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ORIGINAL ARTICLE

Multimodal flight display of a neotropical songbird predicts social pairing but not extrapair mating success

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Abstract

Models of sexual selection predict that socially monogamous females may gain direct or indirect (genetic) benefits by mating with multiple males. We addressed current hypotheses by investigating how, in the socially monogamous blue-black grassquit (*Volatinia jacarina*), male courtship and territory quality varied with social and extrapair paternity. Males of this tropical granivorous passerine exhibit multimodal displays integrating motor (leap displays) and acoustic components.

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Across 3 years, we found that extrapair paternity ranged from 8 to 34 % of all nestlings and from 11 to 47 % of all broods. Extrapair and socially paired male territories had similar seed densities. Females preferred to pair socially with males executing higher leaps, but no other male display characteristic associated with paternity loss and extrapair fertilizations. Extrapair and social mates did not differ in genetic similarity to female partners nor in inbreeding levels. Additionally, inbreeding and body condition of extrapair and within-pair nestlings did not differ. Thus, not only did we reject the direct benefits hypothesis for extrapair copulations, but our results also did not support the additive and nonadditive genetic benefits hypotheses. Instead, we found support for benefits through selection of potentially "good fathers," specifically for females that chose to pair socially with males exhibiting enhanced performance in their displays.

Significance statement

Multiple mating by females is intriguing because resulting advantages seem improbable. However, access to resources, genetic compatibility with the sexual partner and good gene transmission to the offspring are possible explanations for this behavior in several animals, including socially monogamous species. We investigated potential benefits in a socially monogamous neotropical bird, the blue-black grassquit. Males attract females using a sexual display of repeated leap flights synchronized with a song. We found that when selecting social mates, females favor higher-leaping males, an attribute associated with enhanced body condition that could indicate the capacity for better parenting and also be inherited by the offspring. Yet, when choosing extrapair males, females did not appear to base choices on leap parameters, vocal attributes, and genetic compatibility. These results do not suggest benefits for multiple mating by females, but show that selection of males in good physical condition can influence choice for social mates.

Introduction

A question of ongoing debate in evolutionary biology is why females of many socially monogamous species choose to mate with multiple males. Although polygyny can enhance reproductive success in males, sexual polyandry for females would seem to be nonadaptive primarily because, in theory, female reproductive success should increase with egg viability but not with the number of mates (Bateman 1948; Westneat and Stewart 2003; Arnqvist and Kirkpatrick 2005). One potential explanation for this conundrum is that only a few females can pair with the most attractive or preferred males (Wagner 1998), so that extrapair copulations help other females compensate for prior unfavorable choices (Gowaty 1996; review in Jennions and Petrie 2000; Simmons 2005). Adaptive benefits of mating with multiple males in this scenario have been explored and verified across a number of taxa including insects (Arnqvist and Nilsson 2000; Michalczyk et al. 2011), birds (Foerster et al. 2003; Tarvin et al. 2005; Gerlach et al. 2011; Reid et al. 2011; Varian-Ramos and Webster 2012), and mammals (Cohas et al. 2007; Nichols et al. 2015).

Benefits of mating with nonsocial pairs can be direct (nongenetic), such as when extrapair males provide resources that benefit female ability to care for their offspring, or indirect (genetic). In the latter case, genetic benefits may be associated with obtaining "good genes" for offspring, which contribute to trait evolution (additive genetic benefits). Alternatively, when females choose extrapair males with whom they are genetically more compatible, as compared to their social mates, they may increase offspring heterozygosity and avoid inbreeding depression (nonadditive genetic benefits) (review in Griffith et al. 2002; Neff and Pitcher 2005; examples in Gray 1997; Foerster et al. 2003; Arnqvist and Kirkpatrick 2005; Tarvin et al. 2005; Tryjanowski and Hromada 2005; Hoffman et al. 2007; Rubenstein 2007; Varian-Ramos and Webster 2012; Arct et al. 2015; Reid et al. 2015; Winternitz et al. 2015; see Akçay and Roughgarden 2007; Forstmeier et al. 2014; Hsu et al. 2014 for a contrary view).

In a wide range of animal taxa, male mating signals (ornaments and displays) are the main cues females use to assess potential partners relative to esthetic features or the types of benefits they might provide (Darwin 1871; Andersson 1994; Candolin 2003; Byers and Waits 2006; Byers et al. 2010). One theoretical proposal is that mating signals evolve to provide reliable indicators of a signaler quality, with only the highest quality males able to produce the most elaborate or stereotyped signals (Johnstone 1997; Searcy and Nowicki 2005). Furthermore, in some species, males have evolved multimodal signals that feature combinations of signal attributes and modalities. Such may combine intense colors, visual patterns, vocalizations, vibrational cues, chemical cues, and stereotyped exaggerated visual displays (Møller and Pomiankowski 1993; Bradbury and Vehrencamp 1998; Cooper and Goller 2004; Hebets and Papaj 2005; Partan and Marler 2005; DuVal 2007; Byers et al. 2010; O'Loghlen and Rothstein 2010a). Different components of a multimodal signal may reveal distinct signaler properties (multiple message hypothesis) or may convey overlapping information about signaler quality (redundant signal or backup signals hypothesis, Møller and Pomiankowski 1993; Johnstone 1996; Bradbury and Vehrencamp 1998; Candolin 2003).

Females might rely on different components of multimodal signals to guide social pairing versus extrapair mating. In the common yellowthroat (*Geothlypis trichas*), for example, plumage ornaments predict within-pair mating success while song consistency predicts extrapair mating success (Taff et al. 2012). Yet overall, little is known about the association in nature of multimodal display components and reproductive fitness (Elias et al. 2006; O'Loghlen and Rothstein 2010b; Barske et al. 2011; Gray et al. 2014; Lehtonen and Kvarnemo 2015; review in Candolin 2003). This lack of information is especially significant given that "selection never operates on a single trait in isolation" (p. 421, Brooks and Griffith 2010). This scarcity of empirical information constrains our capacity to evaluate multiple parameters of fitness that may influence the production of within-pair versus extrapair offspring (Møller et al. 1998; Freeman-Gallant et al. 2009; Taff et al. 2012; Grunst and Grunst 2014).

Our study focuses on male sexual displays, and their fitness correlates in a wild population of a neotropical passerine, the blue-black grassquit (*Volatinia jacarina*). This species provides an excellent opportunity to document associations between motor and acoustic display components and fitness in a socially monogamous species. Males execute two types of courtship displays: a short, buzzy vocalization while perched, and leaps coupled with a forward and reversed rotation in body axis and synchronized with the same vocalization (leap display, hereafter; Alderton 1963; Almeida and Macedo 2001; Sick 2001; Manica et al. 2013; see Online Resource 1). Male vocalization is composed invariably of a single note, lasting no more than 0.5 s, and with a striking descending pattern in frequency (from ~13 to 2 kHz, Fandiño-Mariño and Vielliard 2004, present study). Despite being acoustically complex, this sound is consistent across time, showing low within-individual variation (Manica et al. 2014, 2016) and a high level of stereotypy (Fandiño-Mariño and Vielliard 2004; Dias 2009). Because the short vocalization is a male-specific signal and intrinsic to a leap display clearly associated with female attraction and territorial defense (Almeida and Macedo 2001), we refer to it as a "song" hereafter.

During approximately 5 months of the breeding season, males settle within small-sized territories (13 to 72.5 m², Almeida and Macedo 2001) and use a few specific (two to four) perches from which to conduct displays. The male

display may demand extraordinary energy, as individuals may spend up to a half-hour leaping uninterruptedly (Manica et al. 2013). Individuals in surrounding territories may easily detect males carrying out displays, since grassquits occupy natural grasslands and human-disturbed open areas (Almeida and Macedo 2001; Sick 2001). Males conducting leap displays may also be exposed to high predation risk as well as draw the attention of nest predators searching for food within their territories (Dias et al. 2010). Display investment is somewhat biased toward nonnesting periods, but males can be detected executing leap displays even after having paired with a female (LTM and RHM, unpublished data). During the breeding season, male nuptial plumage is blue-black and iridescent with white shoulder and underwing patches that are exposed during leap displays (Sick 2001).

Males vary in all of these ornamental parameters, which in turn have been shown to correlate with measures of male health or condition. For example, coccidian parasitism negatively influenced nuptial plumage coverage as well as leap frequency in males in the wild (Costa and Macedo 2005) and display traits for males in captivity (Aguilar et al. 2008). Additionally, plumage color quality is also associated with male condition during molt (Doucet 2002). In a previous study with a small sample of grassquits (11 nests and 20 nestlings), extrapair fertilizations were recorded in 50 % of broods and 64 % of nestlings, and male pairing and nesting success were associated with leap height and leap display rate (Carvalho et al. 2006). However, the importance of acoustic and plumage display components in mate choice, especially as they relate to within-pair versus extrapair mate choice, remains unknown.

Our most general hypothesis in this study is that females will prefer elaborate male motor and acoustic display traits in both within-pair and extrapair mating contexts. Within-pair preference for elaborate males is an expectation of classic sexual selection theory (Andersson 1994; Andersson and Simmons 2006; Kokko et al. 2006). A number of factors might drive preferences for extrapair males. One such factor is that mating with extrapair males could provide females with access to more abundant or higher-quality resources (i.e., higher food density) in comparison to those available through their social mates. Blue-black grassquits are granivorous, and a seed-provisioning experiment with the species showed that extrapair females visited males in food-rich territories more frequently, and that socially paired females in such territories produced larger clutches (Dias and Macedo 2011). Females seeking extrapair mates might also gain indirect genetic benefits for their young. Choice for extrapair mates might be based upon the execution of high-performance display components, in both leaps and vocal structure, since such males might sire higher-quality offspring. Additionally, extrapair mating might improve offspring genetic heterozygosity; if so, we would expect females to be less genetically similar to extrapair males than to their social mates, and extrapair

nestlings to be less inbred, and in better body condition compared to within-pair nestlings. We tested these hypotheses by determining whether success in social pairing, loss of paternity in social broods, and extrapair paternity success are associated with male display characteristics. We also tested these hypotheses by comparing socially paired males with the extrapair males to which they lost paternity, relative to food resources in their territories, display components, genetic similarity to the female, and heterozygosity levels.

Methods

Study area and sampling

We conducted this study at Fazenda Água Limpa (15° 56′ S, 47° 56′ W), a property of the Universidade de Brasília located 28 km from Brasília, DF, Brazil, during three breeding seasons (October to February, 2008–2009, 2009–2010, and 2010–2011; years 1, 2, and 3, hereafter). Our main study site was approximately 6.56 ha, and consisted of an abandoned orchard, altered grasslands, and shrubby vegetation within a matrix of natural cerrado, i.e., tropical savanna.

Beginning in October annually, blue-black grassquits migrate from different parts of northern to central Brazil, where our field site is located and where breeding occurs. We captured birds with mist nets from 0700 hours to 1200 hours, three times weekly from October to December each year. Birds were marked with a combination of three colored plastic bands and one numbered aluminum band supplied by the Brazilian Bird Banding Agency (CEMAVE/ICMBio). From each bird, we collected, via brachial venipuncture, approximately 60 μl of blood that was then stored in Queen's lysis buffer solution (Seutin et al. 1991) and refrigerated at 4 °C.

Nest monitoring

We conducted extensive searches for nests by following birds carrying nesting material and by inspecting all potential nesting sites, especially in areas close to perches of displaying males. Once located, we monitored active nests every 2 or 3 days for the number of eggs/nestlings and for hatching and fledging dates. We collected approximately 20 μl of blood from nestlings 4 days post-hatch or older. When possible, we assigned social parents to each nest, by identifying leg bands of individuals brooding or feeding nestlings. Our parentage analyses (see below) only included social parents confirmed through direct observation. We measured nestling body mass at 1–3 days post-hatching with a spring scale (0.1 g resolution), and tarsus length with calipers (0.1 mm resolution), and calculated a body condition index as body mass divided by tarsus length (as in Costa and Macedo 2005; Aguilar et al. 2008).

Male mating displays

We conducted 30-min focal observations of territorial males, to document motor and acoustic aspects of their mating displays. Focal observations were conducted between 0700 hours and 1000 hours, during one ($n = 31$), two ($n = 13$), or three ($n = 11$) different days over a period of 2 weeks in each breeding period. Focal observations did not overlap with the nesting periods of each male to avoid sampling biased display rates (Alderton 1963; LTM and RHM, unpublished data). We recorded each occurrence of a leap display and calculated the leap display rate as the number of leaps relative to the total time of focal observation. When we monitored males in more than one observation period in the same breeding season, we used mean values of all measurements in subsequent statistical analyses.

We recorded songs of all focal males with a Marantz PMD 660 digital recorder (16-bit precision and 44.1-Hz sampling rate) coupled to either a Sennheiser K6/ME66 unidirectional or Sennheiser K6/ME62 omni-directional microphone with Telinga parabola. Recordings were made between 0700 hours and 1200 hours over one ($n = 30$), two ($n = 20$), three ($n = 4$), or four ($n = 1$) different days for each male. We selected five high-quality song samples from each bird for analysis using the software program Audacity® v. 2.0.0 (http://audacity.sourceforge.net). Using the software program Cool Edit® v. 2.1 (Syntrillium Software Corp.), we then measured song duration (ms) from oscillograms and spectrograms, and minimum and maximum frequencies (kHz) from power spectra, using a −24 dB amplitude cutoff criterion (Podos 1997). We calculated frequency bandwidth for each song as the difference between maximum and minimum frequencies.

In years 2 and 3, we also video-recorded leap displays of a subset of our focal males. In year 2, we used a mini-dv Canon XL1 digital camcorder (from which recordings were digitized using iMovie v. 7.1, Apple Inc.), and in year 3, we used a Casio digital camera EX-FH25 recording in HD. Video recordings were made between 0700 hours and 1200 hours over one ($n = 16$), two ($n = 15$), three ($n = 1$), or four ($n = 1$) different days for each male. To quantify a prominent display parameter, leap height, we identified 5 to 12 leap displays for each male (mean ± standard deviation = $9.6 ± 1.4$, $n = 316$ samples). Leap height in this population of grassquits is known to correlate with other motor display variables such as leap duration and launch velocity (Manica et al. 2016), and can thus be considered a good index of leap performance. Leap height in grassquits has also been shown to correlate with presumed measures of biological significance, including breeding status (paired versus unpaired males), parasite load, plumage condition, and molt stage (Costa and Macedo 2005; Carvalho et al. 2006; Aguilar et al. 2008; Maia and Macedo 2011; see leap height variation in the Online Resource 2). From each clip, we used the program ImageJ® v. 1.45s (Schneider et al. 2012) to measure leap height

as the distance between the beak at the peak of the leap and a horizontal line where the bird has been perched prior to the leap. We calibrated measurements from video clips to head height, a measure that was clearly visible in video frames of leaping birds and that we measured from a sample of grassquit specimens in hand (mean head height ± standard deviation = $13.5 ± 0.87$ mm, $n = 6$). We used averaged values of all male mating display traits repeatedly recorded within breeding seasons, considering that males show highly repeatable display traits (ranging from 0.36 to 0.92, Manica et al. 2016). It was not possible to record data blind because our study involved focal animals in the field.

Territory quality in food resources

To avoid disturbance of breeding activities, we estimated seed density in male territories toward the end of the season, when most nestlings had already fledged. We counted the number of stalks with seeds as well as stalks showing evidence of previous seed production (i.e., inflorescences without seeds) within four 0.5 m × 0.5 m quadrats placed 5 m from the nest in each cardinal direction (as in Manica et al. 2014). Seed density of each territory was estimated as the average of the four quadrats. Counts of grass stalks bearing seeds or seedless inflorescences offer a reliable estimate of seed density within territories, given the continuous crop throughout the rainy season.

Molecular parentage analysis

We used the Puregene® DNA Isolation Kit protocol to extract DNA from blood samples. We amplified 17 microsatellites in four groups, according to their fragment size range and dye color used (Online Resource 3), in multiplex polymerase reaction chains (PCR) using QIAGEN® Multiplex PCR kits. Multiplex PCR reactions of 10 μl contained 5 μl of 2× QIAGEN Multiplex PCR Master Mix, 2 μl of primer mix, 1 μl of 5× Q-Solution, and ~20 ng of DNA template. Final primer concentrations in primer mix solutions were 2 μM for forward and reverse and from 0.4 to 3.0 μM of fluorescently labeled forward primer WellRED™ Oligos Sigma-Aldrich® (for specific concentrations of each marker see Online Resource 3). The PCR program used was as follows: 95 °C for 15 min, 30 cycles of 94 °C for 30 s, 54 °C for 90 s, 72 °C for 90 s, and 72 °C for 10 min. We scored individual genotypes using a Beckman Coulter CEQ™ sequencer with a 400-bp size standard and deionized formamide, and CEQ 8000™ Genetic Analysis System software. We repeated sequencing analyses for 99 samples to calculate genotyping error as 3 %.

We then used the R package "MsatAllele" (Alberto 2009) to bin microsatellite fragment sizes. We tested for Hardy-Weinberg equilibrium and linkage disequilibrium using Genepop 4.1.1 (Rousset 2008) and tested for the presence of null alleles using Micro-Checker software (van Oosterhout

et al. 2004). Since birds in different breeding seasons were mostly different individuals and may have originated in different populations, we separated data for each breeding season to conduct parentage analyses. From the 17 markers amplified, *Gf05* and *ThP1-014* did not fit the assumptions of Hardy-Weinberg equilibrium, linkage disequilibrium, and less than 5 % of null alleles in any of the 3 years, and were thus excluded from final analyses, leaving 15 microsatellites to include in parentage assignments (not all of them in any one year; Table 1). The mean expected heterozygosity across all loci used in analyses of years 1, 2, and 3 was 0.61, 0.53, and 0.58, respectively, and mean observed heterozygosity was 0.61, 0.52, and 0.58, respectively (Table 1). The combined probabilities of excluding unrelated candidate parents from parentage assignments of given nestlings at all loci were 0.9837, 0.9479, and 0.9413, for years 1, 2, and 3, respectively;

when the genotype of a parent of the opposite parent sex was known, exclusion probabilities were even higher, 0.9991, 0.9950, and 0.9931, respectively (Table 1).

We then used Cervus 3.0.3 (Kalinowski et al. 2007) to assign parentage to the most likely candidate parent under relaxed (80 %) and strict (95 %) levels of confidence, by calculating the likelihood ratio scores. Critical values of these scores were estimated through simulations in Cervus using the following parameters: (i) 10,000 simulated nestling genotypes; (ii) number of candidate parents; (iii) proportion of loci typed at 98.1, 97.6, and 98.1 % for years 1, 2, and 3, respectively; (iv) genotyping error set at 3 % (see above); and (v) minimum typed loci as half of total number of loci (default parameter in Cervus). We ran two simulations, "A" and "B," that varied in numbers of candidate parents and thus in the degree to which the simulations can be regarded as

Table 1 Characteristics of 15 microsatellite loci used in maternity and paternity analyses in Cervus 3.0.3 (Kalinowski et al. 2007) for grassquits monitored during three breeding seasons

Locus	Number of alleles	Heterozygosity		NE-1P	NE-2P	P(HWE)	Null allele frequency
		H_O	H_E				
Year 1							
GF01	7	0.404	0.391	0.917	0.772	0.292	−0.022
GF12	22	0.879	0.901	0.332	0.200	0.081	0.007
GF14	8	0.219	0.259	0.964	0.852	0.050	0.059
GF16	6	0.145	0.155	0.988	0.918	0.419	0.015
TG01-148	8	0.769	0.775	0.616	0.437	0.956	0.002
TG02-088	4	0.496	0.519	0.865	0.763	0.275	0.017
TG03-002	5	0.650	0.618	0.800	0.651	0.291	−0.028
TG04-061	10	0.462	0.501	0.856	0.685	0.277	0.053
TG11-011	9	0.757	0.712	0.704	0.534	0.013	−0.039
TG13-017	15	0.755	0.754	0.622	0.439	0.012	−0.005
TG22-001	6	0.629	0.547	0.844	0.701	0.060	−0.089
VJJ13	18	0.798	0.876	0.401	0.250	0.026	0.041
Year 2							
GF01	8	0.400	0.438	0.897	0.750	0.171	0.045
GF11	10	0.503	0.513	0.864	0.742	0.282	0.015
GF12	25	0.842	0.901	0.326	0.195	0.166	0.033
GF14	8	0.148	0.166	0.986	0.911	0.032	0.045
GF16	6	0.117	0.127	0.992	0.934	0.134	0.039
TG01-148	8	0.772	0.754	0.641	0.462	0.043	−0.011
TG02-088	4	0.460	0.467	0.891	0.795	0.215	0.001
TG03-002	4	0.584	0.569	0.837	0.703	0.033	−0.020
TG03-098	9	0.650	0.771	0.623	0.444	0.004	0.078
TG04-061	10	0.588	0.574	0.804	0.623	0.593	−0.011
TG11-011	10	0.652	0.731	0.678	0.503	0.227	0.054
TG22-001	7	0.605	0.597	0.810	0.665	0.711	−0.008
Year 3							
GF11	9	0.497	0.524	0.855	0.718	0.402	0.022
GF16	5	0.088	0.085	0.996	0.956	1.000	−0.044
TG01-148	8	0.743	0.743	0.656	0.479	0.004	−0.004
TG02-088	4	0.503	0.473	0.888	0.786	0.184	−0.041
TG03-098	8	0.692	0.735	0.670	0.494	0.634	0.025
TG04-061	10	0.480	0.513	0.850	0.679	0.123	0.042
TG11-011	10	0.789	0.737	0.667	0.491	0.604	−0.040
TG22-001	6	0.568	0.520	0.861	0.735	0.198	−0.045
VJE5	29	0.835	0.949	0.196	0.109	0.000	0.058
VJJ13	15	0.882	0.891	0.362	0.220	0.063	0.002

H_O observed heterozygosity, H_E expected heterozygosity, *NE-1P* exclusion probability for one candidate parent, *NE-2P* exclusion probability for one candidate parent given the genotype of the opposite sex parent, *P(HWE)* probability of Hardy-Weinberg equilibrium

conservative. In simulation A, we used the number of individuals monitored in each breeding season, which included all banded birds video-recorded, audio-recorded, or registered in the area as breeders or floaters. These birds constituted 85.0, 82.9, and 77.3 % of females genotyped and 78, 79.4, and 67.6 % of males genotyped for years 1, 2, and 3, respectively. In simulation B, we used only the number of individuals genotyped (maternity analysis: $n = 17, 29$, and 17; paternity analysis: $n = 39, 50$, and 48 in years 1, 2, and 3, respectively).

Maternity analyses confirmed that, save a few rare cases of quasi-parasitism and intraspecific parasitism (see "Results"), all females incubating or feeding nestlings were indeed biological mothers of the tended offspring, and confirmed mothers were included in paternity analyses. We accepted all maternity and paternity assignments by Cervus at both strict (95 %) and relaxed (80 %) confidence levels when candidate parents were among the most likely parents in both simulations. When social females or males were unassigned by Cervus but were among the most likely parents in all simulations, we included an additional locus with 6 % (year 1: *Gf14*, and year 3: *VjE5*) or 8 % (year 2: *TG03-098*) of null alleles to assign parentage. Additionally, we assigned maternity and paternity to social parents, even when unassigned by Cervus, when the following conditions were met: the observed social parent was one of the two most likely mothers/fathers in all simulations; no more than one loci mismatch between the parent-nestling pair; and exhibited parentage for multiple siblings in the same nest.

Genetic similarity and inbreeding

We estimated relatedness between females and both social and extrapair males by calculating Queller and Goodnight's (1989) *r* relationship coefficient, using the program SPAGeDi 1.3 (Hardy and Vekemans 2002). We conducted analyses for each year separately, since allele frequencies of the reference populations differed across years. We also estimated *r* coefficients for 28 mother-nestling pairs (mean ± standard deviation 0.49 ± 0.10) and found no significant difference between the observed and expected *r* coefficients (*t* test: $t_{27} = 0.16, P = 0.87$), a result that supports our assignments of mothers to nestlings. We estimated heterozygosity weighted by locus (hereafter heterozygosity; Aparicio et al. 2006) to measure levels of inbreeding and individual genetic quality (Brown 1997; Amos et al. 2001). When a female had multiple extrapair partners, we used the average of her *r* coefficient and heterozygosity values in analyses.

Statistical analyses

We estimated male breeding success using three parameters: (1) success in social pairing (pairing success), which measured whether a male attracted a mate and built a nest in which at least one egg was laid; (2) loss of paternity of at least one nestling in his social brood (within-pair paternity (WPP) loss); and (3) success in siring an extrapair nestling (EPP gain). We used logistic regressions with a complementary log-log link function to test for associations between motor and acoustic display variables and pairing success, WPP loss, and EPP gain. Our models included the predictor variables: leap height, leap display rate, song duration, and frequency bandwidth; we also included the covariate "year" to control for possible differences among breeding seasons. We found no evidence of collinearity among predictors in models, as shown by variance inflation factors lower than 3 and correlation coefficients among pairs of predictors lower than 0.50 or higher than −0.50 (Zuur et al. 2010). All continuous predictors showed normal distributions, except for leap display rate, which was log-transformed to achieve or approximate to a normal distribution. All models were validated through goodness-of-fit tests. Few males were repeatedly sampled in multiple years ($n = 11$); thus, our sample size was small to test for within-individual variation in breeding success over the years. Furthermore, we lacked complete data of motor and acoustic display variables for seven of these males. Therefore, when an individual was sampled in more than one breeding season, we used data only from the year for which we had the most complete information.

We calculated second-order Akaike's information criteria (AICc), using the R package AICcmodavg (Mazerolle 2010), to identify the models that best predicted male breeding success, based upon the three parameters defined above (Burnham and Anderson 2002). We fitted a set of candidate models using the following combinations of predictor (input) variables: (a) all variables, (b) each variable alone, (c) only variables within a single sensorial modality (acoustic: song duration and frequency bandwidth; motor: leap display rate and leap height) including year as a covariate or not, and (d) an intercept-only model (null model). As in Burnham et al. (2011), models with ΔAICc ≥14 were considered as empirically implausible, 14 > ΔAICc ≥ 7 as models with relatively little support, 7 > ΔAICc ≥ 4 as models with moderate support, and ΔAICc <4 as models both plausible and supported by empirical evidence. The relative ranking of models based on ΔAICc values also provides an estimate of each model's relative explanatory value. We also estimated the strength of evidence for each model by calculating Akaike weights (w_i, the probability that model i is the best model) and evidence ratios (ER) between any two models (ratio of w_i of one model against the other, Burnham et al. 2011). When model selection uncertainty was high, i.e., when w_i of the most plausible model was lower than 0.90, we used model averaging to draw inferences about the importance of predictors in the most plausible models. Toward this aim, we calculated weighted averages of beta parameters for predictors across all the models using model probabilities as weight, and unconditional

standard errors and 95 % confidence intervals (CI) of each predictor (Burnham and Anderson 2002). We applied logarithm transformation to leap display rates in all models to achieve normalization or to approximate a normal distribution.

We used *t* tests to assess differences between social males and extrapair males to which he lost paternity, in relation to territorial seed density, song duration and frequency bandwidth, leap height, leap display rate, relatedness with female, and heterozygosity. We compared relatedness between females and all males in the population with relatedness between females and social males or extrapair males using a Wilcoxon test. We next compared within-pair and extrapair nestlings' heterozygosity and body condition indices (body mass divided by tarsus length) with a paired *t* test. When our comparisons included more than one within-pair or extrapair nestling from the same nest, we first averaged values within each category for each nest. All statistical analyses were performed in R (R Core Team 2014) with the statistical significance threshold set at 0.05. All values are presented as mean ± standard deviation, unless noted otherwise.

Results

Parentage analysis

During the three breeding seasons, we monitored 174 grassquit nests. From 131 of these, we collected genetic material of 280 nestlings for parentage analyses. Field observations allowed us to identify social mothers of 126 nestlings from 56 nests and social fathers of 208 nestlings from 95 nests (for 48 of these nestlings we identified both social mothers and fathers). We were able to assign, with confidence, genetic maternity to 119 of the 126 (94.4 %) social mother-nestling pairs. The remaining unmatched nestlings ($n = 7$) were found in five nests (Table 2). For one of these nests, the female was not the genetic mother of any nestling in her nest, while in the other four nests, females were the genetic mothers of at least one nestling in the nest (Table 2). For the seven unmatched nestlings, we interpret four as having resulted from quasi-parasitism, in which social males were the nestlings' biological fathers, and three as resulting from intraspecific brood parasitism, in which neither the social fathers nor mothers had sired the nestlings. Among these three latter cases, we identified two biological fathers: one was probably a floater, not registered breeding or displaying in the study area, and the other had established a territory and nested approximately 50 m from the parasitized nest that contained his nestling.

We also attempted to produce maternity assignments of 154 additional nestlings from 75 nests with unknown social mothers. Of these, we were able to assign maternity with confidence to eight nests, data that were later included in the paternity analysis.

We found that social fathers were also genetic fathers for 164 of 208 nestlings. For the remaining 44 nestlings, we identified 20 extrapair genetic fathers (Table 2). Thirteen extrapair fathers were socially mated to other females, and eight of these had sired at least one within-pair nestling in their own nests. Extrapair paternity rates varied greatly among years, ranging from 8.2 to 34.7 % of all nestlings and 11.4 to 47.1 % of all broods analyzed (Table 2). The second breeding season (year 2) had the highest extrapair paternity rate, and the third season (year 3) the lowest rate (Table 2). Complete loss of paternity within a single brood occurred for social males in 13.7 % of nests (44.8 % of broods with extrapair nestlings), but social males sired at least one nestling in the nest in 16.8 % of nests monitored (55.2 % of broods with extrapair nestlings).

Display correlates of male breeding success

We associated motor and acoustic display traits with breeding success for 35 males, using different subsets of individuals for each display parameter given that we lacked complete display datasets for some individuals.

Pairing success We analyzed social pairing success for 31 males (year 2 = 16 and year 3 = 15). Parameters that predicted pairing success with substantial empirical evidence (ΔAICc < 4) included leap display rate, leap height, frequency bandwidth, and year (Table 3; sum of $w_i = 0.93$). Within this array, we found the strongest evidence for the predictive value of leap height, as supported by five considerations. First, although the null model was classified as plausible, it had a low probability ($w_i = 0.067$, Table 3) and strength of evidence almost four and three times lower than those of the two best models (ER = 4.28 and 3.21, respectively). Second, the removal of leap display rate and year from the model with the lowest AICc, thus retaining leap height only, barely changed model fit (ΔAICc = 0.58; Table 3). Third, when considering model selection uncertainty and calculating averaged estimates of predictors, leap height was the only variable with the strength of evidence to predict pairing success. This is due to leap height values being positively biased ($\beta_{height} = 0.71 \pm 0.36$, CI = −0.002, 1.42, Fig. 1) in contrast with the broader CI of estimates for leap display rate ($\beta_{rate} = -0.50 \pm 0.32$, CI = −1.14, 0.14) and year (estimate for the third breeding season relative to the second breeding season: $\beta_{year3} = 1.08 \pm 0.69$, CI = −0.27, 2.43). Fourth, after calculating ER between the two best models and the model including frequency bandwidth (5.63 and 4.22, respectively), we concluded that this predictor also had reduced support in explaining pairing success ($\beta_{freq.band} = 0.30 \pm 0.29$, CI = −0.27, 0.88). Fifth, the predictor song duration was

Table 2 Extrapair paternity and maternity rates

Analysis type/period	Nestlings analyzed	EP (%)	Broods analyzed	Broods with EP nestlings (%)		
				Complete EP broods	Mixed broods (EP + WP)	Total
Maternity analyses						
Year 1	29	1 (3.4)	14	0 (0.0)	1 (7.1)	1 (7.1)
Year 2	51	4 (7.8)	23	1 (4.3)	2 (8.9)	3 (13.0)
Year 3	46	2 (4.3)	19	0 (0.0)	1 (5.3)	1 (5.3)
Total	126	7 (5.5)	56	1 (1.8)	4 (7.1)	5 (8.9)
Paternity analyses						
Year 1	51	12 (23.5)	26	4 (15.4)	5 (19.2)	9 (34.6)
Year 2	72	25 (34.7)	34	7 (20.6)	9 (26.5)	16 (47.1)
Year 3	85	7 (8.2)	35	2 (5.7)	2 (5.7)	4 (11.4)
Total	208	44 (21.1)	95	13 (13.7)	16 (16.8)	29 (30.5)

EP extrapair nestling, *WP* within-pair nestling

included only in models with moderate or little support, and its model-averaged estimate ($\beta_{\text{song duration}} = 0.10 \pm 0.29$, CI = −0.47, 0.67) was thus considered weak as a predictor in models.

Within-pair paternity loss No male display parameters predicted whether males lost paternity in their social broods (WPP loss: year 2 = 12 males, year 3 = 11 males; Table 4). More specifically, while the entire candidate set is considered empirically plausible, except for the full model (ΔAICc = 6.28, Table 4), the two best models including song characteristics

Table 3 Model selection for male probability of pairing with a female (pairing success) in relation to display traits

Models[a]	K	AICc	ΔAICc	w_i
Leap rate + height + year	4	32.33	0.00	0.287
Height	2	32.91	0.58	0.215
Leap rate + height	3	33.21	0.88	0.185
Leap rate	2	34.85	2.52	0.081
Null	1	35.26	2.92	0.067
Fband	2	35.77	3.44	0.051
Year	2	36.08	3.75	0.044
Sdur	2	36.91	4.57	0.029
Leap rate + height + sdur + fband + year	6	38.07	5.74	0.016
Sdur + Fband	3	38.19	5.86	0.015
Sdur + Fband + year	4	39.38	7.05	0.008

K number of parameters, *AICc* second-order Akaike's information criteria, w_i Akaike's weight

[a] Explanatory variables: leap rate = number of leap displays per unit time; height = leap height; sdur: song duration, fband: frequency bandwidth; and year = 2009–2010 and 2010–2011 breeding seasons

provided little empirical support, as their strength of evidence was only 1.34 and 1.09 times greater than the null model (Table 4). Song duration and frequency bandwidth also had wide CI and low effect sizes ($\beta_{\text{song duration}} = 0.90 \pm 0.65$, CI = −0.38, 2.19; $\beta_{\text{freq.band.}} = -0.95 \pm 0.54$, CI = −2.01, 0.11). Leap display rate and leap height were only included in models with low probability (0.098 and 0.078, respectively). Also, model-averaged estimates of motor display traits had CIs encompassing zero ($\beta_{\text{height}} = 0.65 \pm 0.46$, CI = −0.26, 1.56; $\beta_{\text{rate}} = 0.79 \pm 0.56$, CI = −0.31, 1.89), indicating that their value in predicting paternity loss was weak. We found a low variation in the probabilities of WPP loss across years since the best model including this predictor had a low probability (0.105, Table 4) and since the model-averaged estimate of this predictor had wide CI ($\beta_{\text{year3}} = -1.57 \pm 1.18$, CI = −3.90, 0.75, estimate relative to the second breeding season).

Extrapair paternity gain Evidence was also weak for a relationship between display parameters and EPP gain for 21 males (year 1, $n = 4$ males; year 2, $n = 17$ males, Table 5). The null model ranked lowest in AICc values and showed a probability of 0.349 considering all models. All model-averaged estimates also indicated only weak contributions of display predictors, as follows: $\beta_{\text{height}} = 0.12 \pm 0.36$, CI = −0.59, 0.82; $\beta_{\text{rate}} = -0.18 \pm 0.36$, CI = −0.89, 0.53; $\beta_{\text{song duration}} = -0.25 \pm 0.38$, CI = −0.99, 0.49; $\beta_{\text{freq.band.}} = 0.09 \pm 0.37$, CI = −0.65, 0.82. These results indicate that the probability of extrapair fertilization was not strongly associated with any motor or acoustic display traits we measured. Although year was included in a model with ΔAICc <4, model probability was low (0.118) and model-averaged estimate indicated its weak importance as a predictor in models ($\beta_{\text{year2}} = -0.46 \pm 0.84$, CI = −2.12, 1.19, estimate relative to the first breeding season).
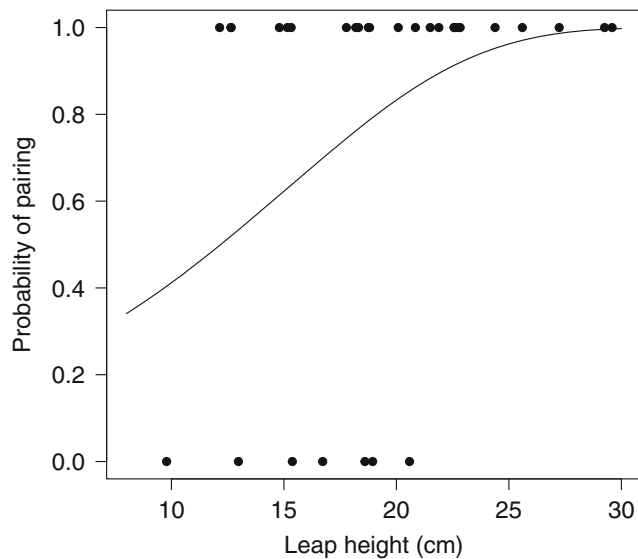
Fig. 1 Probability of blue-black grassquit males pairing socially with a female (and having at least one nesting attempt) as a function of that male's average leap height during leap displays. The *points* represent observed values, and the *line* represents a fitted function of a GLM with a binomial distribution

Pairwise contrasts between socially paired versus extrapair males

We found no evidence of higher seed density in extrapair male territories as compared to social male territories (Table 6). Extrapair and social males did not differ in song duration, frequency bandwidth, leap height, or leap display rate (Table 6).

Table 4 Model selection for male probability of losing paternity in the social brood (WPP loss) in relation to display traits

Models[a]	K	AICc	ΔAICc	w_i
Sdur + fband	3	25.70	0.00	0.159
Fband	2	26.11	0.41	0.130
Null	1	26.28	0.57	0.119
Sdur + fband + year	4	26.53	0.83	0.105
Year	2	26.58	0.88	0.102
Leap rate	2	26.67	0.96	0.098
Height	2	27.12	1.42	0.078
Sdur	2	27.15	1.44	0.077
Leap rate + height	3	27.43	1.73	0.067
Leap rate + height + year	4	27.67	1.97	0.059
Leap rate + height + sdur + fband + year	6	31.98	6.28	0.007

K number of parameters, *AICc* second-order Akaike's information criteria, w_i Akaike's weight

[a] Explanatory variables: leap rate = number of leap displays per unit time; height = leap height; sdur: song duration, fband: frequency bandwidth; and year = 2009–2010 and 2010–2011 breeding seasons

Table 5 Model selection for male probability of siring extrapair nestling (EPP gain) in relation to display traits

Models[a]	K	AICc	ΔAICc	w_i
Null	1	30.12	0.00	0.349
Sdur	2	32.15	2.03	0.126
Year	2	32.29	2.17	0.118
Leap rate	2	32.34	2.22	0.115
Height	2	32.47	2.35	0.108
Fband	2	32.54	2.42	0.104
Sdur + fband	3	34.76	4.64	0.034
Leap rate + height	3	35.02	4.90	0.030
Leap rate + height + year	4	37.65	7.53	0.008
Sdur + fband + year	4	37.78	7.65	0.008
Leap rate + height + sdur + fband + year	6	44.90	14.78	0.000

K number of parameters, *AICc* second-order Akaike's information criteria, w_i Akaike's weight

[a] Explanatory variables: leap rate = number of leap displays per unit time; height = leap height; sdur: song duration, fband: frequency bandwidth; and year = 2008–2009 and 2009–2010 breeding seasons

Genetic similarity and heterozygosity among adults

Genetic similarity of females and their social mates did not differ between broods with extrapair nestlings ($r = 0.0006 \pm 0.20$) and broods with within-pair nestlings only ($r = 0.02 \pm 0.20$, $t_{47} = 0.39$, $P = 0.70$). Females and their social mates were not more closely related to each other than were females and extrapair males (Table 6). In three cases, we found that extrapair males were closely related to females with whom they mated ($r = 0.55$, 0.42, and 0.43). Relatedness values between females and social males that lost paternity and between females and extrapair partners were similar to relatedness values between females and all males in the population ($r_{\text{social male}} = 0.02 \pm 0.19$, $r_{\text{population}} = -0.005 \pm 0.08$, $W = 60$, $P = 0.66$, $n = 15$; $r_{\text{EP male}} = 0.07 \pm 0.23$, $r_{\text{population}} = 0.005 \pm 0.10$, $W = 32$, $P = 0.30$, $n = 9$). Extrapair and social males were equally heterozygous (Table 6), as were mothers of broods with within-pair nestlings only (0.36 ± 0.12) when compared with mothers of broods with extrapair nestlings (0.37 ± 0.15, $t_{46} = -0.34$, $P = 0.73$).

Nestling characteristics

Extrapair and within-pair nestlings did not differ in heterozygosity (EPY 0.35 ± 0.11 and WPY 0.32 ± 0.17, respectively, $t_{16} = 0.56$, $P = 0.58$). Extrapair and their within-pair half-sibs did not differ in body condition (extrapair 0.34 ± 0.08 g/mm and within-pair nestling 0.31 ± 0.05 g/mm, mean of the differences = -0.03, $t_{12} = 1.39$, $P = 0.19$).

Table 6 Pairwise comparisons of quality traits in social versus extrapair males

Trait	Social pair Mean (SD)	Extrapair Mean (SD)	n (male pairs)	t	P value
Territory					
Seed density (stalks/m^2)	62.5 ± 33.7	58.9 ± 36.3	7	0.22	0.83
Song					
Duration (s)	0.40 ± 0.02	0.39 ± 0.05	6	0.54	0.61
Frequency bandwidth (kHz)	7.30 ± 0.43	6.90 ± 0.90	6	0.74	0.50
Motor display					
Leap height (cm)	21.8 ± 8.2	21.2 ± 5.7	3	0.12	0.91
Leap display rate (leaps/min)	6.22 ± 4.24	11.00 ± 1.72	3	3.26	0.08
Genetic parameters					
Relatedness with female (r)	0.01 ± 0.23	0.07 ± 0.21	9	0.62	0.55
Heterozygosity (HL)	0.33 ± 0.16	0.31 ± 0.17	9	0.22	0.83

Discussion

Extrapair paternity rates are markedly high in blue-black grassquits (30.5 % of broods) relative to socially monogamous passerines in general (average of 18.7 % of broods, Griffith et al. 2002). Such frequent extrapair copulation behavior is puzzling, and in this study, we investigated this issue using an adaptive framework. We designed our study to test prevailing hypotheses that suggest that female preference could be based upon indirect (genetic) or direct benefits (territorial resources). With regard to indirect benefits, we further subdivided the category into female preference based upon male attributes potentially signaling good genes (additive genetic effects) or genetic compatibility (nonadditive genetic effects). We were able to exclude several alternative predictions of these classical hypotheses but found support for direct benefits through pairing with potentially "good fathers," in that our data show that females chose to pair socially with males exhibiting enhanced performance in their motor displays. Specifically, females chose to pair socially with males exhibiting higher leaps.

One prediction of the indirect genetic benefits hypothesis is that females would prefer social or extrapair mates exhibiting cues indicative of better physical condition. Given that a multimodal display may easily transmit information of different types, we broke down the display into several components. In our analyses, we thus considered rates and height of the leap display as well as acoustic traits as potential targets of female choice, a pattern documented for several species (Byers et al. 2010; O'Loghlen and Rothstein 2010b; Barske et al. 2011; Lukianchuk and Doucet 2014).

Our data revealed that males with higher leaps were indeed more successful in forming social bonds with females, consistent with previous preliminary findings for this species (Carvalho et al. 2006). Preference for social males exhibiting higher leaps suggests females benefit directly, but not indirectly through transmission of "good genes" to the offspring, because they were not as selective when seeking for extrapair partners. Female choosiness could have direct implications for their fitness, since superior leaping ability in males may indicate older, vigorous, and healthy social partners that may be able to provide females with suitable assistance, for example, by defending the nest as well as females against predators (Almeida and Macedo 2001). Thus, males leaping higher may indicate a high probability of developing into good social partners and fathers in the future. Evidence for covariance between male attractiveness and paternal feeding effort in blue-black grassquit exists only for plumage coloration (Diniz et al. 2015). In addition, it is important to highlight the fact that while motor performance traits and song may be intrinsically associated with genetic quality of males, such traits may be influenced by other factors. Bird song, for example, has been shown to be affected by developmental stress (Nowicki et al. 1998) and motor performance in courtship displays might be positively affected by age.

By contrast, the limited predictive value of leap display rate in pairing success suggests that this trait, in isolation, may not necessarily signal relevant male properties for social bonding or extrapair mating. We highlight, however, that further studies investigating female assessment of more detailed aspects of the motor display may bring new insights about mate choice in blue-black grassquits. We have also shown trade-offs among leap attributes and an influence of male body condition upon motor displays (Manica et al. 2016). Future studies with larger sample sizes associating such trade-offs with male breeding success will allow us to assess whether the combination of these attributes is under female choice. Our examination of the acoustic display also suggested a weak importance in the mating context, which is puzzling, given that male song is a key target for mate choice in many bird species (Andersson 1994; Catchpole and Slater 2008). Blue-black grassquit song structure is unique: males produce acoustically complex elements during a single

expiratory movement (Fandiño-Mariño and Vielliard 2004). It is possible that song duration or frequency bandwidth do not challenge male performance capacities and are thus unrelated to their health or general condition. Alternatively, song traits may offer redundant information at a weaker level relative to male condition. Further studies on the importance of acoustic elements of the single note (as detailed in Fandiño-Mariño and Vielliard 2004) could also bring new insights relative to song relevance for mate choice.

We also tested for another hypothetical genetic benefit for extrapair mating in the blue-black grassquit: the genetic similarity between mates and the inbreeding level of adults and offspring. In contrast to findings for a few other species (Tarvin et al. 2005; Whittingham and Dunn 2010; Varian-Ramos and Webster 2012), we found no evidence supporting potential advantages of mating with more dissimilar or more heterozygous males. Social and extrapair males did not differ in relatedness to females, nor when compared with the average relatedness of all males in the population. Moreover, extrapair and social males did not differ in heterozygosity, and broods of females with and without extrapair nestlings did not differ in terms of heterozygosity or body condition.

Our results suggest that potential inbreeding is not a factor that influences mate choice in the blue-black grassquit, and that extrapair copulation is not a strategy to increase offspring heterozygosity or condition (at least during the nestling period). Inbreeding is likely to occur when natal dispersal is low or when opportunities for mate choice are restricted (Cockburn et al. 2003; Foerster et al. 2003; Tarvin et al. 2005) and affect population persistence (Keller and Waller 2002). We suspect, therefore, that females in our studied group are not constrained when seeking less inbred males. Alternatively, when inbreeding does occur, offspring fitness may not necessarily be reduced, and no selection pressure would favor copulations between less genetically similar partners.

Results of studies testing indirect benefits of mate choice through non-additive effects have been contradictory. Since the landmark Griffith et al. (2002) review, numerous studies have failed to support the "compatible genes" hypothesis (e.g., Hsu et al. 2015). Additional study cases include the house wren (*Troglodytes aedon*), in which extrapair nestlings did not have greater immune responsiveness than their within-pair half siblings (Forsman et al. 2008), and the song sparrow (*Melospiza melodia*), in which extrapair males were not more heterozygous or less related to the female (Hill et al. 2011). Yet, Schmoll et al. (2009) showed an opposite pattern in which within-pair offspring had higher lifetime numbers of broods and social offspring than their extrapair counterparts.

An alternative and very recent genetic explanation for polyandry in socially monogamous species is that female extrapair mating behavior is not adaptive at all but may evolve instead through sexual conflict, since multiple matings may enhance male fitness resulting in positive selection of alleles for this behavior (Forstmeier et al. 2014; Hsu et al. 2015). However, longer-term quantitative investigations of extrapair nestling fitness are necessary to fully consider the contributions of additive and nonadditive genetic benefits (e.g., Reid et al. 2015) or an alternative nonadaptive explanation. Most studies to date consider only the very short-term fitness benefits seen during the nesting stage.

We predicted that if females gain direct benefits from extrapair copulations, they would seek extrapair males in territories with higher food resources. High seed abundance, which may be indicated by male singing rates (Manica et al. 2014), indeed appears to favor high fertility in female blue-black grassquits (Dias and Macedo 2011). As such, polyandrous behavior could be explained by such direct benefits leading to higher female fitness. However, our results do not support this hypothesis, as seed density was equivalent in the territories of the extrapair and social males. Among other possible explanations, the constraint on food availability may be negligible in our study area such that the amount of seed in social partner territories suffices for adult maintenance during breeding. Alternatively, females may have unlimited access to neighboring territories regardless of whether they copulate with the territory owner. Ultimately, to understand the importance of resource benefits in the polyandrous behavior of females, it would be desirable to track female movements and food exploitation in extrapair male territories.

In conclusion, our data suggest that females may accrue benefits through a social alliance with partners in good physical condition. Our results also provide indirect evidence in favor of the "multiple message hypothesis" (Møller and Pomiankowski 1993), because females may be using different cues to evaluate particular properties of males, whether in a social or extrapair context. Laboratory or field studies that focus on single attributes to test for fitness effects through mate choice may be severely constrained in the interpretation of results. Apparently, females are exquisitely sensitive to subtle cues encoded in male displays and can use those cues to guide their decisions about mating.

Compliance with ethical standards

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