thought these instances might be the result of bigamous mating. Skutch (1954) further categorized the species as nonterritorial because of the lack of aggression shown by mated pairs towards other adults near their nests. Breeding in Cherrie's Tanager is relatively asynchronous, extending over as many as seven or eight months of the year, in the populations studied by Skutch (1954) and by us (Krueger 2005), respectively.

One of the factors that best explains interspecific variation in extra-pair paternity is phylogeny: over 50% of the variation in extra-pair paternity occurs among families or orders (Griffith et al. 2002). Thus, to understand the evolution of extra-pair paternity, it is important to analyze genetic mating systems in disparate families and orders. Cherrie's Tanager is a member of the tanager family, Thraupidae, and as far as we know, this is the first analysis of the genetic mating system of any member of that family.

METHODS

Our study was conducted in the vicinity of La Gamba Biological Station, in Puntarenas Province in southwestern Costa Rica, at 8°42′N, 83°12′W. The 15 ha study site consisted of a mixture of secondary forest edge, open woodland, cattle pasture, and marsh. The population density of Cherrie's Tanagers in the study area was approximately 12 individuals per ha, and the adult sex ratio was approximately 1:1 (Krueger 2005). Fieldwork was conducted in the most active part of the 2000 and 2001 breeding seasons, from late February through mid-July in 2000, and from late January through late May in 2001.

Adults were captured using mist nets, banded with unique combinations of color bands, sampled, and released. Nestlings were sampled in the nest at 7–12 days posthatching. Blood samples (<250 µl for adults, <50 µl for nestlings) for DNA extraction were obtained via jugular venipuncture. The amounts of blood taken are below the limits recommended for nonharmful sampling based on the body mass of sampled individuals (Gaunt and Oring 1997). We observed and captured color-banded individuals in areas adjacent to nests, using the birds' locations to assign them as social parents to particular nestlings.

We extracted DNA from blood using a modified salt-extraction method (Bruford et al. 1998). DNA was diluted to ~50–100 ng per µl. All individuals in female plumage were sexed using the PCR-based approach developed by Kahn et al. (1998), since one-year-old males look similar to females. We then genotyped all individuals at seven microsatellite loci (Table 1) using the methods described in Krueger and Williams (2006). We tested all loci for Hardy-Weinberg and genotypic linkage equilibrium using GENEPOP Version 3.3 (Raymond and Rousset 1995). All seven loci were in Hardy-Weinberg equilibrium, and none of the pairwise tests between loci for linkage was significant after a Bonferroni correction for multiple tests. The average number of alleles at a locus (16.9), heterozygosity (0.82), and polymorphic information content (0.81) were high (Table 1). As a result of the high observed polymorphism, the exclusionary power of the combined loci was 0.9970 assuming no parent was known and 0.9999 assuming one parent was known. We also assessed our genotyping error rate by amplifying all loci two times in 24 individuals. All genotypes were identical, suggesting our genotyping error rate was low.

TABLE 1. Genetic diversity at seven microsatellite loci in 349 Cherrie's tanagers sampled in 2000–2001 in southwestern Costa Rica. Na indicates the number of alleles; H_O, the observed heterozygosity; H_E, the expected heterozygosity; and PIC, the polymorphic information content.

Locus	Na	H_O	H_E	PIC
RcGT257	12	0.57	0.57	0.54
RcAAAG406	10	0.86	0.85	0.84
RcAAAG410	8	0.73	0.76	0.73
Asµ15	16	0.92	0.88	0.87
RcAAT11	20	0.88	0.89	0.88
RcAAAG14	33	0.87	0.91	0.91
RcAAT12	19	0.91	0.92	0.91
Average	16.9	0.82	0.83	0.81

We used CERVUS 3.0 (Kalinowski et al. 2007), a likelihood-based program, to determine genetic parentage of the nestlings. The likelihood ratio is the ratio between the likelihood that a candidate parent is the true parent over the likelihood that the candidate parent is not the true parent, given the observed frequency of alleles in the population. The LOD score is the natural logarithm of the likelihood ratio. Positive LOD scores indicate a candidate parent is more likely to be the true parent than a randomly chosen individual, while a zero or negative number indicates the candidate is as likely or less likely to be the true parent as a randomly chosen individual. CERVUS simulates the critical difference in LOD scores (delta score) between the most-likely candidate parent and the second-most-likely candidate that is required to assign parentage at a specified confidence level. In this study, we assigned a candidate parent to a nestling if the critical difference exceeded the 95% confidence level. To simulate the critical delta scores, we assumed a genotyping error rate of 0.0184 (determined from the number of nestling-mother mismatches) and that 80% of candidate parents were sampled. First, we assigned the social mother to nestlings in each nest. We then used each assigned mother as a known candidate and 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.

RESULTS

We obtained DNA samples from 67 nestlings from 36 nests. Brood sizes were 1 (n = 7), 2 (28), or 4 (1), with mean brood size of 1.9. In 2000, we sampled 15 broods. In two cases, we did not observe a female in social association with a brood. The other 13 broods were associated with 10 females, 3 of whom were double brooded. In 2001, we sampled 21 broods. Again, we could not identify a social mother for two of the broods. The other 19 broods were associated with 17 females, 2 of whom were double-brooded. Females who were double brooded had the same social mates between broods in four of five instances.

Of the 59 young from a total of 32 broods for which we identified and sampled a social mother, the microsatellite data excluded her as the genetic mother in only one instance. In other words, the microsatellite data indicated that the female we identified from behavior as the social mother was also the genetic mother in almost every case. The one exception occurred in the single observed brood of four nestlings, which thus was likely to have been

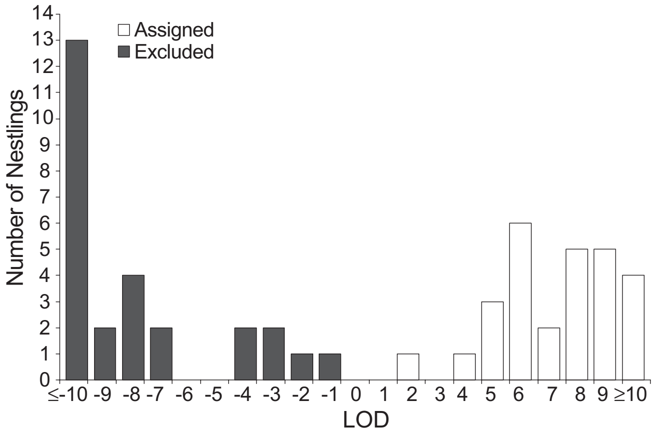

FIGURE 1. Distribution of LOD scores (natural log of the likelihood ratio), calculated in CERVUS 3.0 (Kalinowski et al. 2007), of male Cherrie's Tanagers in southwestern Costa Rica in 2000–2001. Scores are of males that were assigned genetic paternity and those that were excluded from genetic paternity for nestlings in their own nests.

produced by two females laying eggs in the same nest. Only one of the attending females was captured, and she was the most likely mother of two of the nestlings and was clearly excluded from each of the other two nestlings at a total of four mismatched loci.

Combining both years, 27 social fathers were genetically matched with 55 nestlings from 31 broods. The microsatellite data excluded the social father as the genetic sire for 27 nestlings, or 49% of the total. In 14 broods, all young were within-pair; in 12 broods, all young were extra-pair; and in five broods, paternity was mixed. Thus, some extra-pair paternity occurred in 55% of the broods for which we had data. The open analysis assigned the same social fathers as sires as the analysis using only the social pair. Social fathers that were excluded as sires mismatched young at an average of 4.00 ± 0.25 SE loci (range: 2–6) and thus all had negative LOD scores (Fig. 1). For the two males that were double brooded in 2000, both sired all the young in both of their nests. In contrast, the two males that were double brooded in 2001 did not sire any of the young in either of their nests.

We were able to assign seven extra-pair sires at the 95% confidence level to 13 of the 27 nestlings from which the social father had been excluded as the genetic sire. All of these assigned sires were sexually mature males (greater than one year of age, based on plumage). Four of these males sired a combination of extra-pair young, sometimes with multiple females, and young within social pairings, suggesting that the genetic mating system of the species is complex (Table 2). For example, male 320 sired two extra-pair young with female 322 in 2000. He then paired socially with her in 2001, siring two young with her, and one extra-pair young with female 450.

DISCUSSION

Given our sample size, the 95% confidence interval around our estimate of 49% extra-pair young is ±13%, or 36% to 62%. Although this confidence interval is fairly wide, the lower limit falls well above the mean of 11% extra-pair paternity estimated by Griffith et al. (2002) for socially monogamous species. Thus, we are justified in concluding that Cherrie's Tanager shows a high frequency of extra-pair paternity relative to other socially monogamous birds. Cherrie's Tanager has one of the highest percentages of extra-pair young described so far in a Neotropical passerine, and our data suggest that paternity patterns within a population can be highly variable, as was found in a recent study of another Neotropical species, the Blue-black Grassquit (*Volatinia jacarina*; Carvahlo et al. 2006). We also found one instance of multiple maternity within a nest, a phenomenon that Skutch (1954) also believed was occurring in rare nests he found containing four young.

TABLE 2. Patterns of paternity for male Cherrie's Tanagers siring extra-pair young in southwestern Costa Rica in 2000–2001. Numbers refer to number of young sired; zeros indicate that the paired male lost paternity to an extra-pair male. Numbers separated by commas indicate numbers of young with different females.

Year	2000		2001	
Male	Within-pair	Extra-pair	Within-pair	Extra-pair
366		1, 1	0	
314	0	1	1	
538				1, 2
320		1	2	1
305		1		
241				2
455				1

We could not calculate a quantitative index of breeding synchrony (Kempenaers 1993) for our population because we did not follow breeding through entire breeding seasons. Nevertheless, with breeding extending through as many as seven or eight months of the year (Skutch 1954, Krueger 2005), Cherrie's Tanager clearly fits the paradigm of extended seasonal breeding in tropical birds. Our results, then, do not fit well with the prediction of low extra-pair paternity in asynchronously breeding birds (Stutchbury and Morton 1995). The one data point provided by Cherrie's Tanager is not enough, however, to negate the interspecific relationship between breeding synchrony and extra-pair paternity frequency, which has been demonstrated with sample sizes as large as 34 species (Stutchbury 1998a).

Our results also do not fit well with the generalization that the frequency of extra-pair paternity is low in tropical species. This generalization has been supported by a comparative analysis of extra-pair paternity in 186 species (Spottiswoode and Møller 2004), which showed that rates of extra-pair paternity tend to increase with increasing latitude. The latitudinal trend, however, explains only a small part of the variance in extra-pair paternity (Spottiswoode and Møller 2004); a great deal of variance remains even among strictly tropical species. Thus, some monogamous tropical landbirds including Clay-colored Robins (*Turdus grayi*; Stutchbury et al. 1998), Blue-black Grassquits (Carvahlo et al. 2006), and Lesser Elaenias (*Elaenia chiriquensis*; Stutchbury et al. 2007), show high levels (>30%) of extra-pair paternity. In others, including Cactus Finches (*Geospiza scandens*; Petren et al. 1999), Capricorn Silvereyes (*Zosterops lateralis*; Robertson et al. 2001), and Monteiro's Hornbills (*Tockus monteiri*; Stanback et al. 2002), extra-pair paternity appears not to occur at all. Some of this variance may be explained by details of species' life histories. For example, the high rate of extra-pair paternity found in Cherrie's Tanager may be explained in part by the absence of territorial defense, which promotes frequent association between adults not paired with one another. At the same time, however, it may be that much of the variance in extra-pair paternity among birds is explained, not by contemporary ecology, but by past evolutionary history (Arnold and Owens 2002, Griffin et al. 2002). More genetic parentage studies of tropical species will be needed to resolve these issues.

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