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Dangerous love? Predation risk does not affect female mate choice in blue-black grassquits

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Abstract

Predation risk may be an important factor affecting female mate choice. Hypothetically, females could choose extravagantly ornamented males that survive in high predation risk environments. However, this decision could be different if choosing a conspicuous male under high predation risk is costly for females or results in reduced offspring survival. In such contexts, females could become indifferent to male quality or prefer inconspicuous males. We tested this idea using captive blue-black grassquits (*Volatinia jacarina*, Linnaeus, 1766), a species in which males perform conspicuous leap displays coupled with songs during the breeding season, which presumably subjects females and offspring to higher predation risk. Females were placed in an arena with speakers on opposite sides emitting male courtship songs. One speaker emitted songs at a high rate (proxy for a conspicuous male) while the other speaker broadcast songs at a low rate (proxy for a less conspicuous male). While the female evaluated the two male songs, a third speaker emitted vocalizations characterizing three levels of risk: adult predator, nest predator, and no-risk control. Females showed no preference for either male stimuli across the predation risk treatments. This lack of preference relative to frequency of male vocal displays suggests that leap-song frequency is not used by females during mate choice. We suggest that in addition to its role in courtship, male grassquit displays also signal status to other males when competing for territories. Thus, we propose that predation risk does not directly influence blue-black grassquit intersexual selection and that females in this species may exercise indirect mate choice, choosing social mates based on male ability to establish and defend a territory, and relying secondarily upon other aspects of male display attributes, such as its visual components.

KEYWORDS
indirect mate choice, intersexual selection, multimodal display, passive mate choice, survival cost, *Volatinia jacarina*

1 | INTRODUCTION

The intersexual component of sexual selection commonly ascribes to females the role of choosing among males the one that will sire their offspring (Darwin, 1871). Female mate choice may depend on a myriad of intrinsic factors, such as genotype (Brooks & Endler, 2001), hormones (Lynch, Crews, Ryan, & Wilczynski, 2006), sensory capabilities (Ronald, Fernández-Juricic, & Lucas, 2012), physical condition (Hunt, Brooks, & Jennions, 2005), as well as environmental factors including the social environment (Madden & Whiteside,

2013), and the climate (Conrad, Stocker, & Ayasse, 2017). Predation risk may also influence female mate choice via two distinct possibilities. First, in risky environments, females may prefer males that present elaborate ornaments and conspicuous displays, since such males are able to do so even at the risk of drawing predator attention to themselves. This is the case of green swordtails (*Xiphophorus helleri*), a species where females prefer males with elongated caudal fins (also known as swords; MacLaren, 2017). However, this trait also increases male predation risk as predators direct more attacks toward individuals with intact swords when compared with those that had their swords experimentally removed (Hernandez-Jimenez & Rios-Cardenas, 2012). In scenarios like this one, males that survive in a risky environment bearing detrimental but striking ornaments probably have high-quality genes (Zahavi, 1975). Thus, females that choose to mate with conspicuous males may increase the survival probability of their offspring by indirectly selecting genes that encode for traits that increase predator evasion.

A different scenario predicts that females may suffer a high survival cost by associating with conspicuous males (Marzal et al., 2016). However, there are few examples of avian studies assessing this female predicament during mate choice. Most avian examples in the literature concern the effect of predation risk on lek dynamics (Boyko, Gibson, & Lucas, 2004), nest site selection and clutch size (Eggers, Griesser, Nystrand, & Ekman, 2006), and parental care (Ghalambor & Martin, 2000; Massaro, Starling-Windhof, Briskie, & Martin, 2008). In other taxa, there are many more experimental studies assessing the cost for females of choosing conspicuous males in varying levels of predation risk. Female guppies (*Poecilia reticulata*), for example, have a strong preference for conspicuous ornaments displayed by males in risk-free environments, but they decrease sexual activity and choose their mates randomly under high predation risk (Godin & Briggs, 1996). Female green swordtails may go even further and completely avoid conspicuous males in risky scenarios, preferring less ornamented mates (Johnson & Basolo, 2003). Females of a different swordtail species (*Xiphophorus birchmanni*) may also show no preference between conspecific and heterospecific (*Xiphophorus malinche*) males in risky environments (Willis, Rosenthal, & Ryan, 2012), possibly bearing the reproductive costs of producing hybrids to increase their own survival chances.

Sexual selection through female choice may enhance conspicuousness of a signal (Darwin, 1871), but the above examples illustrate that natural selection may drive signal evolution in the opposite direction if females associate with less conspicuous males under high predation risk (Godin & Briggs, 1996; Johnson & Basolo, 2003). Given the natural diversity of forms and behaviors used by males for mate attraction across different taxa, it is important to ask how females from different species modify their mate choice under different levels of predation risk.

To develop this concept, we used a Neotropical bird, the blue-black grassquit (*Volatinia jacarina*, Linnaeus, 1766), which exhibits an array of traits that make it an excellent subject with which to explore questions about predation risk and mate choice. This socially monogamous passerine has elevated rates of extrapair fertilization associated with a complex repertoire of courtship behaviors and ornaments (Macedo, Manica, & Dias, 2012; Manica, Graves, Podos, & Macedo, 2016), and breeds in regions of high levels of predation risk (Diniz, Ramos, & Macedo, 2015; Macedo et al., 2012; Skutch, 1985). During the breeding season, grassquit males molt from a brownish cryptic plumage to a blue-black iridescent nuptial plumage (Maia & Macedo, 2011), which contrasts sharply with the savanna vegetation of their breeding grounds (Sicsú, Manica, Maia, & Macedo, 2013). Additionally, males perform long bouts of a multimodal display consisting of repeated leaps that include high-speed wingbeats, and the emission of a high-pitched song at the peak of the leap (Macedo et al., 2012). This sexual display is emitted throughout the day and is more frequently performed when sunlight shines directly over the male's territory (Sicsú et al., 2013). Males maintain their sexual displays even after establishing a socially monogamous breeding pair (Macedo et al., 2012), probably to attract neighboring females to obtain extrapair copulations (Carvalho, Macedo, & Graves, 2006; Manica et al., 2016). Males perform these conspicuous displays despite having an active nest in their territories, and this has been shown to increase predation risk for nests (Dias, Castilho, & Macedo, 2010).

Because females associate with males for most of the breeding season (Carvalho, Macedo, & Graves, 2007), predator attention that males draw to themselves and their nests may possibly also affect females. This raises the question of whether grassquit females benefit by associating with vigorous and conspicuous males (e.g., by ensuring that their offspring will inherit "good genes"), or whether this association may be costly in situations of high predation risk, wherein the female and her offspring are at a greater risk due to intense male sexual displays. Previous studies showed that grassquit females do not consider male territory quality and resource availability (Almeida & Macedo, 2001) or even male health (Aguilar, Maia, Santos, & Macedo, 2007) during mate choice. However, we still do not know how females choose a mate in different or suboptimal contexts such as immediate perceived threat for females and offspring vs. low-risk situations.

Therefore, in this study we experimentally tested whether blue-black grassquit females modify mate choice preferences under different predation risk regimes. We simulated different levels of predation risk and used a simple mate choice experiment to test this hypothesis, predicting three possible outcomes from our experiment (Table 1). The first outcome assumes that predation risk has no influence in female mate choice and, therefore, female preference (if such occurs) should be similar in situations of low and high predation risk (i.e., female presents the same behavioral pattern across all treatments). The second outcome assumes that females benefit from choosing conspicuous males as their mates when predation risk is high. Males that perform intense sexual displays in situations of greater predation risk are probably of higher quality and should be chosen by females (Zahavi, 1975), despite the possible costs associated with the risk of predation. In this scenario, females should favor conspicuous males in situations of greater risk (simulation of a predator of adult birds) than

TABLE 1 Summary of possible female mate preference outcomes resulting from the experiments in this study. Blue-black grassquit females were used as experimental subjects

Outcome	Experimental treatment	Male conspicuousness	
		High	Low
Predation risk has no effect on female mate choice	Predation risk	− or +	−
	Control	− or +	−
Associating with conspicuous males: greater reproductive benefits to females[a]	Predation risk	++	−
	Control	+	−
Associating with conspicuous males: greater survival costs to females and offspring[b]	Predation risk	−	− or +
	Control	+	−

Notes. Within each experimental treatment context, females would be expected to favor males strongly (++), moderately (+) or not at all (−), as assessed by time spent in close proximity with the specific male stimulus (high or low song rate).
[a]Based on Zahavi (1975). [b]Based on Dias et al. (2010) and Marzal et al. (2016).

in situations of no risk (control treatment). The third outcome assumes that females do not benefit from choosing conspicuous males in high predation risk environments. The greater reproductive costs endured by females with the increased predation risk to themselves (Marzal et al., 2016), and their offspring (Dias et al., 2010) cancel the benefits of pairing with a conspicuous male. In this scenario, females should favor conspicuous males during the no-risk control treatment (assuming that male conspicuousness is sexually selected by females in optimal conditions—Godin & Briggs, 1996) and in contrast, should either be non-responsive to male display conspicuousness or show a greater response to less conspicuous males when predation risk is high (simulation of adult and/or nest predators).

2 | METHODS

2.1 | Study subjects and aviary maintenance protocol

Blue-black grassquits are small, granivorous passerines that breed in central Brazil during the local rainy season (Nov.–Apr.; Carvalho et al., 2007). After establishing their territories, males perform conspicuous multimodal displays for most of the day; natural variation in display rate ranges from 5.0 to 20.8 displays/min ($\bar{x} \pm SD$; 14.2 ± 3.2; Manica, Maia, Dias, Podos, & Macedo, 2014). With the establishment of the breeding pair, both parents build an open cup nest in herbaceous vegetation (10–50 cm off the ground) and care for broods of 2–3 nestlings (Almeida & Macedo, 2001). Extrapair fertilization rates in this species are exceptionally high, ranging from 11% to 47% of all broods (Manica et al., 2016). Nest predation is very high, with up to 80% of nests being depredated in some breeding seasons (Diniz et al., 2015). Predation is exacerbated because males continue to perform sexual displays during the nesting period within their typically extremely small territories (as small as 13.0 m^2 and average of 72 m^2; Almeida & Macedo, 2001; Carvalho et al., 2006). These displays apparently are excellent indicators of the location of male territories and nests, increasing nest predation risk (Dias et al., 2010).

We used mist nets (2.5 × 12 m, 10 mm mesh) to capture 26 female blue-black grassquits in savanna habitat patches within the Universidade de Brasília campus (15°46′S, 47°52′W) throughout the breeding season of 2014/2015. The females were banded with a unique combination of two plastic color bands and housed together in an outdoor aviary (2.56 × 1.38 × 2.06 m). We kept the females in the aviary for 10 days before using them in any experimental trial to minimally standardize their reproductive status, since it was impossible to know their reproductive condition prior to capture. This 10-day period was estimated as being adequate because under field conditions, new clutches are initiated on average 13 days after nest predation events (P. Diniz, unpublished data). Before each experimental trial, we measured female body mass to the nearest 0.2 g with a scale, and the length of the left tarsus with calipers (accuracy: ±0.01 mm). We then created a body mass index (body mass divided by tarsus length) and used it as a proxy of female condition relative to body fat deposition (Aguilar et al., 2007; Costa & Macedo, 2005; Magalhães, Diniz, & Macedo, 2014; Santos, Maia, & Macedo, 2009). We also visually counted the number of feather lice on both wings (data pooled) and used it to estimate female ectoparasite load (Magalhães et al., 2014).

2.2 | Ethical note

Our capturing protocol and subsequent aviary maintenance of individuals were in accordance with the ethical standards of the Brazilian Ministry of the Environment (permit #42365-3) and the Universidade de Brasília (permit #92808/2014). As capture and handling can cause distress to the birds, we limited handling time to <3 min, and no individual died during this procedure. In captive conditions, the birds were supplied with ad libitum water and a diet of selected seeds (Nutrópica®).

2.3 | Experimental stimuli and design

The experiment took place in a second outdoor aviary containing the experimental arena (100 × 35 × 65 cm), which included two wooden
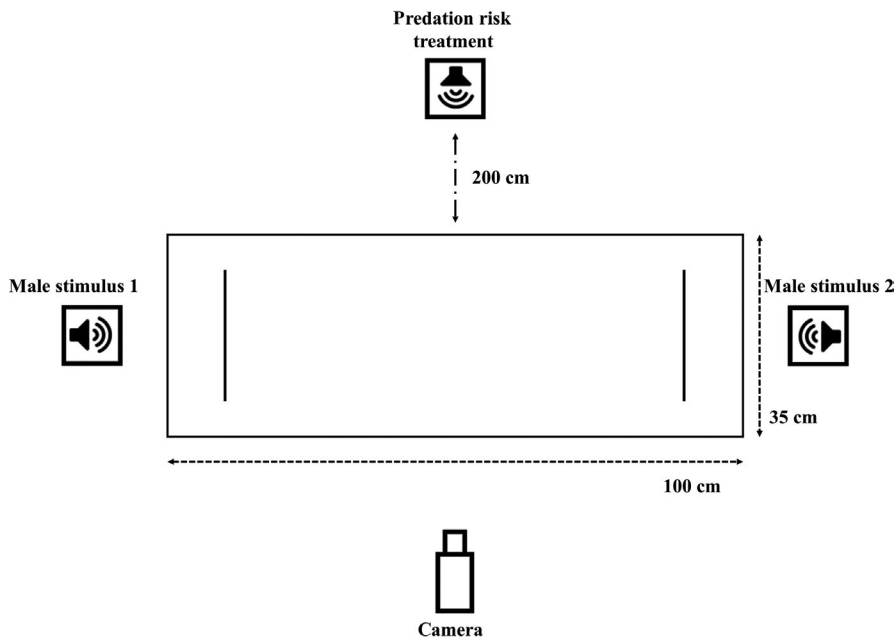
FIGURE 1 Schematics of the experimental apparatus, viewed from above. The experimental arena (100 × 35 × 65 cm) contained two wooden perches, each one at a 10 cm distance from each side of the arena. Two sound-speakers placed at each side of the arena broadcasted male blue-black grassquit songs. The third speaker positioned 2 m from the center of the arena emitted the predator treatment stimuli. A video camera was placed at the front of the arena to record female behavior during the experiments

perches at a height of 10 cm, each one at a 10 cm distance from each side of the arena. Bird seed and water ad libitum were positioned centrally within the arena. Females were placed by themselves in the arena for 12 hr for habituation before starting the experimental trial. We used one female per trial, and the experimental protocol (detailed below) consisted of exposing females to two contrasting types of male song, while simulating three different levels of predation risk by broadcasting predator vocalizations.

Two sound-speakers (Kaiyue KY-907, frequency response: 0.15–18 kHz) were placed outside but close to each side of the arena. These two speakers were used to broadcast male blue-black grassquit songs. A third similar speaker was positioned 2 m at the front of and aligned with the center of the arena. This centrally located speaker was used to broadcast the predator treatment types (Figure 1).

To create the contrasting grassquit songs, we recorded males in the field with a Marantz PMD660 recorder (WAV, sampling rate of 48 kHz, 24 bits of resolution, mono) and a unidirectional microphone (Sennheiser ME66). We edited the audio files from each male with Audacity® to create two categories of male song rates: (a) high song rate (20 songs per minute), simulating a more acoustically conspicuous male; and (b) low song rate (10 songs per minute), simulating a more acoustically discrete male. These high and low song repetition rates are naturally observed in the wild and have been found to be biologically relevant since they reflect both motor display investment and territory quality: Each song is coupled with a leap and males with greater seed density in their territories produce leap-songs at higher rates (Manica et al., 2014). To control for male identity and song attributes other than rate, we created both stimuli audios (high and low song rates) from the songs of the same male, and these were presented simultaneously to each female. We filtered acoustic frequencies below 2 kHz to minimize noise interference in stimuli creation, and standardized stimuli amplitude. The final 1-hour audio files were

comprised of 1-min bouts of male songs (at either high or low song rates) intercalated by 30 s of silence. We prepared one pair of male stimuli for each female subject, totaling 26 pairs of male stimuli. Hence, we used the same pair of stimuli for the three treatments within an experimental trial (see below) and there was no repetition of male stimuli between trials (i.e., between females).

The potential predator vocalizations broadcast from the centrally located and farthest speaker from the experimental arena were taken from an online library of avian vocalizations (Xeno-Canto Foundation©, https://www.xeno-canto.org/). We used audios of native birds to create three treatments with different levels of acoustically simulated predation risk: (a) a predator of adult grassquits (aplomado falcon, *Falco femoralis*; Bó, 1999; Hector, 1985; Xeno-Canto file XC53279), which represents a great risk to females; (b) a grassquit nest predator (guira cuckoo, *Guira guira*; Menezes & Marini, 2017; Xeno-Canto file XC114982), representing a greater risk to the offspring, but still somewhat dangerous to the female; and (c) a no-risk control (sayaca tanager, *Tangara sayaca*; Xeno-Canto file XC116274), that offers no predation risk to either female or offspring. The aplomado falcon stimulus consisted of a sequence of five alarm calls (Bierregaard & Kirwan, 2018) that lasted 46 s, the guira cukoo stimulus consisted of two glacis songs (Macedo, 1992) that lasted 36 s, and the sayaca tanager stimulus consisted of a song bout lasting 55 s. Although some studies suggest the use of multiple playback stimuli, we have chosen a single recording for each predation risk stimulus for two reasons (Pettinga, Kennedy, & Proppe, 2016). High-quality recordings for standardized vocalization types were not available to produce 26 stimuli (to match the number of female subjects). In addition, we have chosen relatively stereotyped and frequently emitted vocalization types (Bierregaard & Kirwan, 2018; Macedo, 1992), reducing the information lost by using a single instead of multiple stimuli for each species (Pettinga et al., 2016). All stimuli were broadcast

in looping during the whole treatment. We performed pilot experiments to observe the effect of these playback calls on captive blue-black grassquits that were not used in this study. Playback calls of the aplomado falcon and the guira cuckoo resulted in noticeable changes in grassquit behavior, such as decreased feeding behavior and socialization, and an increase in alarm call emission. Therefore, we believe that these acoustic predator playbacks were appropriate options to simulate predation risk to blue-black grassquits.

Experimental treatments lasted 2 hours and were conducted separately at fixed times (0900 and 1500 hr) each day, totaling 6 hours of observation per female (three treatments, 2 hours each, completed in two consecutive days). During experimental trials, we recorded female activity within the arena, while the vocalizations of potential predators or non-predator (control) were broadcast by the central speaker, and male songs with different attributes (low vs. high rates) were broadcast from the lateral speakers. We changed the positions of the lateral speakers after 1 hour of each treatment, thus inverting the emission site of each category of male song to avoid any possible biases in female preference for sides of the arena. The day shift (morning or afternoon) for execution of the experimental treatments, the order of control and predator stimuli presentation (adult predator, nest predator), and the initial position of male song rate categories (left or right side of the arena) varied randomly across trials.

Before each experimental treatment, we used a decibel meter (SEW® 2310 SL) to calibrate all three speakers to a standard amplitude of 69 dB. We calculated this value using the same equipment to measure the singing amplitude of grassquits in the wild and also in captivity. After sampling the grassquit singing amplitudes, we used a measuring tape to calculate the distance between the sampled grassquits and the decibel meter. We applied these two values in the formula $L1 = L_r + 20 \times \log_{10}(r)$, which calculates the amplitude that should be sampled by a decibel meter when it is one meter away from a sound-speaker (van den Heuvel, Cherry, & Klump, 2012). In this formula, L_r represents the singing amplitude measured at distance r, resulting in the singing amplitude measured at one meter from the emitter L1 (regarding the grassquits, L1 = 69 dB).

We used a video camera (Kodak Z×1) to record experimental trials for the 26 females used as experimental subjects (156 hr of observation). We used a virtual line dividing the arena into two halves and then identified which side corresponded to which male song rate category. We used scan sampling (Altmann, 1974) to score the position of the female in the arena at 2-min intervals. We created two mate preference scores: "mate preference score 1" was the proportion of scans where the female was observed in the "high male song rate side" of the arena; "mate preference score 2" consisted of the difference in the number of scans between "high male song rate" side and "low male song rate" side. A high value in both mate preference scores indicates that the female spent more time closer to the high male song rate stimulus, while a low value meant that the female spent more time in close proximity to the low male song rate stimulus.

2.4 | Data analysis

We analyzed data with R version 3.4.1 (R Development Core Team, 2017). We used generalized linear mixed models (GLMM, package lme4) to analyze variation in the mate preference scores. To avoid model overfitting, we conducted the analyses in two steps. First, we investigated whether grassquit females under predation risk expressed a preference for either high or low male song rate, using a GLMM with a binomial error distribution and observation-level random effect (to deal with overdispersion; Harrison, 2014). We used the proportion of the number of scans in which a female was recorded in the "high male song rate side" of the arena (mate preference score 1) as a response variable. We included the main effect of the predation risk treatment and female identity as random effects.

Since female condition can affect mate choice behavior (Hunt et al., 2005), we performed a second analysis to control for confounding effects on mate choice unrelated to predation risk. This time, we used a global model to test whether variation in female preference for the variable male song rate varied with female body mass index (scaled) and ectoparasite load (scaled). For this analysis, the "mate preference score 2" was the response variable. Accordingly, we used a GLMM with a Gaussian error distribution. We included the main effects of predation risk treatment, female body mass index, and female ectoparasite count. We also added the interactions predation risk treatment * female body mass index, and predation risk treatment * female ectoparasite count, as fixed effects in this global model. Finally, we included female identity as a random factor in this model.

We conducted likelihood ratio tests in a backward stepwise approach for model selection. For each variable, we present the significance of these tests for the last model in which the variable occurred.

We performed a power analysis (package simr) for mate preference 1 to detect the probability of rejecting our null hypothesis given our sample size and the model structure (Green & MacLeod, 2016). We set the effect sizes of predator levels (aplomado falcon and guira cuckoo) to −0.4 (Green & MacLeod, 2016; Hoenig & Heisey, 2001), corresponding to approximately 10% of decline in the proportion of scans in the "high song rate" side of each predator treatment relative to the control (sayaca tanager). We used a negative effect size in power analysis to match the observed negative estimates for predator levels in the original model. Number of simulations and alpha value were set to 1,000 and 0.05, respectively.

3 | RESULTS

Grassquit females did not show any preference for playbacks of either high or low song rate bouts (Table 2). This pattern of no preference was not influenced by simulated predation risk offered by adult or nest predator vocalizations (Figure 2), female body mass index or female ectoparasite load (Table 2). In other words, females spent equal amounts of time in the high and low song rate sides of the arena, irrespective of predation risk regimes. Power analysis indicates

TABLE 2 Model selection through backward stepwise procedure to assess variation in mate preference scores by blue-black grassquit females for variable male song rates

Global model	Family	Response variable	Variable	Model selection step	Likelihood ratio test χ^2	df	p
1	Binomial	Mate preference score 1	Predation	1	2.44	2	0.30
2	Gaussian	Mate preference score 2	Predation * ectoparasite	1	0.66	2	0.72
			Ectoparasite	2	0.79	1	0.37
			Predation * body mass index	3	2.41	2	0.30
			Predation	4	2.37	2	0.31
			Body mass index	5	2.47	1	0.12

Note. Female identity is included in all models. Mate preference score 1 is the proportion of scans wherein the females was scored in the "high male song rate" side of the cage. Mate preference score 2 is the difference in the number of scans recorded for the female between cage sides ("high male song rate" minus "low male song rate"). Variables considered include the following: body mass index (mass/tarsus), ectoparasite load (ectoparasite count for female), predation (predation risk treatment = control, adult predator, nest predator), and song (high and low male song rate).
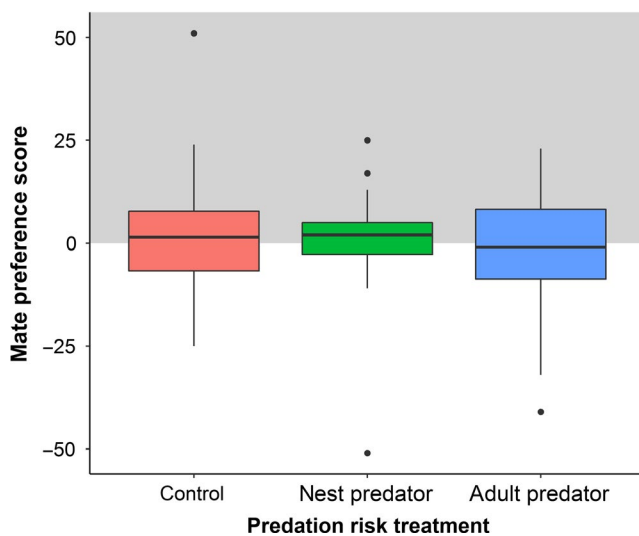

FIGURE 2 Boxplot showing variation in female mate preference scores for the playback of variable male song rates (high vs. low) under different scenarios of predation risk (N = 26 females, three treatments per female). Gray area corresponds to preference for high male song rate, whereas white area corresponds to preference for low male song rate. Scenarios of manipulated predation risk by playback: control = sayaca tanager, *Tangara sayaca*; nest predator = guira cuckoo, *Guira guira*; adult predator = aplomado falcon, *Falco femoralis* [Colour figure can be viewed at wileyonlinelibrary.com]

that sample size was enough to detect a difference as small as 10% in female preference (score 1; power, CI: 95.8%, 94.4–97.0) between simulated predation risk (nest or adult predator) and the no-risk control. The number of scans for females in the three predator risk regimes exhibited very similar scores for the alternative male song rate categories ($\bar{x} \pm SD$): (a) control high song rate side = 32.6 ± 7.4 vs. low song rate side = 30.2 ± 7.4; (b) adult predator high song rate side = 30.0 ± 7.7 vs. low song rate side = 32.9 ± 7.3; and (c) nest predator high song rate side = 31.9 ± 6.5 vs. low song rate side = 31.0 ± 6.5.

4 | DISCUSSION

We experimentally tested whether predation risk influences mate choice by female blue-black grassquits and predicted three possible scenarios resulting from our experiments. The first scenario predicted that females would not be affected by predation risk during mate choice, presenting similar mating preferences across all levels of perceived risk. For the second scenario, we expected that females would choose more conspicuous male stimuli across every predation risk treatment. That is to say that female preference would interact with predation risk and females would favor more conspicuous male stimuli under higher predation risk than in the no-risk situation (control treatment). Our final scenario predicted that females would select more conspicuous male stimuli only in situations of low risk, and under high predation risk, they would choose male stimuli at random or even prefer more muted stimuli. This latter outcome assumed that association with vigorously displaying males might impose a great survival cost on females or their potential offspring (Dias et al., 2010; Marzal et al., 2016). Our results confirmed the first predicted scenario, given that females were non-responsive to male song stimuli, whether at low or high rates, across all experimental treatments. Therefore, our question about whether blue-black grassquit females modify their partner choice preferences under different predation risk regimes received a negative answer.

This non-responsive pattern presented by our data confirms some previous findings for this species, suggesting alternative explanations that advance our understanding of how sexual selection operates. Initial studies of this species indicated that, regardless of male attributes, grassquit females did not appear to choose their mates based on male territory quality and resource availability (Almeida & Macedo, 2001). This led to a series of experiments designed to explore female choice based upon male phenotype. A previous mate choice experiment using captive grassquits exposed females to two male groups: healthy males treated with coccidiostatic drugs and parasite-infested males (Aguilar et al., 2007). Results showed that

the coccidian infection significantly affected several sexual display attributes, and that healthy males performed higher leaps and longer display bouts than parasitized males. However, grassquit females did not prefer the healthy males over the parasitized ones, although male performance honestly reflected male health. A subsequent experiment investigated how socially paired females reacted to potential extrapair mates (Dias, Oliveira, Podos, & Macedo, 2014). Females were interested in the novel males even when the familiar male attributes were of higher quality.

Taken together, these previous findings and our results suggest that female grassquits may exhibit passive or indirect mate choice, that is, dependent on male competition (Wiley & Poston, 1996). During the breeding season, males compete to defend small territories with available substrate for nests (Almeida & Macedo, 2001). Females then pair with territorial males, apparently ignoring most male attributes (Dias et al., 2014). They also appear to choose their mates disregarding male health (Aguilar et al., 2007), territory quality (Almeida & Macedo, 2001; Carvalho et al., 2006), and predation risk (present study). Successfully paired males in the wild have been shown to spend more time in their territories, display for longer periods and at greater rates, and produce higher leaps in their displays (Carvalho et al., 2006; Manica et al., 2016).

Possibly, female grassquits are attracted to males that are successfully established in territories. In this context, male attributes such as persistence in displaying and performing higher leaps may determine male status in intrasexual competition. Males that defend territories with greater seed density have higher singing rates (Manica et al., 2014), suggesting that these males invest greater amounts of time in territory defense. There is also experimental evidence showing that intrasexual competition may be strongly associated with male blue-black ornamentation. Males living in all-male settings, in contrast to those in mixed-sex groups or in pairs, are more aggressive and have higher levels of plasmatic testosterone (Lacava, Brasileiro, Maia, Oliveira, & Macedo, 2011). Such males also develop nuptial plumage faster, earlier, and with more ultraviolet reflectance (Maia, Brasileiro, Lacava, & Macedo, 2012), which may signal male quality (Keyser & Hill, 1999). Therefore, mating patterns in blue-black grassquits may not result primarily from female choice for male attributes, but could also strongly depend on male status and intrasexual competition (Arak, 1988; Okamura & Goshima, 2010; Rebout et al., 2017).

An alternative reason for the lack of female grassquit preference in the context of elevated predation risk may be related to our use of only one male display attribute (song rate) as the experimental stimulus. Blue-black grassquit males perform multimodal displays that involve not only acoustic components, but also visual cues: males leap at heights of 25 ± 9 cm (Costa & Macedo, 2005) while clapping their wings behind their heads and presenting white underwing plumage patches (Maia & Macedo, 2011). At the peak of the leap, males emit a high-pitched song and return to the perched position to leap again a few seconds later (Manica, Macedo, Graves, & Podos, 2017). Previous studies have shown that the height of the leap is important for male pairing success (Carvalho et al., 2006; Manica et al., 2016). Given that avian predators can capture males during

their leap display (R. Macedo, pers. observ.) and that grassquit males often sing without performing the leap display (Manica et al., 2017), singing without leaping may be a low-risk male strategy for mate attraction. However, the singing without leaping strategy may have low appeal to females, which may explain the lack of female responsiveness to male song in our experiments.

In conclusion, we found that blue-black grassquit females do not respond to a specific set of nest and adult predation risk stimuli under specific experimental conditions of mate choice, and do not show any preference for contrasting male song rates under controlled conditions. We discussed two alternative explanations for these results (passive or indirect mate choice, and multimodal signaling), although we cannot substantiate them without further studies. To further advance our understanding about the relevance of male stimuli for female choice, we suggest that mate choice studies of species that exhibit multimodal displays use the complete display as experimental stimulus. This may present a challenge depending on the species being studied, but video playbacks with sufficient visual resolution would be an alternative, in addition to high-quality vocal playbacks.

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REFERENCES

Aguilar, T. M., Maia, R., Santos, E. S. A., & Macedo, R. H. (2007). Parasite levels in blue-black grassquits correlate with male displays but not female mate preferences. *Behavioral Ecology*, 19, 292–301. https://doi.org/10.1093/beheco/arm130

Almeida, J. B., & Macedo, R. H. (2001). Lek-like mating system of the monogamous blue-black grassquit. *The Auk*, 118, 404–411. https://doi.org/10.1642/0004-8038(2001) 118[0404:LLMSOT]2.0.CO;2

Altmann, J. (1974). Observational study of behavior: Sampling methods. *Behaviour*, 49, 227–266. https://doi.org/10.1163/156853974X00534

Arak, A. (1988). Female mate selection in the natterjack toad: Active choice or passive attraction? *Behavioral Ecology and Sociobiology*, 22, 317–327. https://doi.org/10.1007/BF00295100

Bierregaard Jr, R. O., & Kirwan, G. M. (2018). Aplomado falcon (*Falco femoralis*). In J. del Hoyo, A. Elliott, J. Sargatal, D. A. Christie & E. de Juana (Eds.), *Handbook of the birds of the world alive*. Barcelona, Spain: Lynx Edicions.

Bó, M. S. (1999). Dieta del halcón plomizo (*Falco femoralis*) en el sudeste de la província de Buenos Aires, Argentina. *Ornitologia Neotropical*, 10, 95–99.

Boyko, A. R., Gibson, R. B., & Lucas, J. R. (2004). How predation risk affects the temporal dynamics of avian leks: Greater sage grouse versus golden eagles. *The American Naturalist*, 163, 154–165. https://doi.org/10.1086/380419

Brooks, R., & Endler, J. A. (2001). Female guppies agree to differ: Phenotypic and genetic variation in mate-choice behavior and the consequences for sexual selection. *Evolution*, 55, 1644–1655. https://doi.org/10.1111/j.0014-3820.2001.tb00684.x

Carvalho, C. B. V., Macedo, R. H., & Graves, J. A. (2006). Breeding strategies of a socially monogamous neotropical passerine: Extra-pair fertilizations, behavior, and morphology. *The Condor*, 108, 579–590. https://doi.org/10.1650/0010-5422(2006)108[579:BSOASM]2.0.CO;2

Carvalho, C. B. V., Macedo, R. H., & Graves, J. A. (2007). Reproduction of blue-black grassquits in central Brazil. *Brazilian Journal of Biology*, 67, 275–281. https://doi.org/10.1590/S1519-69842007000200012

Conrad, R., Stocker, C., & Ayasse, M. (2017). The effect of temperature on male mating signals and female choice in the red mason bee, *Osmia bicornis* (L.). *Ecology and Evolution*, 7, 8966–8975. https://doi.org/10.1002/ece3.3331

Costa, F., & Macedo, R. H. (2005). Coccidian oocyst parasitism in the blue-black grassquit: Influence on secondary sex ornaments and body condition. *Animal Behaviour*, 70, 1401–1409. https://doi.org/10.1016/j.anbehav.2005.03.024

Darwin, C. (1871). *The descent of man and selection in relation to sex*. London, UK: John Murray.

Dias, R. I., Castilho, L., & Macedo, R. H. (2010). Experimental evidence that sexual displays are costly for nest survival. *Ethology*, 116, 1011–1019. https://doi.org/10.1111/j.1439-0310.2010.01817.x

Dias, R. I., Oliveira, R. F., Podos, J., & Macedo, R. H. (2014). The importance of novelty: Male-female interactions among blue-black grassquits in captivity. *Behavioural Processes*, 103, 211–217. https://doi.org/10.1016/j.beproc.2013.12.016

Diniz, P., Ramos, D. M., & Macedo, R. H. (2015). Attractive males are less than adequate dads in a multimodal signalling passerine. *Animal Behavior*, 102, 109–117. https://doi.org/10.1016/j.anbehav.2015.01.006

Eggers, S., Griesser, M., Nystrand, M., & Ekman, J. (2006). Predation risk induces changes in nest-site selection and clutch size in siberian jay. *Proceedings of the Royal Society B*, 275, 701–706. https://doi.org/10.1098/rspb.2005.3373

Ghalambor, C. K., & Martin, T. E. (2000). Parental investment strategies in two species of nuthatch vary with stage-specific predation risk and reproductive effort. *Animal Behaviour*, 60, 263–267. https://doi.org/10.1006/anbe.2000.1472

Godin, J. G. J., & Briggs, S. E. (1996). Female mate choice under predation risk in the guppy. *Animal Behaviour*, 51, 117–130. https://doi.org/10.1006/anbe.1996.0010

Green, P., & MacLeod, C. (2016). simr: An R package for power analysis of generalized linear mixed models by simulation. *Methods in Ecology and Evolution*, 7, 493–498. https://doi.org/10.1111/2041-210X.12504

Harrison, X. A. (2014). Using observation-level random effects to model overdispersion in count data in ecology and evolution. *PeerJ*, 2, e616. https://doi.org/10.7717/peerj.616

Hector, P. (1985). The diet of the aplomado falcon (*Falco femoralis*) in Eastern Mexico. *The Condor*, 87, 336–342. https://doi.org/10.2307/1367212

Hernandez-Jimenez, A., & Rios-Cardenas, O. (2012). Natural versus sexual selection: Predation risk in relation to body size and sexual ornaments in the green swordtail. *Animal Behaviour*, 84, 1051–1059. https://doi.org/10.1016/j.anbehav.2012.08.004

Hoenig, J. M., & Heisey, D. M. (2001). The abuse of power: The pervasive fallacy of power calculations for data analysis. *The American Statistician*, 55, 19–24. https://doi.org/10.1198/000313001300339897

Hunt, J., Brooks, R., & Jennions, M. D. (2005). Female mate choice as a condition-dependent life-history trait. *The American Naturalist*, 166, 79–92. https://doi.org/10.1086/430672

Johnson, J. B., & Basolo, A. L. (2003). Predator exposure alters female mate choice in the green swordtail. *Behavioral Ecology*, 14, 619–625. https://doi.org/10.1093/beheco/arg046

Keyser, A. J., & Hill, G. E. (1999). Condition–dependent variation in the blue–ultraviolet coloration of a structurally based plumage ornament. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 266(1421), 771–777. https://doi.org/10.1098/rspb.1999.0704

Lacava, R. V., Brasileiro, L., Maia, R., Oliveira, R. F., & Macedo, R. H. (2011). Social environment affects testosterone level in captive male blue-black grassquits. *Hormones and Behavior*, 59, 51–55. https://doi.org/10.1016/j.yhbeh.2010.10.003

Lynch, K. S., Crews, D., Ryan, M. J., & Wilczynski, W. (2006). Hormonal state influences aspects of female mate choice in the túngara frog (*Physalaemus pustulosus*). *Hormones and Behavior*, 49, 450–457. https://doi.org/10.1016/j.yhbeh.2005.10.001

Macedo, R. H. (1992). Reproductive patterns and social organization of the communal guira cuckoo (*Guira guira*) in Central Brazil. *The Auk*, 109, 786–799. https://doi.org/10.2307/4088153

Macedo, R. H., Manica, L., & Dias, R. I. (2012). Conspicuous sexual signals in a socially monogamous passerine: The case of neotropical blue-black grassquits. *Journal of Ornithology*, 153, S15–S22. https://doi.org/10.1007/s10336-012-0815-5

MacLaren, R. D. (2017). Effects of male apparent length on female preference for absolute body size in *Xiphophorus helleri*. *Acta Ethologica*, 20, 27–46. https://doi.org/10.1007/s10211-016-0245-0

Madden, J. R., & Whiteside, M. A. (2013). Variation in female mate choice and mating success is affected by sex ratio experienced during early life. *Animal Behaviour*, 86, 139–142. https://doi.org/10.1016/j.anbehav.2013.05.003

Magalhães, R. B., Diniz, P., & Macedo, R. H. (2014). Plumage coverage is related to body condition and ectoparasitism in blue-black grassquits. *The Wilson Journal of Ornithology*, 126, 581–584. https://doi.org/10.1676/13-197.1

Maia, R., Brasileiro, L., Lacava, R. V., & Macedo, R. H. (2012). Social environment affects acquisition and color of structural nuptial plumage in a sexually dimorphic tropical passerine. *PLOS One*, 7, e47501. https://doi.org/10.1371/journal.pone.0047501

Maia, R., & Macedo, R. H. (2011). Achieving luster: Prenuptial molt pattern predicts iridescent structural coloration in blue-black grassquits. *Journal of Ornithology*, 152, 2243–2252. https://doi.org/10.1007/s10336-010-0576-y

Manica, L. T., Graves, J. A., Podos, J., & Macedo, R. H. (2016). Multimodal flight display of a neotropical songbird predicts social pairing but not extrapair mating success. *Behavioral Ecology and Sociobiology*, 70, 2039–2052. https://doi.org/10.1007/s00265-016-2208-x

Manica, L. T., Macedo, R. H., Graves, J. A., & Podos, J. (2017). Vigor and skill in the acrobatic mating display of a Neotropical songbird. *Behavioral Ecology*, 28, 164–173. https://doi.org/10.1093/beheco/arw143

Manica, L. T., Maia, R., Dias, A., Podos, J., & Macedo, R. H. (2014). Vocal output predicts territory quality in a neotropical songbird. *Behavioural Processes*, *109*, 21–26. https://doi.org/10.1016/j.beproc.2014.07.004

Marzal, J. C. S., Rudh, A., Rogell, B., Odeen, A., Løvlie, H., Rosher, C., & Qvarnstrom, A. (2016). Cryptic female strawberry poison frogs experience elevated predation risk when associating with an aposematic partner. *Ecology and Evolution*, *7*, 744–750. https://doi.org/10.1002/ece3.2662

Massaro, M., Starling-Windhof, A., Briskie, J. V., & Martin, T. E. (2008). Introduced mammalian predators induce behavioural changes in parental care in an endemic New Zealand bird. *PLoS One*, *3*, 1–7. https://doi.org/10.1371/journal.pone.0002331

Menezes, J. C. T., & Marini, M. A. (2017). Predators of bird nests in the Neotropics: A review. *Journal of Field Ornithology*, *88*, 99–114. https://doi.org/10.1111/jofo.12203

Okamura, S., & Goshima, S. (2010). Indirect female choice mediated by sex pheromones in the hermit crab *Pagurus filholi*. *Journal of Ethology*, *28*, 323–329. https://doi.org/10.1007/s10164-009-0188-2

Pettinga, D., Kennedy, J., & Proppe, D. S. (2016). Common urban birds continue to perceive predator calls that are overlapped by road noise. *Urban Ecosystems*, *19*, 373–382. https://doi.org/10.1007/s11252-015-0498-9

R Development Core Team (2017). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. https://www.R-project.org/

Rebout, N., Thierry, B., Sanna, A., Cozzolino, R., Aujard, F., & De Marco, A. (2017). Female mate choice and male-male competition in tonkean macaques: Who decides? *Ethology*, *123*, 365–375. https://doi.org/10.1111/eth.12605

Ronald, K. L., Fernández-Juricic, E., & Lucas, J. R. (2012). Taking the sensory approach: How individual differences in sensory perception can influence mate choice. *Animal Behaviour*, *84*, 1283–1294. https://doi.org/10.1016/j.anbehav.2012.09.015

Santos, E. S. A., Maia, R., & Macedo, R. H. (2009). Condition-dependent resource value affects male-male competition in the blue-black grassquit. *Behavioral Ecology*, *20*, 553–559. https://doi.org/10.1093/beheco/arp031

Sicsú, P., Manica, L. T., Maia, R., & Macedo, R. H. (2013). Here comes the sun: Multimodal displays are associated with sunlight incidence. *Behavioral Ecology and Sociobiology*, *67*, 1633–1642. https://doi.org/10.1007/s00265-013-1574-x

Skutch, A. F. (1985). Clutch size, nesting success, and predation on nests of neotropical birds, reviewed. *Ornithological Monographs*, *36*, 575–603. https://doi.org/10.2307/40168306

van den Heuvel, I. M., Cherry, M. I., & Klump, G. M. (2012). Individual identity, song repertoire and duet function in the crimson-breasted shrike (*Laniarius atrococcineus*). *Bioacoustics*, *22*, 1–15. https://doi.org/10.1080/09524622.2012.701041

Wiley, R. H., & Poston, J. (1996). Indirect mate choice, competition for mates, and coevolution of the sexes. *Evolution*, *50*, 1371–1381. https://doi.org/10.1111/j.1558-5646.1996.tb03911.x

Willis, P. M., Rosenthal, G. G., & Ryan, M. J. (2012). An indirect cue of predation risk counteracts female preference for conspecifics in a naturally hybridizing fish *Xiphophorus birchmanni*. *PLOS One*, *7*, e34802. https://doi.org/10.1371/journal.pone.0034802

Zahavi, A. (1975). Mate selection – A selection for a handicap. *Journal of Theoretical Biology*, *53*, 205–214. https://doi.org/10.1016/0022-5193(75)90111-3