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Seleção sexual, características sexuais multimodais e cópulas extra-par em tizius (*Volatinia jacarina*)

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SUMÁRIO

INTRODUÇÃO GERAL

RESUMO EXPANDIDO

CAPÍTULO 1 - Trade-offs and constraints within an acrobatic mating display

CAPÍTULO 2 - Multiple benefits of social and sexual mating choice in the blue-black grassquit

CAPÍTULO 3 - Leaping higher, arriving earlier: advantages in a clustered breeding system

LISTA DE FIGURAS

INTRODUÇÃO GERAL

RESUMO EXPANDIDO

CAPÍTULO 1

CAPÍTULO 2

CAPÍTULO 3

LISTA DE TABELAS

RESUMO EXPANDIDO

CAPÍTULO 1

CAPÍTULO 2

CAPÍTULO 3

APÊNDICE

RESUMO

A poliandria sexual em espécies socialmente monogâmicas é mais comum do que se pensava e fêmeas podem aumentar sua aptidão ao buscarem por múltiplos parceiros sexuais. Em aves, a expressão de sinais sexuais nos machos (e.g. plumagem colorida e canto) é a principal informação utilizada pelas fêmeas para avaliar a qualidade genética ou não-genética de potenciais parceiros. Esse estudo teve como foco o tiziu (*Volatinia jacarina*), uma espécie socialmente monogâmica e sexualmente poligâmica que forma agregações territoriais durante o período reprodutivo. Os machos desta espécie executam exibições multimodais: repetição de uma vocalização curta enquanto empoleirados (exibição incompleta), e vocalização sincronizada com voos verticais ("saltos") e rotações do eixo corporal no ápice do salto (exibição completa). Nossos objetivos foram testar: 1) demandas conflitantes entre componentes das exibições que potencialmente indicam a qualidade individual; 2) benefícios diretos (recursos) e indiretos (bons genes e compatibilidade genética) da escolha das fêmeas por parceiros sociais e sexuais, e 3) a hipótese do "lek escondido" (modelos de preferência das fêmeas e machos atraentes "hotshot"), cuja principal predição é de que agregações ocorram para maximizar a oportunidade de cópulas extra par. Em três estações reprodutivas, observamos e gravamos vocalizações e saltos de machos em exibições. Monitoramos a atividade reprodutiva e utilizamos marcadores microssatélites para determinar a paternidade genética dos filhotes. A duração do salto reduziu com a rotação do corpo de machos que saltam mais baixo, e a altura do salto reduziu com as taxas de exibições completas de machos em condição corporal inferior, indicando demandas conflitantes. Os níveis de paternidade extra par variaram de 8% a 34% dos filhotes e de 11% a 47% das ninhadas analisadas. Encontramos fraca evidência de benefício direto pelo maior acesso a alimento, porém encontramos suporte para benefício indireto (bons genes) considerando que as fêmeas selecionaram machos sociais com saltos mais altos, favorecendo a qualidade genética da prole. Fêmeas preferiram machos com cantos mais curtos e, portanto, a duração do canto também deve ser um sinal indicativo de qualidade. No entanto, a dissimilaridade genética entre fêmeas e machos extra par não foi maior do que entre fêmeas e machos sociais, assim como filhotes extra e intra par não diferiram em diversidade alélica e condição corporal. Machos que estabelecem territórios em uma agregação mais cedo na estação reprodutiva saltaram mais alto e produziram canto mais longo, porém não houve relação entre essas características e sua posição espacial dentro da agregação. Machos adiantados também obtiveram mais fertilizações extra par, mas não houve evidência de aumento no sucesso em pareamento e de cópulas extra par em agregações maiores. Em conclusão, nosso estudo mostrou que a) as exibições são provavelmente custosas e a produção simultânea de múltiplos componentes pode ser limitada; b) a escolha das fêmeas por parceiros sociais e sexuais não foi aleatória, sendo que machos que exibiram melhores características sexuais tiveram maior sucesso em pareamento e fertilizações, e c) agregações podem formar-se por um processo hierárquico de estabelecimento dos territórios, iniciado por machos mais atraentes e seguido por machos em condições inferiores, provavelmente devido à preferência das fêmeas por atributos dos machos.

Palavras-chave: cópulas extra par, sinais multimodais, monogamia social, agregação de territórios, *Volatinia jacarina*

ABSTRACT

Sexual polyandry in socially monogamous species is more common than previously thought and females may increase their fitness by having multiple sexual partners. In birds, male expression of multimodal sexual signals (e.g. plumage coloration and song) is a cue females use to assess the genetic or non-genetic qualities of potential partners. We studied the blue-black grassquit (*Volatinia jacarina*), a socially monogamous and sexually polygamous species that forms territorial clusters during the breeding season. Males of this species execute multimodal displays, which comprise either only vocalizations (incomplete displays) or vocalization with a synchronized "leap" flight and body axis rotation at the peak of the flight (complete display). We tested for: 1) trade-offs in display components that could indicate individual quality; 2) direct (resources) and indirect (good genes or compatible genes) benefits to females from social and sexual mating choice, and 3) the "hidden-lek" hypothesis (female preference and "hotshot" models) for which the main expectation is that aggregations is related to increased opportunities for extrapair copulations. In three breeding seasons, we observed and audio/video recorded males in displays. We monitored breeding activities and used microsatellites markers to access genetic paternity of nestlings. Leap duration reduced with body axis rotation for males leaping lower, and leap height reduced with rates of complete displays for males with lower body condition, indicating trade-offs. Extrapair paternity levels ranged from 8% to 34% of all nestlings and 11% to 47% of broods analyzed. Direct benefits of female choice through increased access to resources were unlikely, but we found support for indirect benefits, as females preferred social males with higher leaps and should guarantee genetic benefits to the offspring. Females preferred males with shorter songs, suggesting that song length also indicates male quality. However, genetic dissimilarity between females and extrapair males is not greater than females and social males, nor did extrapair and within-pair young differ in quality. Males establishing territories in a cluster earlier in a breeding season had higher leaps and longer songs; however, there was no relationship between these traits and male spatial position within the cluster. Earlier males obtained more extrapair fertilizations, but we found no support for increased pairing success and increased chance for extrapair copulations in larger clusters. Overall, our study showed that a) displays are probably costly and the combination of multiple cues may be under trade-offs, b) female choice for social and sexual partners is non-random, with males bearing better sexual traits showing increased success in paring and fertilizations and c) clustering may develop through a hierarchical settlement process, initialized by more attractive males and followed by low quality males, probably because of female preference for male traits.

Keywords: extrapair copulation, multimodal signals, social monogamy, territorial clustering, *Volatinia jacarina*

INTRODUÇÃO GERAL

Seleção sexual e características sexuais secundárias

Características sexuais secundárias, como ornamentos e exibições elaboradas, ocorrem em muitas espécies e mais frequentemente em machos do que em fêmeas. Em *The Descent of Man and Selection in Relation to Sex* (1871), Darwin propôs que essas características evoluiriam por aumentarem o sucesso reprodutivo dos indivíduos que as tivessem na competição por parceiros sexuais ("seleção sexual"). Tal mecanismo poderia explicar a persistência e exagero desses atributos que a princípio seriam custosos e estariam sujeitos às pressões negativas da seleção natural (Darwin 1871; Andersson 1994). Atualmente, a seleção sexual é um dos temas mais abordados na biologia evolutiva e, embora diversos estudos já demonstrem evidências corroborando a teoria (Andersson 1994), mecanismos genéticos responsáveis por esse fenômeno ainda são debatidos (Kokko et al. 2003, 2006; Andersson e Simmons 2006).

A seleção sexual pode resultar da diferença na taxa potencial reprodutiva (TPR, número potencial de descendentes produzidos por unidade de tempo) entre os sexos (Clutton-Brock e Parker 1992). Machos e fêmeas diferem, por definição, na qualidade e quantidade de gametas produzidos ("anisogamia"): machos produzem gametas pequenos e numerosos enquanto fêmeas produzem gametas grandes e em menor número (Trivers 1972). A anisogamia implica em TPR maiores em machos e menores em fêmeas e, sendo assim, machos devem maximizar seu sucesso reprodutivo com o aumento no número de fertilizações, enquanto que as fêmeas dependem da viabilidade de seus gametas (Bateman 1948). Diferenças na TPR entre os sexos resultam em um maior número de machos sexualmente ativos em relação ao número de fêmeas em um determinado tempo (razão sexual operacional, RSO) (Emlen e Oring 1977; Clutton-Brock e Parker 1992). Consequentemente, há um aumento na competição entre machos pela conquista de fêmeas, que por sua vez tendem a ser seletivas na escolha de parceiros de melhor qualidade (Andersson 1994). No entanto as relações entre investimento parental, TPR e RSO podem ser ainda mais complexas e os "papéis sexuais" (competidor ou seletivo) em alguns casos são invertidos (e.g., cavalo-marinho, *Hippocampus subelongatus*, Kvarnemo et al. 2007) (revisão em Andersson 2004; Clutton-Brock 2009). Por exemplo, outros fatores além da anisogamia podem também influenciar a RSO, como a razão sexual no nascimento (razão sexual primária) e a taxa de mortalidade diferencial entre machos e fêmeas (Clutton-Brock e Parker 1992; Clutton-Brock 2007).

A competição (ou seleção intrassexual) pode configurar-se em diversos aspectos: disputas por condições ou locais que favoreçam o encontro com fêmeas (e.g. territórios, recursos); manutenção da capacidade reprodutiva por maior tempo em relação aos rivais; combates diretos entre competidores (e.g. lutas corporais); coerção sobre as fêmeas; competição espermática ou, ainda, o infanticídio (revisão em Andersson e Iwasa 1996). Dessa forma, a seleção intrassexual deve favorecer a evolução de atributos que beneficiem a persistência, rivalidade ou vigor dos machos e que maximizem seu sucesso reprodutivo. Exemplos incluem grandes tamanhos corporais e estruturas especializadas para o combate, como galhada em cervídeos e chifres em besouros (Andersson 1994).

Evidências da escolha de parceiros pelas fêmeas (ou seleção intersexual) são também abundantes na literatura (Andersson 1994). Esse mecanismo tem sido um dos principais enfoques no âmbito da seleção sexual devido à inconsistência nas relações entre escolha e benefícios para fêmeas encontrados até o momento (Kokko et al. 2003; Andersson e Simmons 2006; Kotiaho e Puurtinen 2007). A explicação da busca das fêmeas por machos com características aparentemente custosas ("paradoxo de lek") é uma discussão antiga. Darwin (1871) já havia proposto o argumento da "apreciação pelo belo" desconsiderando que tal escolha poderia conferir benefícios às fêmeas ao serem seletivas (revisão em Prum 2012). Em contraposição, seu contemporâneo A. R. Wallace já defendia que tais atributos poderiam estar correlacionados com o vigor ou qualidade de um indivíduo (Prum 2012). Atualmente, as principais hipóteses baseiam-se em vantagens adaptativas diretas que aumentam a aptidão das fêmeas: hipótese do benefício direto (Møller e Jennions 2001) e da exploração sensorial (Ryan 1998), ou em vantagens adaptativas indiretas que resultam em benefícios genéticos para a prole: hipótese de Fisher-Zahavi (Zahavi 1975; Eshel et al. 2000; Kokko et al. 2002) e da compatibilidade genética (Zeh e Zeh 1996).

A hipótese dos benefícios diretos postula que alguns machos provisionam benefícios materiais para fêmeas ou filhotes, conquistam e defendem melhores territórios, oferecem menor risco de transmissão direta de doenças, ou ainda, reduzem custos das fêmeas associados ao cuidado parental (Kirkpatrick e Ryan 1991; Andersson 1994). Dessa forma, a escolha por parceiros de boa qualidade favorece diretamente a sobrevivência ou fecundidade das fêmeas (Møller e Jennions 2001). Por outro lado, o conflito sexual (divergência de interesses reprodutivos entre machos e fêmeas) muitas vezes impõem restrições diretas às fêmeas na escolha por machos com adornos extravagantes, pois podem reduzir a própria longevidade ou o valor adaptativo de suas filhas (considerando que apenas filhos expressam a característica sexual) (coevolução antagonista, revisão em Arnqvist e Nilsson 2000; Chapman et al. 2003).

A hipótese da exploração sensorial (ou do viés pré-existente) propõe que a preferência das fêmeas pode evoluir primeiramente sob pressões da seleção natural sobre outra atividade (Enquist e Arak 1993; Ryan 1998), tais como forrageamento e proteção contra predadores. Subsequentemente evoluem as exibições dos machos de maneira a se ajustarem ao comportamento das fêmeas. Um exemplo é o caso do ácaro *Neumania papillator*, no qual a exibição do macho durante o cortejo pode mimetizar as vibrações produzidas por presas (copépodos), estimulando o comportamento predatório das fêmeas (Proctor 1991). O mecanismo da exploração sensorial, no entanto, não exclui a possibilidade de que a preferência resulte também em outras vantagens adaptativas para as fêmeas, como por exemplo a transferência de genes de atratividade ou qualidade dos filhotes (ver abaixo).

Na ausência de benefícios diretos, o aumento da aptidão das fêmeas torna-se menos evidente e, alternativamente, a preferência pode evoluir devido à qualidade genética da prole. A escolha por machos mais atraentes pode garantir a herança de genes que assegurem melhor condição corporal ("bons genes") ou maior atratividade ("filhos atraentes") para os filhotes (hipótese de Fisher-Zahavi; Eshel et al. 2000; Kokko et al. 2002). Essa hipótese inclui duas ideias controversas na literatura: a de que a característica do macho indica ou não sua condição corporal. No entanto, atributos dos machos podem sinalizar qualidade independentemente do componente adaptativo (condição corporal ou atratividade) ao qual está geneticamente correlacionado uma vez que poderão favorecer a sobrevivência ou o sucesso reprodutivo, respectivamente (Kokko 2001). Em alguns casos, o efeito negativo da expressão de um ornamento sobre a chance de sobrevivência pode ser compensado pelo maior sucesso em pareamento próprio ou de seus filhotes (Kokko 2001).

Seleção sexual e sistemas de acasalamento em aves

Por muito tempo acreditou-se que a maioria das aves eram monogâmicas (estimadas em mais de 90% por Lack 1968), e que portanto as consequências da anisogamia (machos competidores e fêmeas seletivas) eram improváveis nesse grupo (Emlen e Oring 1977). Tal expectativa fundamentava-se na reduzida oportunidade para múltiplas cópulas devido ao vínculo social entre machos e fêmeas. Entretanto, com o advento de ferramentas moleculares nos estudos de paternidade, revelou-se que o sistema genético de acasalamento das aves socialmente monogâmicas é na realidade sexualmente poligâmico na grande maioria das espécies estudadas até o momento (revisão em Griffith et al. 2002). Revisões mostram que 86% dos passeriformes socialmente monogâmicos apresentam fertilizações extra par (FEP), sendo a frequência média nessas espécies de 11,1% dos filhotes e 18,7% das ninhadas (Griffith et al. 2002). Tais resultados provocaram uma revolução no entendimento dos sistemas de acasalamento das aves e, consequentemente, nos estudos sobre mecanismos evolutivos da seleção sexual em espécies socialmente monogâmicas.

Há uma grande variação inter e intraespecífica nas frequências de FEP e diversas hipóteses têm surgido para explicar tal padrão (Petrie e Kempenaers 1998; Griffith et al. 2002). Uma explicação foi proposta por Griffith et al. (2002) em uma compilação de aproximadamente 150 publicações, com fatores ecológicos, genéticos e sociais atuando em diferentes níveis taxonômicos. Características da história de vida, como taxa de mortalidade dos adultos e tipo de cuidado parental, parecem melhor explicar variações entre famílias e ordens (Bennet e Owens 2002), enquanto que fatores ecológicos, como densidade de ninhos (Møller e Birkhead 1993), sincronia reprodutiva (Stutchbury et al. 1998) e nível de variação genética na população (Petrie e Kempenaers 1998), são mais adequados para explicar diferenças entre espécies filogeneticamente próximas e entre populações ou indivíduos em uma espécie.

A variação nos níveis de FEP entre espécies e populações pode resultar, ainda, de um balanço entre os custos e os benefícios para indivíduos (Petrie e Kempenaers 1998; Westneat e Stewart 2003). Ainda é largamente discutido se múltiplas cópulas de fato são vantajosas para as fêmeas, uma vez que benefícios diretos seriam improváveis e os riscos de aquisição de ectoparasitas, doenças sexualmente transmissíveis (Sheldon 1993), ou deserção/retaliação do parceiro social (Cezilly & Nager 1995) são custosos (Westneat e Stewart 2003; Arnqvist e Kirkpatrick 2005). Entretanto, os machos que buscam por cópulas extra par também sofrem risco de depleção de esperma, aquisição de doenças e retaliação das fêmeas, embora sejam beneficiados pelo aumento no sucesso reprodutivo (Petrie & Kempenaers 1998). Estudos mostram que fêmeas buscam ativamente por cópulas extra par (Kempenaers et al. 1992; Gray 1996; Double e Cockburn 2000), ou aceitam solicitações de cópulas por múltiplos machos sem resistência (Akçay et al. 2011) sugerindo que fêmeas devem se beneficiar desse comportamento. Fêmeas em aves possuem, geralmente, controle sobre a transferência de esperma (mecanismos pós-copulatórios de controle da paternidade, Birkhead e Møller 1993; Birkhead 1998; Petrie e Kempenaers 1998), indicando ainda que mecanismos como a seleção críptica ou competição espermática permitem a escolha de parceiros reprodutivos de melhor qualidade. Além disso, embora em alguns grupos (em geral, aves aquáticas) as fêmeas frequentemente sofram coação dos machos, a ausência de um órgão copulatório intromitente sugere pouca ocorrência de cópulas forçadas (Gowaty 1996).

Analogamente aos modelos evolutivos tradicionais de seleção intersexual, a busca por múltiplos parceiros sexuais pelas fêmeas pode resultar em benefícios diretos e indiretos. Fêmeas podem aumentar sua área de forrageio ou ganhar auxílio na defesa do ninho ou no cuidado parental pelo macho extra par (hipótese dos benefícios diretos, e.g., Burke et al. 1989), aumentar a atratividade ou qualidade dos filhotes pela herança de “bons genes” (hipótese Fisher-Zahavi, Eshel et al. 2000) e aumentar a chance de cópulas com machos mais geneticamente compatíveis do que seus parceiros sociais (Zeh e Zeh 1996; Neff e Pitcher 2005). Portanto, a poliandria em sistemas socialmente monogâmicos pode ser uma estratégia reprodutiva alternativa a uma escolha anterior “indesejada” ou que seja menos favorável à aptidão das fêmeas (Gowaty 1996). Nesses sistemas reprodutivos, é comum a rápida redução na disponibilidade de potenciais parceiros sociais de melhor qualidade (ou de maior preferência) ao longo do período reprodutivo, uma vez que os “melhores” machos tem maiores chances de formarem pares sociais.

Existem ainda muitas lacunas no nosso conhecimento sobre vias evolutivas da poligamia em espécies socialmente monogâmicas, especialmente para a região tropical. Grande parte do que se sabe atualmente sobre seleção sexual e FEP é baseada em estudos conduzidos na região temperada (Stutchbury e Morton 2001; Macedo et al. 2008; Tori et al. 2008). Há uma expectativa de que menores níveis de FEP ocorram nos trópicos em relação às regiões temperadas devido, principalmente, à diferença na sazonalidade climática e nas características da história de vida entre espécies dessas regiões (Macedo et al. 2008). É esperado, por exemplo, que a sincronia e densidade reprodutiva sejam menores nos trópicos devido à estações reprodutivas prolongadas reduzindo a oportunidade de comparação simultânea de parceiros sexuais por parte das fêmeas e a ocorrência de cópulas extra par (Møller e Birkhead 1993; Stutchbury e Morton 1995). No entanto, ainda é muito cedo para que padrões sejam propostos nesse sentido, dada a escassez de estudos nos trópicos e a vasta diversidade biológica e de condições climáticas que podem ser encontradas nessa região (Macedo et al. 2008). Por exemplo, diversas espécies de aves na região tropical apresentam períodos reprodutivos mais curtos do que o padrão prolongado esperado para a região (e.g. Marini et al. 2009, 2012).

Espécie de estudo

O tiziu (*Volatinia jacarina*) é um Passeriforme da família Emberizidae, migratório no Brasil Central, onde se reproduz de dezembro a abril (Almeida e Macedo 2001; Sick 2001; Carvalho et al. 2006). É uma espécie granívora que ocorre em áreas abertas naturais ou alteradas, onde os territórios de alimentação e nidificação são relativamente pequenos (13 a 72 m), espacialmente distribuídos em agregações ou isolados (Almeida e Macedo 2001). Os machos possuem uma plumagem nupcial preto-azulada iridescente (Doucet 2002; Maia e Macedo 2010) e manchas brancas subaxilares, enquanto as fêmeas, jovens e machos fora da estação reprodutiva são amarronzados com o peito branco estriado (Fig. 1) (Sick 2001).

A ornamentação da plumagem nupcial dos machos torna-se ainda mais evidente durante exibições comportamentais (Sicsu et al. submetido) compostas por múltiplas modalidades sensoriais, visual e auditiva (Webber 1985; Almeida e Macedo 2001; Sick 2001). As exibições podem ser de dois tipos: a) completas, nas quais repetidos voos verticais (similares a “saltos”) acompanhados de uma rotação vertical ou horizontal do eixo do corpo são aparentemente sincronizados com uma curta vocalização emitida a cada salto (Fig. 2), e b) incompletas, durante as quais permanecem empoleirados e produzem repetidamente a mesma vocalização emitida em exibições completas. O canto, embora tipicamente simples e curto, composto por uma única nota, exibe uma grande variação interindividual nos elementos acústicos que o compõem e permitem, portanto, sua individualização (Fandiño-Mariño e Vielliard 2004; Dias 2008).

Estudos prévios sugerem que as características morfológicas e comportamentais de machos de tizius devem ser custosas e indicar qualidade e, portanto, devem estar sob avaliação de outros indivíduos em contextos competitivos ou na escolha de parceiros reprodutivos. Já foi demonstrado que a plumagem nupcial (Doucet 2002; Aguilar et al. 2008) e a execução de exibições (Costa e Macedo 2005; Aguilar et al. 2008) podem estar relacionadas à condição corporal dos machos (revisão em Macedo et al. 2012). O componente acústico da exibição é aparentemente importante na indicação da qualidade do território, sendo que machos que executam maiores taxas de canto possuem territórios com maior densidade de sementes (Dias et al. em preparação). No entanto, pouco se sabe da importância dessas características na escolha de parceiros (veja abaixo).

Figura 1. Fêmea e macho (esquerda e direita, respectivamente) adultos de tiziu (*Volatinia jacarina*). (foto: J. Podos, esq., L. Manica, dir.).

Figura 2. Composição de imagens instantâneas de um salto da exibição de um macho de tiziu (*Volatinia jacarina*).

O sistema social de acasalamento dos tizius é monogâmico, machos e fêmeas formam pares e cooperam no cuidado da prole (Almeida e Macedo 2001; Carvalho et al. 2006). Em contraste, o sistema sexual de acasalamento é poligâmico, apresentando altas taxas de FEP (50% dos filhotes e 64% das ninhadas) e, em menor proporção, parasitismo de ninho intraespecífico e quasiparasitismo (Carvalho et al. 2006). Sabe-se que fêmeas preferem parceiros sociais com saltos mais altos e mais frequentes (Carvalho et al. 2006), porém a preferência por parceiros extra par é desconhecida até o presente momento. O sistema reprodutivo assemelha-se superficialmente ao sistema de *lek*, uma vez que há uma agregação de territórios onde os machos executam as exibições comportamentais (Almeida e Macedo 2001; Carvalho et al. 2006). Padrões semelhantes já foram documentados em outras espécies, sendo que uma das explicações para esse fenômeno é a de que fêmeas preferem machos cujos territórios estão localizados próximos a parceiros sexuais potenciais e de melhor qualidade com os quais poderiam obter cópulas extra par (hipótese do "*lek* escondido", Wagner 1998). Embora essa hipótese já tenha sido proposta para explicar o sistema de acasalamento em tizius (Murray 1982; Webber 1985; Almeida e Macedo 2001; Tarof et al. 2004; Dias et al. 2009) tal resposta ainda permanece desconhecida.

Objetivos

Nesse trabalho nós avaliamos a influência das características sexuais secundárias de machos de tiziu nos sistemas social e sexual de acasalamento. No primeiro capítulo, nós testamos se parâmetros do componente motor e acústico das exibições (altura do salto, rotação vertical do corpo e canto) seriam indicadores de qualidade corporal dos machos. Com essa finalidade, testamos a ocorrência de demanda conflitante na produção sincronizada desses parâmetros, predizendo que sinais custosos e indicadores de qualidade estariam correlacionados negativamente entre si devido à restrições energéticas, biomecânicas ou fisiológicas impostas ao organismo. No segundo capítulo, testamos a escolha das fêmeas por parceiros sociais e extra par em função de suas características sexuais. Mais especificamente, testamos se a escolha está associada a benefícios diretos (acesso a recursos) ou indiretos ("bons genes" e compatibilidade genética). Finalmente, no terceiro capítulo, testamos a influência da distribuição espacial e temporal dos machos dentro das agregações nos sistemas de acasalamento social e sexual. O objetivo específico nesse capítulo foi testar se o modelo evolutivo de "lek escondido" explica a agregação dos territórios dos machos e a ocorrência de cópulas extra par no grupo de indivíduos estudados.

REFERÊNCIAS

Aguilar TM, Maia R, Santos ESA, Macedo RH (2008) Parasite levels in blue-black grassquits correlate with male displays but not female mate preference. Behavioral Ecology 19:292–301

Akçay C, Searcy W, Campbell S, Reed V, Templeton C, Hardwick K, Beecher M (2011) Who initiates extrapair mating in song sparrows? Behavioral Ecology 23:44–50

Almeida JB, Macedo RH (2001) Lek-like mating system of the monogamous blue-black grassquit. Auk 118:404–411

Andersson M (1994) Sexual selection. Princeton University Press, Princeton.

Andersson M (2004) Social polyandry, parental investment, sexual selection, and evolution of reduced female gamete size. Evolution 58:24–34

Andersson M, Iwasa Y (1996) Sexual selection. Trends in Ecology & Evolution 11:53–58

Andersson M, Simmons LW (2006) Sexual selection and mate choice. Trends in Ecology & Evolution 21:296–302

Arnqvist G, Kirkpatrick M (2005) The evolution of infidelity in socially monogamous passerines: the strength of direct and indirect selection on extrapair copulation behavior in females. American Naturalist 165:S26–S37

Arnqvist G, Nilsson T (2000) The evolution of polyandry: multiple mating and female fitness in insects. Animal Behaviour 60:145–164

Bateman AJ (1948) Intra-sexual selection in *Drosophila*. Heredity 2:349–368

Bennet PM, Owens IPF (2002) Evolutionary ecology of birds: life histories, mating systems and extinction. Oxford University Press, Oxford.

Birkhead TR (1998) Sperm competition in birds : mechanisms and function. In: Birkhead TR, Møller AP (eds.) Sperm competition and sexual selection. Academic Press, London. pp. 579–622.

Birkhead TR, Møller AP (1993) Female control of paternity. Trends in Ecology & Evolution 8:100–104

Burke T, Daviest N, Bruford M, Hatchwell B (1989) Parental care and mating behaviour of polyandrous dunnocks *Prunella modularis* related to paternity by DNA fingerprinting. Nature 338:249–251

Carvalho CBV, Macedo RH, Graves JA (2006) Breeding strategies of a socially monogamous neotropical passerine: extra-pair fertilizations, behavior, and morphology. Condor 108:579–590

Cezilly F, Nager RG (1995) Comparative evidence for a positive association between divorce and extra-pair paternity in birds. Proceedings of the Royal Society B: Biological Sciences 262:7–12

Chapman T, Arnqvist G, Bangham J, Rowe L (2003) Sexual conflict. Trends in Ecology & Evolution 18:41–47

Clutton-Brock T (2007) Sexual selection in males and females. Science 318:1882–1885

Clutton-Brock T (2009) Sexual selection in females. Animal Behaviour 77:3–11

Clutton-Brock T, Parker G (1992) Potential reproductive rates and the operation of sexual selection. Quarterly Review of Biology 67:437–456

Costa FJV, Macedo RH (2005) Coccidian oocyst parasitism in the blue-black grassquit: influence on secondary sex ornaments and body condition. Animal Behaviour 70:1401–1409

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

Dias A (2008) Comparação e descrição de parâmetros acústicos do canto de *Volatinia jacarina* (Aves: Emberizidae) no contexto de seleção sexual. Dissertação de Mestrado, Universidade de Brasília, Brasília.

Dias RI, Kuhlmann M, Lourenço LR, Macedo RH (2009) Territorial clustering in the blue-black grassquit: reproductive strategy in response to habitat and food requirements? Condor 111:706–714

Double M, Cockburn A (2000) Pre-dawn infidelity: females control extra-pair mating in superb fairy-wrens. Proceedings of the Royal Society B: Biological Sciences 267:465–470

Doucet SM (2002) Structural plumage coloration, male body size, and condition in the blue-black grassquit. Condor 104:30–38

Emlen ST, Oring L (1977) Ecology, sexual selection, and the evolution of mating systems. Science 197:214–223

Enquist M, Arak A (1993) Selection of exaggerated male traits by female aesthetic senses. Nature 361:446–448

Eshel I, Volovik I, Sansone E (2000) On Fisher–Zahavi's handicapped sexy son. Evolutionary Ecology Research 2:509–523

Fandiño-Mariño H, Vielliard JME (2004) Complex communication signals: the case of the Blue-black Grassquit *Volatinia jacarina* (Aves, Emberizidae) song. Part I-A structural analysis. Anais da Academia Brasileira de Ciências 76:325–334

Gowaty PA (1996) Battles of the sexes and origins of monogamy. In: Black JM (ed.) Partnerships in birds: the study of monogamy. Oxford University Press, Oxford, pp. 21–52.

Gray EM (1996) Female control of offspring paternity in a western population of red-winged blackbirds (*Agelaius phoeniceus*). Behavioral Ecology and Sociobiology 38:267–278

Griffith SC, Owens IPF, Thuman KA (2002) Extra pair paternity in birds: a review of interspecific variation and adaptive function. Molecular Ecology 11:2195–2212

Kempenaers B, Verheyen G, Van den Broeck M, Burke T, Van Broeckhoven C, Dhondt AA (1992) Extra-pair paternity results from female preference for high-quality males in the blue tit. Nature 357:494–496

Kirkpatrick M, Ryan MJ (1991) The evolution of mating preferences and the paradox of the lek. Nature 350:33–38

Kokko H (2001) Fisherian and "good genes" benefits of mate choice: how (not) to distinguish between them. Ecology Letters 4:322–326

Kokko H, Brooks R, Jennions MD, Morley J (2003) The evolution of mate choice and mating biases. Proceedings of the Royal Society B: Biological Sciences 270:653–664

Kokko H, Brooks R, McNamara JM, Houston AI (2002) The sexual selection continuum. Proceedings of the Royal Society B: Biological Sciences 269:1331–1340

Kokko H, Jennions MD, Brooks R (2006) Unifying and testing models of sexual selection. Annual Review of Ecology, Evolution, and Systematics 37:43–66

Kotiaho JS, Puurtinen M (2007) Mate choice for indirect genetic benefits: scrutiny of the current paradigm. Functional Ecology 21:638–644

Kvarnemo C, Moore GI, Jones AG (2007) Sexually selected females in the monogamous Western Australian seahorse. Proceedings of the Royal Society B: Biological Sciences 274:521–525

Lack D (1968) Ecological adaptations for breeding in birds. Methuen, London.

Macedo RH, Karubian J, Webster MS (2008) Extrapair paternity and sexual selection in socially monogamous birds: are tropical birds different? The Auk 125:769–777

Macedo RH, Manica L, Dias RI (2012) Conspicuous sexual signals in a socially monogamous passerine: the case of neotropical blue-black grassquits. Journal of Ornithology 153:15–22

Maia R, Macedo RH (2010) Achieving luster: prenuptial molt pattern predicts iridescent structural coloration in blue-black grassquits. Journal of Ornithology 152:243–252

Marini MÂ, Sousa N, Borges F, Silveira M (2009) Biologia reprodutiva de *Elaenia cristata* (Aves: Tyrannidae) em cerrado do Brasil central. Neotropical Biology and Conservation 4:2–12

Marini MÂ, Borges F, Lopes L, Sousa N, Gressler D, Santos L, Paiva L, Duca C, Manica LT, Rodrigues SS, França L, Costa PM, França LC, Heming NM, Silveira MB, Pereira ZP, Lobo Y, Medeiros RCS, Roper JJ (2012) Breeding biology of birds in the cerrado of Central Brazil. Ornitología Neotropical, 23:385–405

Murray BGJ (1982) Territorial behavior of the blue-black grassquit. Condor 84:119–119

Møller AP, Birkhead TR (1993) Cuckoldry and sociality: a comparative study of birds. American Naturalist 142:118–140

Møller AP, Jennions MD (2001) How important are direct fitness benefits of sexual selection? Naturwissenschaften 88:401–415

Neff BD, Pitcher TE (2005) Genetic quality and sexual selection: an integrated framework for good genes and compatible genes. Molecular Ecology 14:19–38

Petrie M, Kempenaers B (1998) Extra-pair paternity in birds: explaining variation between species and populations. Trends in Ecology & Evolution 13:52–58

Proctor HC (1991) Courtship in the water mite *Neumania papillator*: males capitalize on female adaptations for predation. Animal Behaviour 42:589–598

Prum RO (2012) Aesthetic evolution by mate choice: Darwin’s really dangerous idea. Philosophical Transactions of the Royal Society of London, Series B 367:2253–2265

Ryan MJ (1998) Sexual selection, receiver biases, and the evolution of sex differences. Science 281:1999–2003

Sick H (2001) Ornitologia brasileira. Editora Nova Fronteira, Rio de Janeiro.

Sicsu P, Manica LT, Maia R, Macedo RH Here comes the sun: display behavior is influenced by sunlight incidence Behavioral Ecology and Sociobiology (submetido)

Sheldon BC (1993) Sexually transmitted disease in birds: occurrence and evolutionary significance. Philosophical Transactions of the Royal Society of London, Series B 339:491–497

Stutchbury BJM, Morton E (1995) The effect of breeding synchrony on extra-pair mating systems in songbirds. Behaviour 132:675–690

Stutchbury BJM, Morton ES (2001) Behavioral ecology of tropical birds. Academic Press, San Diego.

Stutchbury BJM, Morton ES, Piper WH (1998) Extra-pair mating system of a synchronously breeding tropical songbird. Journal of Avian Biology 29:72–78

Tarof SA, Ratcliffe LM, Kasumovic MM, Boag PT (2004) Are least flycatcher (*Empidonax minimus*) clusters hidden leks? Behavioral Ecology 16:207–217

Tori WP, Durães R, Ryder TB, Anciães M, Karubian J, Macedo RH, Uy JAC, Parker PG, Smith TB, Stein AC, Webster MS, Blake JG, Loiselle BA (2008) Advances in sexual selection theory: insights from tropical avifauna. Ornitologia Neotropical 19:151–163

Trivers R (1972) Parental investment and sexual selection. In: Campbell BG (ed.) Sexual selection and the descent of man, 1871-1971. Aldine Press, Chicago, pp. 136–179.

Wagner RH (1998) Hidden leks: sexual selection and the clustering of avian territories. In: Parker P, Burley N (eds.) Avian reproductive tactics: female and male perspectives, Ornithological Monographs No 49. American Ornithologists' Union, Washington, pp. 123–145.

Webber T (1985) Songs, displays, and other behavior at a courtship gathering of blue-black grassquits. Condor 87:543–546

Westneat DF, Stewart IRK (2003) Extra-pair paternity in birds: causes, correlates, and conflict. Annual Review of Ecology, Evolution, and Systematics 34:365–396

Zahavi A (1975) Mate selection-A selection for a handicap. Journal of Theoretical Biology 53:205–214

Zeh J, Zeh D (1996) The evolution of polyandry I: intragenomic conflict and genetic incompatibility. Proceedings of the Royal Society B: Biological Sciences 263:1711–1717

RESUMO EXPANDIDO

INTRODUÇÃO

Sinais comunicativos em muitas espécies são complexos e envolvem múltiplas modalidades que estimulam diferentes canais sensoriais, como a visão, audição e quimiorrecepção (Partan e Marler 2005). Em sinais multimodais, cada componente pode revelar um aspecto distinto e particular do estado geral do emissor, ou podem juntamente revelar aspectos comuns da qualidade do emissor (Møller e Pomiankowski 1993; Johnstone 1996; Candolin 2003; Hebets e Papaj 2004). É esperado que apenas indivíduos em boa qualidade sejam capazes de expressar e manter sinais elaborados (Zahavi 1975). No entanto, a relação entre qualidade e sinais sexuais pode ser mais complexa (e.g. Griffith 2000; Andersson et al. 2002). Por exemplo, uma característica pouco elaborada não necessariamente indica baixa qualidade de um individuo *per se*, mas que ele tenha feito "escolhas" sobre a alocação de recursos disponíveis entre vários traços sexuais.

Demandas conflitantes em sinais multimodais devem ocorrer devido a conflitos energéticos, biomecânicos ou fisiológicos (e.g. Lahti et al. 2011; Cardoso et al. 2012; Wagner et al. 2012). Múltiplos movimentos acrobáticos em exibições comportamentais, por exemplo, são possivelmente influenciados por conflitos de alocação de recursos devido à combinação simultânea de sinais energeticamente custosos. Embora muitas espécies de aves apresentem exibições elaboradas (Byers et al. 2010), poucos estudos investigaram possíveis demandas conflitantes na produção sincrônica dos múltiplos componentes do sinal (e.g. Patricelli e Krakauer 2010) e nenhum estudo, do nosso conhecimento, já registrou demandas conflitantes entre componentes motores em acrobacias aéreas.

A influência da sinais multimodais no sucesso reprodutivo e no sistema de acasalamento é também ainda pouco compreendida nas aves (Byers et al. 2010, Patricelli e Krakauer 2010). Fêmeas devem avaliar a qualidade genética ou não-genética de seus potenciais parceiros reprodutivos pela expressão e condição desses sinais sexuais (Candolin 2003, Byers et al. 2010). Mais intrigante ainda é a influência desses atributos na escolha de múltiplos parceiros sexuais em espécies socialmente monogâmicas. A poliandria sexual nestas espécies pode ser explicada pela busca de parceiros "extra par" que propiciem benefícios diretos (não-genéticos) ou indiretos (genéticos) para fêmeas que estabelecem vínculos sociais com machos de qualidade inferior (revisões em Griffith et al. 2002; Arnqvist e Kirkpatrick 2005; Akçay e Roughgarden 2007). Ao copularem com múltiplos parceiros, fêmeas podem ganhar maior acesso a recursos (Gray 1997), auxílio no cuidado parental (Burke et al. 1989; Rubenstein 2007) ou ainda garantir a fertilidade do parceiro sexual (benefícios diretos, Wetton e Parkin 1991; Sheldon 1994). Alternativamente, os benefícios podem estar associados à qualidade genética da prole (benefícios indiretos, Griffith et al. 2002; Neff e Pitcher 2005). Na seleção de parceiros extra par, fêmeas podem garantir a herança de "bons genes" que favoreçam a qualidade corporal ou atratividade dos seus filhotes (processo de Fisher-Zahavi, Zahavi 1975; Eshel et al. 2000; Kokko et al. 2006), ou de "genes compatíveis" que aumentem a heterozigosidade (i.e., nível de endogamia) e a viabilidade da prole (Neff e Pitcher 2005; Varian-Ramos e Webster 2012).

Nesse trabalho estudamos o tiziu (*Volatinia jacarina*), uma espécie cujos machos apresentam exibições comportamentais multimodais, compostas por sinais acústicos e visuais (Almeida e Macedo 2001; Sick 2001). As exibições de corte podem ser de dois tipos: completas, repetidos voos verticais ("saltos") sincronizados com rotações verticais e horizontais do eixo do corpo e uma curta vocalização, ou incompletas, apenas vocalizações emitidas enquanto o macho permanece empoleirado (Sick 2001). Durante a estação reprodutiva, os machos também adquirem uma plumagem negra iridescente que contrasta com manchas brancas subaxilares e evidenciadas durante as exibições completas.

O sistema de acasalamento é a monogamia social, porém fertilizações extra par são comuns (Almeida e Macedo 2001; Carvalho et al. 2006). Durante o período reprodutivo, os machos defendem territórios pequenos (13-72 m^2) e agregados, onde realizam as exibições comportamentais (Almeida e Macedo 2001; Sick 2001). Estudos anteriores propuseram que o sistema de acasalamento nessa espécie é semelhante a um "lek escondido" (Wagner 1998), onde fêmeas visitam agregações para escolha de parceiros mais atraentes ou para buscarem cópulas extra par. Os tizius cumprem todos os critérios para um lek escondido (Fletcher e Miller 2006): 1) agregação territorial, 2) formação de vínculo social entre macho e fêmea, 3) ocorrência de cópulas extra par, e 4) ausência de contribuição no cuidado parental e de monopolização de recursos pelo macho extra par. Entretando, essa questão ainda é pouco compreendida, sendo que trabalhos anteriores averiguaram apenas a importância de características do ambiente (disponibilidade de alimento e estrutura da vegetação) ou do risco predatório dos ninhos sobre a distribuição espacial dos tizius (Almeida e Macedo 2001, Aguilar et al. 2008a, Dias et al. 2009).

Nesse trabalho nós avaliamos a influência das características sexuais secundárias de machos de tiziu nos sistemas social e sexual de acasalamento. Inicialmente testamos a presença de demanda conflitante nas exibições de corte de machos de tiziu i) em uma mesma modalidade de sinal, relacionando a duração do salto, altura do salto e rotação vertical do corpo e ii) entre diferentes modalidades e frequências de exibição, relacionando altura do salto e rotação com parâmetros do canto (duração do canto e banda de frequência) e com a taxa de exibições. Posteriormente, descrevemos os padrões sociais e genéticos de acasalamento em tizius e testamos as hipóteses de benefícios diretos (disponibilidade de alimento) e indiretos (bons genes e compatibilidade genética) na escolha das fêmeas por parceiros sociais e sexuais. Com esse objetivo, utilizamos os atributos motores e acústicos das exibições dos machos como parâmetros de qualidade ou atratividade. Finalmente, testamos a influência da distribuição espacial e temporal dos machos nos sistemas de acasalamento social e sexual. Mais especificamente, testamos as seguintes predições de dois modelos de lek escondido: i) machos em territórios centrais e que se estabelecem primeiro nas agregações são mais atraentes ou mais saudáveis e são bem sucedidos em fertilizações (modelo do macho atraente ou *hotshot*) e ii) o número de machos nas agregações aumenta a chance de pareamento social e a ocorrência de fertilizações extra par (modelo de preferência das fêmeas por agregações).

MÉTODOS

Coletamos os dados em três estações reprodutivas (outubro a fevereiro, 2008-2009, 2009-2010 e 2010-2011; anos 1, 2 e 3, daqui em diante) na Fazenda Água Limpa (15°56'S 47°56'W) localizada a 28 km de Brasília, DF. A área de estudo consistiu em 6,56 ha, aproximadamente, em um pomar inativo e em processo de regeneração natural, e em áreas adjacentes cobertas por gramíneas invasoras e vegetação típica de cerrado.

Dados morfológicos e comportamentais dos machos

Capturamos machos de tizius com redes de neblina e anilhamos cada indivíduo com uma combinação de três anilhas coloridas e uma metálica fornecida pelo CEMAVE/ICMBio. Medimos a massa corporal com balança de mola (precisão 0,2 g) e o comprimento do tarso com paquímetro (precisão 0,1 mm) de cada indivíduo. Calculamos um índice de condição corporal pela razão entre massa corporal e comprimento do tarso, que é um parâmetro indicativo de qualidade corporal em relação à infecção por parasitas (Costa e Macedo 2005; Aguilar et al. 2008b). Coletamos uma amostra de sangue de aproximadamente 60 μl pela punção da veia braquial de cada indivíduo e armazenamos em solução tampão (Seutin et al. 1991) a 4ºC.

Filmamos machos em exibições completas com mini-câmeras digitais (Canon XL1 ou Casio EX-FH25) em três dias diferentes sempre que possível. Após transferir as filmagens para o computador, medimos a duração e altura do salto e o ângulo da rotação vertical do corpo no ápice do salto (rotação, daqui em diante). Calculamos a duração do salto no programa Windows® Movie Maker v. 5.1 (Microsoft Corporation 2007) pelo intervalo de tempo entre o início do salto (momento em que o macho sai do poleiro) e o fim do salto (momento em que o macho pousa de volta no poleiro). Medimos a altura do salto no programa ImageJ® v. 1.45s (Schneider et al. 2012) pela distância entre a ponta do bico do macho no ápice do salto e uma linha horizontal que cruza o local onde ele estava empoleirado (Capítulo 1). Usando o mesmo programa, medimos a rotação como sendo o ângulo formado entre os eixos do corpo no início e no ápice do salto (Capítulo 1).

Gravamos as vocalizações dos machos utilizando um gravador digital Marantz PMD 660 (precisão de 16 bits e taxa de amostragem de 44.1 Hz) acoplado a um microfone unidirecional Sennheiser K6/ME66 ou omnidirecional Sennheiser K6/ME62 e uma parábola Telinga. Utilizando o programa Audacity® v. 2.0.0 (http://audacity.sourceforge.net), selecionamos cinco amostras do canto, excluindo as notas introdutórias que precedem o canto para medição da duração e banda de frequência do canto (Capítulo 1). No programa Cool Edit Pro® v. 2.1 (Syntrillium Software Corporation 2003), medimos a duração do canto a partir dos gráficos de oscilograma e espectrograma, e a banda de frequência a partir do gráfico de espectro de potência pela diferença entre as frequências mínima e máxima obtidas e utilizando -24 dB como linha de corte (método utilizado por Podos 1997).

Em observações focais de aproximadamente 30 minutos e em três dias diferentes sempre que possível, registramos o tempo, a ocorrência e o tipo de exibição (completa ou incompleta) executada. A partir desses dados calculamos a intensidade de exibições (soma de exibições completas e incompletas dividido pelo tempo total em exibições) e a proporção de exibições completas em relação ao total de exibições realizadas.

Monitoramento de ninhos

Procuramos ninhos em toda área de estudo seguindo indivíduos e inspecionando potenciais sítios de nidificação. Checamos o número de ovos e filhotes presentes no ninho a cada dois ou três dias, ou diariamente quando próximo da eclosão ou saída dos filhotes do ninho para definir precisamente a idade dos mesmos. Identificamos os pais sociais dos filhotes pela identificação dos adultos no cuidado parental em observações focais dos ninhos de aproximadamente 15 minutos. Determinamos que um macho obteve sucesso em pareamento quando registramos ao menos uma tentativa de nidificação daquele indivíduo.

Medimos a massa corporal dos filhotes com balança de mola (precisão 0,1 g) e o comprimento do tarso com paquímetro (precisão 0,1 mm) após a eclosão (1 a 3 dias). Calculamos um índice de condição corporal pela razão entre massa corporal e comprimento do tarso. Coletamos uma amostra de sangue de aproximadamente 20 µl pela punção da veia braquial de cada filhote e armazenamos em solução tampão (Seutin et al. 1991) a 4ºC.

Distribuição espacial e temporal dos machos

Procuramos por machos em exibições pelo menos três vezes por semana na área de estudo. Consideramos que um macho estabeleceu território se ele foi regularmente registrado na mesma área por um período mínimo de duas semanas ou se ele obteve ao menos uma tentativa reprodutiva. Consideramos a presença de ninhos cujos machos sociais eram desconhecidos e que estavam ativos em sincronia com ninhos de machos conhecidos para estimar com precisão o tamanho da agregação. Definimos a data de estabelecimento do macho no território como o primeiro dia em que registramos um macho territorial em exibição na área de estudo (dia 1 = 24 de outubro).

Definimos agregações como dois ou mais territórios isolados de outros territórios por vegetação que impedisse o contato visual entre indivíduos ou por áreas desocupadas por coespecíficos a uma distância mínima de 20 m (média ± desvio-padrão = 45,0 ± 15,5 m). Machos solitários foram definidos como aqueles cujos territórios estavam separados de outros territórios por no mínimo 50 m. Utilizando um GPS (*Global Positioning System*), registramos as coordenadas geográficas (*Universal Transverse Mercator coordinates*, *datum* WGS84) de cada território no local de nidificação ou, no caso de machos que não tiveram tentativa reprodutiva, no local onde eram encontrados mais frequentemente em exibições. Definimos as agregações utilizando o programa Google Earth® v. 7.0.2. No programa AutoCad® 2013 traçamos o mínimo polígono convexo unindo os pontos extremos de uma agregação e medimos a distância linear de um território ao centro geométrico do polígono (centralidade, daqui em diante) (Capítulo 3).

Disponibilidade de alimento no território

Estimamos a densidade de sementes no território dos machos em quatro parcelas de 0,5 x 0,5 m posicionadas nas quatro direções cardeais a partir do ninho. Contamos o número de inflorescências com sementes e sem sementes, que são indicativas de produção prévia de sementes. Calculamos a densidade pela média do número de inflorescências nas quatro parcelas.

Análises moleculares e de parentesco

Extraímos o DNA das amostras de sangue utilizando o protocolo do fabricante Puregene® DNA Isolation Kit. Amplificamos 15 marcadores microssatélites, cujos iniciadores foram marcados com fluorescências, distribuídos em quatro grupos de Reações em Cadeia da Polimerase Multiplex (Capítulo 2). Os genótipos dos indivíduos foram registrados utilizando o sequenciador Beckman Coulter CEQ^{TM} e o programa CEQ Genetic Analysis System 8000^{TM}. Agrupamos os tamanhos dos fragmentos utilizando o pacote MsatAllele (Alberto 2009) no programa R (R Development Core Team 2011). Testamos o equilíbrio de Hardy-Weinberg e desequilíbrio de ligação, utilizando o programa Genepop 4.1.1 (Rousset 2008) e a presença de alelos nulos utilizando programa Micro-Checker (van Oosterhout et al. 2004). O polimorfismo dos microssatélites variou de 4 a 29 alelos por loco e a probabilidade combinada de exclusão foi 0,9837, 0,9479 e 0,9413, para os anos 1, 2 e 3, respectivamente, e 0,9991, 0,9950 e 0,9931, respectivamente, quando o genótipo de um dos pais era conhecido (Capítulo 2).

Utilizamos o programa Cervus 3.0.3 (Kalinowski et al. 2007) para atribuir paternidade/maternidade ao macho/fêmea candidatos pelo cálculo da razão de verossimilhança (probabilidade do macho/fêmea candidato(a) ser o verdadeiro parental dividida pela probabilidade de não ser o verdadeiro parental). Inicialmente, realizamos as análises maternidade para confirmar se as fêmeas que incubaram ou alimentaram os filhotes eram realmente as mães biológicas. Quando a maternidade foi confirmada, a identidade da mãe foi incluída nas análises de paternidade. Para estimar o sucesso reprodutivo dos machos em cada estação reprodutiva registramos: a) a perda de fertilização de pelo menos um filhote na prole social (“perda de FIP”, 0 = sem perda, 1 = com perda) e b) o sucesso em fertilização de pelo menos um filhote extra par (“sucesso em FEP”, 0 = sem sucesso, 1 = com sucesso).

Estimamos a relação de parentesco entre fêmeas e parceiros sociais ou extra par pelo coeficiente de parentesco “r” de Queller e Goodnight (1989) no programa SPAGeDi v. 1.3 (Hardy e Vekemans 2002). Estimamos o índice de heterozigosidade ponderado por loco (HL, Aparicio et al. 2006) para medir o nível de endogamia de cada indivíduo.

Análises estatísticas

Relacionamos os parâmetros motores das exibições entre si utilizando regressões lineares múltiplas e incluindo a duração do salto como variável resposta e altura, rotação e a interação entre altura e rotação como variáveis preditoras. Comparamos os parâmetros motores (duração, altura e rotação) de machos monitorados repetidamente em diferentes anos utilizando testes t pareados. Relacionamos os parâmetros motores altura e rotação com duração e banda de frequência do canto, intensidade de exibições, proporção de exibições completas, índice de condição corporal, interação entre índice de condição corporal e intensidade de exibições ou proporção de exibições completas, e ano utilizando regressões lineares múltiplas.

Utilizamos regressões logísticas para relacionar o sucesso em pareamento, perda de FIP e sucesso em FEP com altura do salto, proporção de exibições completas, duração e banda de frequência do canto e ano. Análises prévias indicaram que altura do salto não influenciou a perda de FIP e o sucesso em FEP (resultados não apresentados), portanto, nós excluímos essa variável das análises finais para aumentar o tamanho amostral uma vez que o número de indivíduos com dados completos foi pequeno (22 machos). Comparamos machos sociais e extra par em relação à densidade de sementes no território, atributos motores e acústicos, relação de parentesco com as fêmeas e HL usando testes t pareados. Comparamos índices corporais e HL de filhotes intra e extra par utilizando testes t pareados.

Relacionamos as características das exibições (altura do salto, proporção de exibições completas, duração e banda de frequência do canto), perda de FIP e sucesso em FEP com centralidade e data de estabelecimento dos machos em agregações utilizando regressões lineares múltiplas mistas, incluindo o fator aleatório "agregação aninhada dentro de ano". Relacionamos a proporção de machos bem sucedidos em pareamento e a proporção de ninhos com perda de FIP com o número de machos na agregação utilizando regressões lineares múltiplas mistas, incluindo o fator aleatório ano.

Utilizamos combinações das variáveis preditoras dos modelos de regressão múltipla para criar um conjunto de modelos que foram ranqueados com base no Critério de Informacão de Akaike de segunda ordem (*second-order Akaike's Information Criteria*, AICc, Burnham e Anderson 2002). Calculamos a probabilidade de cada modelo (peso de Akaike), e as estimativas ponderadas pelos pesos de Akaike, o erro-padrão e o intervalo de confiança de 95% (IC) de cada variável preditora para inferir sobre sua importância nos modelos. Para realizar essas análises, utilizamos o pacote AICcmodavg (Mazerolle 2010) no programa R (R Development CoreTeam 2011). Em todos os modelos, testamos as premissas de normalidade na distribuição das variáveis resposta e preditoras, homogeneidade das variâncias e colinearidade. Quando necessário, transformamos as variáveis para melhor ajuste do modelo. Toda as análises foram realizadas no programa R (R Development CoreTeam 2011) com alfa = 0,05. Todos os valores são apresentados como média ± desvio padrão, exceto quando indicado.

RESULTADOS

Demanda conflitante em sinais multimodais

O melhor modelo da duração do salto de 55 machos baseado nos valores de AICc incluiu a interação entre altura do salto e rotação ($\beta \pm$ EP = 0,24 ± 0,11, IC = 0,03, 0,45, Tabela 1), sendo que a duração reduziu com o aumento na rotação apenas entre machos com saltos mais baixos (≤ 19,8 cm, Fig. 1). A duração do salto aumentou com a altura ($\beta \pm$ EP = 0,76 ± 0,09, IC = 0,57, 0,93) independentemente da rotação, enquanto que reduziu com a rotação, embora essa evidência seja mais fraca ($\beta \pm$ EP = -0,14 ± 0,09, IC = -0,32, 0,04, Tabela 1). A duração, altura e rotação do salto não diferiram entre anos em machos monitorados repetidamente em diferentes estações reprodutivas (média das diferenças: duração = 0,02 s, IC = -0,10, 0,06, t_7= 0,46, p = 0,27; altura = 2,3 cm, IC = - 4,6, 9,3, t_7= 0,80, p = 0,23 e rotação = 3,1°, IC = -22,8, 16,7, t_7 = 0,34, p = 0,72).

A altura do salto de 36 machos foi melhor explicada pela interação entre índice de condição corporal e proporção de exibições completas (Tabela 1). Altura do salto reduziu com a proporção de exibições completas em machos com menores índices de condição corporal (< 0,56), enquanto que essa relação foi positiva em machos com maiores índices de condição corporal (≥ 0,56) (Fig. 2). Nenhuma outra variável (duração e banda de frequência do canto, intensidade de exibições e interação índice de condição corporal x intensidade de exibições) foi importante no modelo da altura do salto (Tabelas 1 e 2). A rotação do corpo dos mesmos 36 machos aumentou com as proporções de exibições completas, porém não encontramos evidências da influência de variáveis do canto, intensidade de exibições, índice de condição corporal e interações sobre a rotação (Tabelas 1 e 2).

Tabela 1. Resultado da seleção de modelos da duração do salto em relação a altura e rotação e modelos da altura e rotação em relação a outros parâmetros das exibições e da condição corporal. K = número de parâmetros, AICc = Critério de Informação de Akaike, ΔAICc = diferença entre AICc do modelo e o AICc mínimo encontrado dentre os models e w_i = peso de Akaike.

Modelo/variável preditora[1]	K	AICc	ΔAICc	w_i
Duração do salto				
Altura*rotação	5	114,89	0,00	0,677
Altura+rotação	4	117,75	2,86	0,162
Altura	3	117,77	2,88	0,161
Altura do salto				
Intensidade*ICC + % exibição*ICC	4	86,23	0,00	0,280
% exibição*ICC	3	86,24	0,01	0,279
Nulo	2	88,32	2,08	0,099
% exibição	3	89,59	3,36	0,052
ICC	3	89,91	3,68	0,044
Freq	3	89,95	3,72	0,044
Sdur	3	90,03	3,80	0,042
Rotação				
Ano	4	95,26	0,00	0,644
Intensidade + % exibição + ano	6	97,86	2,60	0,175
Intensidade*ICC + % exibição*ICC + ano	6	99,03	3,77	0,098

Modelos com ΔAICc > 4 foram omitidos (ver Tabela 2, Capítulo 1).

[1] Variáveis preditoras: Duração (sdur) e banda de frequência do canto (freq), intensidade de exibições (intensidade), proporção de exibições completas (% exibição) e índice de condição corporal (ICC).

Tabela 2. Estimativas ponderadas pelos pesos de Akaike dos modelos ± erro padrão e intervalos de confiança de 95% da duração do canto, banda de frequência, intensidade de exibições, proporção de exibições completas, índice de condição corporal, ano e interações nos modelos de altura do salto e rotação das exibições de machos de tiziu.

Modelo/variável preditora	Estimativa ± EP	IC 95%
Altura do salto		
Duração do canto	0,13 ± 0,19	-0,24, 0,50
Banda de frequência	0,12 ± 0,16	-0,18, 0,43
Intensidade de exibições	0,02 ± 0,17	-0,31, 0,34
Proporção de exibições completas	-0,17 ± 0,16	-0,48, 0,15
Índice de condição corporal	0,17 ± 0,19	-0,21, 0,55
Intensidade de exibições * condição corporal	0,02 ± 0,21	-0,38, 0,42
Proporção de exibições completas * condição corporal	0,53 ± 0,24	0,06, 1,00
Ano		
Ano 2	0,64 ± 0,49	-0,32, 1,59
Ano 3	0,64 ± 0,50	-0,34, 1,62
Rotação		
Duração do canto	0,03 ± 0,17	-0,31, 0,37
Banda de frequência	-0,08 ± 0,17	-0,42, 0,25
Intensidade de exibições	-0,08 ± 0,18	-0,45, 0,28
Proporção de exibições completas	0,28 ± 0,18	-0,07, 0,63
Índice de condição corporal	0,01 ± 0,22	-0,42, 0,44
Intensidade de exibições * condição corporal	0,16 ± 0,23	-0,61, 0,29
Proporção de exibições completas * condição corporal	0,16 ± 0,23	-0,30, 0,62
Ano		
Ano 2	-1,63 ± 0,49	-2,59, -0,66
Ano 3	-1,89 ± 0,51	-2,88, -0,90

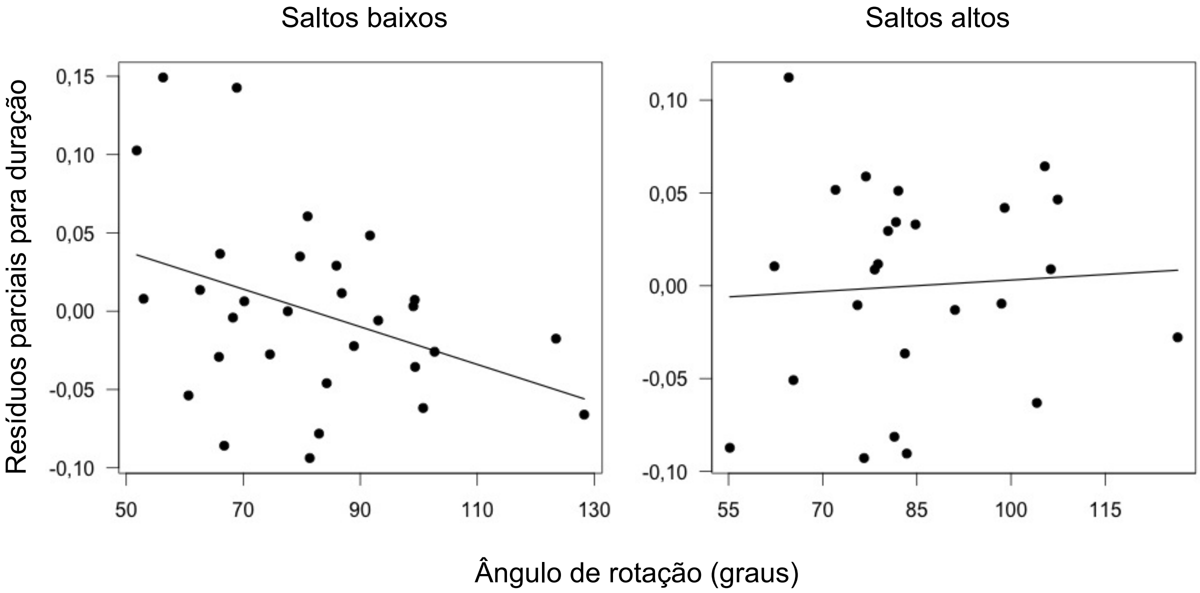

Figura 1. Relação entre duração do salto (representada por resíduos parciais, controlando para a altura do salto) e rotação nas exibições de machos de tizius em saltos baixos (≤ 19,8 cm, à esquerda) e saltos altos (> 19,8 cm, à direita).

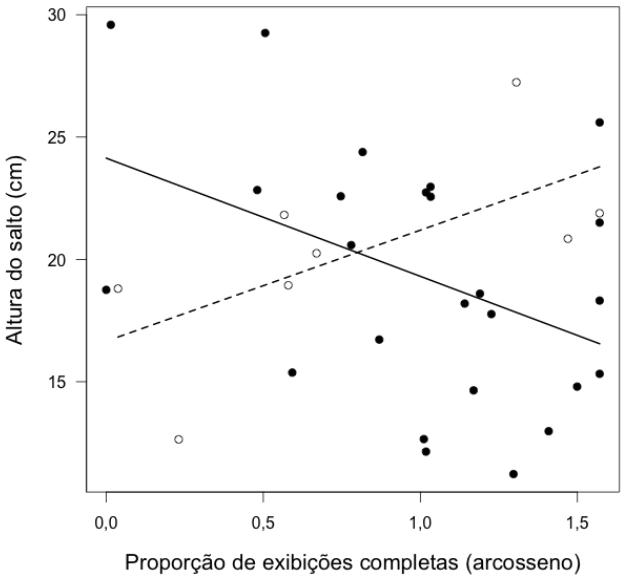

Figura 2. Relação entre altura do salto e proporção de exibições completas (transformada em arcosseno) de machos de tizius com baixos índices de condição corporal (< 0,56, círculos fechados, linha contínua) e altos índices de condição corporal (≥ 0,56, círculos abertos, linha pontilhada).

Sistema de acasalamento

Durante os três anos de estudo monitoramos 174 ninhos de tizius, do quais 131 obtivemos material genético dos filhotes para análises de parentesco. Identificamos as mães sociais de 56 ninhos e pais sociais de 95 ninhos. Confirmamos a maternidade das mães sociais da maioria dos filhotes (94,5% dos filhotes e 91,1% das ninhadas). Registramos parasitismo intraespecífico em três ninhos, cujos machos sociais também não eram pais genéticos dos filhotes, e quasi-parasitismo em dois ninhos, cujos machos sociais eram os pais biológicos dos filhotes extra par. O nível de paternidade extra par variou largamente entre os anos, de 8,2% a 34,2% entre todos os filhotes e de 11,4% a 47,1% entre todas as ninhadas analisadas. Os níveis foram maiores na segunda estação reprodutiva (ano 2) e menores na terceira estação reprodutiva (ano 3). Machos sociais perderam paternidade em toda a ninhada em 13,7% dos ninhos, mas em 16,8% dos ninhos monitorados os machos sociais eram pais biológicos de pelo menos um filhote na ninhada. Identificamos 21 machos extra par, dos quais 17 também formaram vínculo social com uma fêmea e nove produziram ao menos um filhote intra par.

Sucesso reprodutivo e características dos machos

Relacionamos o sucesso em pareamento com características das exibições de 31 machos. Aqueles que saltaram mais alto tiveram maior sucesso em pareamento (β ± EP = 0,70 ± 0,36, IC = -0,002, 1,40, Fig. 3, Tabela 3). A proporção de exibições completas (β ± EP = -0,42 ± 0,42, IC = -1,25, 0,41), a duração do canto (β ± EP = 0,13 ± 0,28, IC = -0,41, 0,67) e a banda de frequência (β ± EP = 0,29 ± 0,29, IC = -0,27, 0,86) não explicaram o sucesso em pareamento (Tabela 3). O sucesso em pareamento foi maior no terceiro ano de estudo, porém a evidência foi fraca (β ± EP = 1,40 ± 0,84, IC = -0,24, 3,05, Tabela 3).

Relacionamos a probabilidade de perda de FIP e o sucesso em FEP com atributos motores e acústicos de 32 e 33 machos, respectivamente. Encontramos uma tendência a maiores perdas de FIP entre machos cujos cantos eram mais longos (β ± EP = 0,66 ± 0,41, IC = -0,15, 1,47, Fig. 4), evidenciada pela razão das probabilidades dos modelos que incluem essa variável em relação ao modelo nulo de 2,4. A perda de FIP não dependeu da proporção de exibições completas (β ± EP = -0,05 ± 0,39, IC = -0,82, 0,71) e da banda de frequência (β ± EP = -0,49 ± 0,35, IC = -1,17, 0,19) (Tabela 3). Não encontramos evidência de importância de nenhuma característica do macho no sucesso em FEP (β ± EP, IC 95%: proporção exibições completas = 0,15 ± 0,38, -0,55, 0,85; duração do canto = -0,26 ± 0,38, -1,01, 0,49; banda de frequência = 0,03 ± 0,36, -0,68, 0,74 e ano 2 = 1,26 ± 0,82, -0,33, 2,86, Tabela 3).

Tabela 3. Resultado da seleção de modelos da probabilidade de sucesso em pareamento, perda de fertilização intra par (FIP) e sucesso em fertilização extra par (FEP) de machos de tizius. K = número de parâmetros, AICc = Critério de Informação de Akaike, ΔAICc = diferença entre AICc do modelo e o AICc mínimo encontrado dentre os models e w_i = peso de Akaike.

Modelo/variável preditora[1]	K	AICc	ΔAICc	w_i
Sucesso em pareamento				
% exibição+altura+ano	4	32,88	0,00	0,291
Altura	2	32,91	0,03	0.287
Nulo	1	35,26	2,38	0,089
% exibição+altura	3	35,33	2,46	0,085
Freq	2	35,77	2,89	0,068
Ano	2	36,08	3,21	0,059
Perda de FIP				
Sdur	2	39,75	0,00	0,181
Ano	3	39,77	0,02	0,179
Sdur+freq	3	39,84	0,10	0,173
Nulo	1	40,16	0,41	0,148
Freq	2	40,39	0,64	0,132
Sdur+freq+ano	5	40,78	1,04	0,108
% exibição	2	42,18	2,43	0,054
% exibição+sdur+freq+ano	6	43,63	3,88	0,026
Sucesso em FEP				
Ano	2	40,24	0,00	0,358
Nulo	1	40,80	0,56	0,270
Sdur	2	42,72	2,48	0,103
% exibição	2	42,93	2,69	0,093
Freq	2	43,06	2,83	0,087

Modelos com ΔAICc > 4 foram omitidos (ver Tabela 5, Capítulo 2).

[1] Variáveis resposta: 0 ou 1, variáveis preditoras: Duração (sdur) e banda de frequência do canto (freq), proporção de exibições completas (% exibição), altura do salto (altura) e ano = 2008-2009, 2009-2010 e 2010-2011.

Ao comparar machos sociais e extra par não encontramos diferença relativas à densidade de sementes nos territórios (62,5 ± 33,7 e 58,9 ± 36,3 sementes/m^2, respectivamente, t_6 = 0,22, p = 0,83); duração do canto (0,40 ± 0,02 e 0,39 ± 0,05 s, respectivamente, t_5 = 0,54, p = 0,61); banda de frequência do canto (7,30 ± 0,43 e 6,90 ± 0,95 kHz, respectivamente, t_6 = 0,22, p = 0,83), altura do salto (21,8 ± 8,2 e 21,2 ± 5,7 cm, respectivamente, t_3 = 0,12, p = 0,91) e proporção de exibições completas (0,65 ± 0,41 e 0,88 ± 0,13, respectivamente, t_2 = 1,35, p = 0,31).

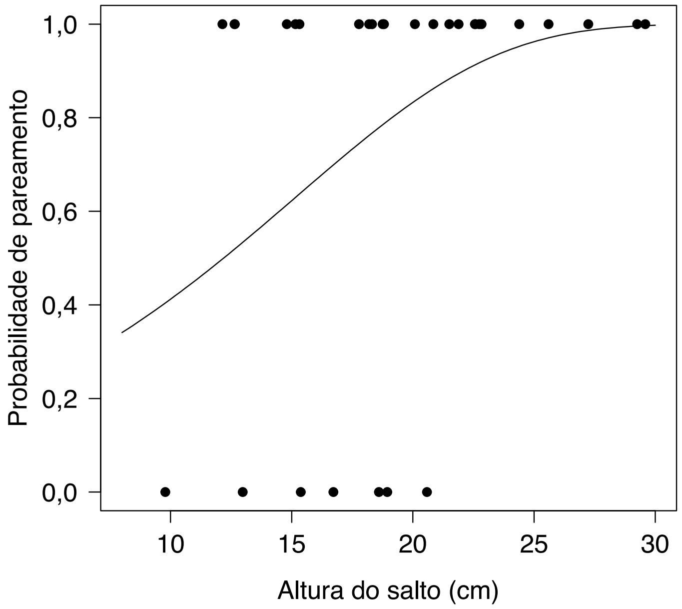

Figura 3. Probabilidade de pareamento de machos de tiziu em relação a altura do salto durante as exibições completas. A linha representa a regressão logística predita pelo modelo e os pontos representam as observações.

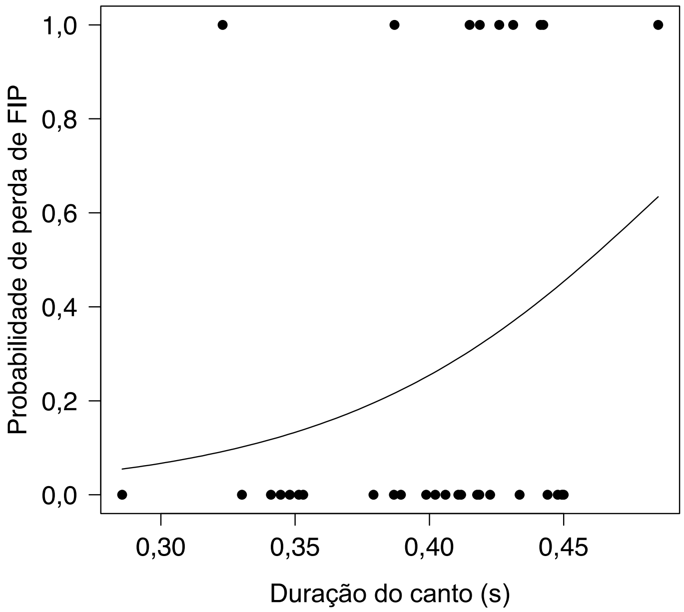

Figura 4. Probabilidade de perda de fertilizações intra par por machos de tiziu em relação à duração do canto. A linha representa a regressão logística predita pelo modelo e os pontos representam as observações

Similaridade genética e heterozigosidade

Em ninhadas com filhotes extra par (r = 0,0006 ± 0,20) fêmeas não eram mais similares geneticamente a seus parceiros sociais em comparação a parceiros sociais de ninhadas com apenas filhotes intra par (r = 0,02 ± 0,20, t_{47} = 0,39, p = 0,70). Fêmeas e machos sociais não eram mais aparentados entre si do que fêmeas e machos extra par (r_{social} = 0,01 ± 0,23, $r_{extra\ par}$ = 0,07 ± 0,21, t_8 = 0,62, p = 0,55). Machos extra par não apresentaram menor nível de endogamia do que machos sociais (HL_{social} = 0,33 ± 0,16, $HL_{extra\ par}$ = 0,31 ± 0,17, t_9 = 0,22, p = 0,83).

Características dos filhotes

Filhotes intra e extra par não diferiram no nível de endogamia (HL: 0,32 ± 0,17 e 0,35 ± 0,11, respectivamente, t_{16} = 0,56, p = 0,58) e no índice de condição corporal (0,31 ± 0,05 e 0,34 ± 0,08 g/mm, respectivamente, t_{12} = 0,19, p = 0,50).

Agregações territoriais

Registramos 224 territórios, dos quais apenas dois eram isolados e os demais, distribuídos em 32 agregações (Capítulo 3). O número de machos nas agregações variou de 2 a 19 (6,9 ± 4,6) e a distância de cada território ao centro da agregação variou de 1,8 a 72,3 m^2 (26,9 ± 9,7).

Estabelecimento de territórios e características dos machos

Relacionamos centralidade e data de estabelecimento nos territórios com as características de 58 machos, porém os tamanhos amostrais em cada análise variaram em função da variável preditora incluída nos modelos (Tabela 4). Não encontramos relação entre centralidade e proporção de exibições completas (β ± EP = 0,20 ± 0,22, IC = -0,22, 0,63) (Apêndice), portanto excluímos essa variável dos modelos finais. Da mesma forma, não encontramos evidências de relação entre centralidade e as demais características (β ± EP, IC 95%: duração do canto = -0,13 ± 0,15, -0,17, 0,42; banda de frequência = 0,08 ± 0,15, = -0,21, 0,38; altura do salto = 0,13 ± 0,15, = -0,17, 0,43, Tabela 4). Encontramos fraco suporte para influência da proporção de exibições completas na data de estabelecimento (Tabela 4), embora o intervalo de confiança da estimativa seja positivo (β ± EP= 0,28 ± 0,12, IC = 0,03, 0,52). A data de estabelecimento relacionou-se negativamente com altura do salto (β ± EP = -0,28 ± 0,12, IC = -0,52, -0,09, Fig. 5) e duração do canto (β ± EP = -0,33 ± 0,12, IC = -0,58, -0,09, Fig. 6) (Tabela 4), indicando que machos que saltam mais alto e têm canto mais longo são os primeiros a estabelecerem territórios. A banda de frequência não foi importante nos modelos de data de estabelecimento (β ±EP = -0,15 ± 0,13, IC = -0,40, 0,10, Tabela 4).

Tabela 4. Resultado da seleção de modelos de centralidade e data de estabelecimento em relação às características das exibições de machos de tiziu. Proporção de exibições completas excluída dos modelos finais de centralidade devido à sua fraca importância (veja Apêndice). Modelo da data de estabelecimento: altura do salto excluída (*n* = 43) e proporção de exibições completas excluída (*n* = 41 machos). K = número de parâmetros, AICc = Critério de Informação de Akaike, ΔAICc = diferença entre AICc do modelo e o AICc mínimo encontrado dentre os models e w_i = peso de Akaike.

Modelo/variável preditora[1]	K	AICc	ΔAICc	w_i
Centralidade[2]				
Nulo	4	123,58	0,00	0,451
Altura	5	125,50	1,92	0,173
Sdur	5	125,57	1,99	0,166
Freq	5	125,93	2,35	0,139
Data de estabelecimento[2]				
Altura excluída				
Sdur+freq+% exibição	7	112,24	0,00	0,344
Sdur	5	112,28	0,05	0,336
Sdur+freq	6	114,09	1,86	0,136
% exibição	5	114,57	2,33	0,107
% exibição excluída				
Sdur+freq+altura	7	109,52	0,00	0,541
Sdur	5	111,90	2,38	0,165
Sdur+freq	6	111,95	2,43	0,161

Modelos com ΔAICc > 4 foram omitidos (ver Tabela 3, Capítulo 3).

[1] Variáveis preditoras: duração do canto (sdur), banda de frequência do canto (freq), proporção de exibições completas (% exibição) e altura do salto (altura).

[2] Variância dos efeitos aleatórios:

Modelo centralidade: agregação/ano = 0,16, ano < 0,001.

Modelo data de estabelecimento, altura excluída: agregação/ano < 0,001, ano = 0,63 e % exibição excluída: agregação/ano < 0,001, ano = 0,80.

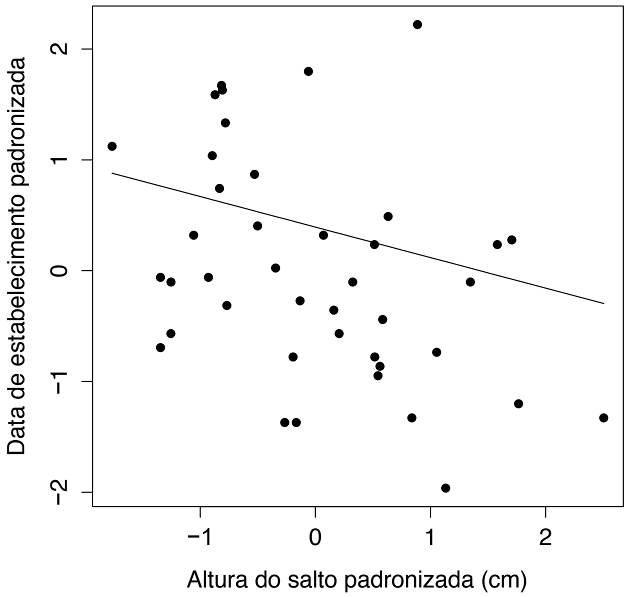

Figura 5. Relação entre a data de estabelecimento (dia 1 = 24 de outubro) e a altura do salto de machos de tiziu. Os eixos representam as variáveis centralizadas em zero e padronizadas pelo desvio padrão.

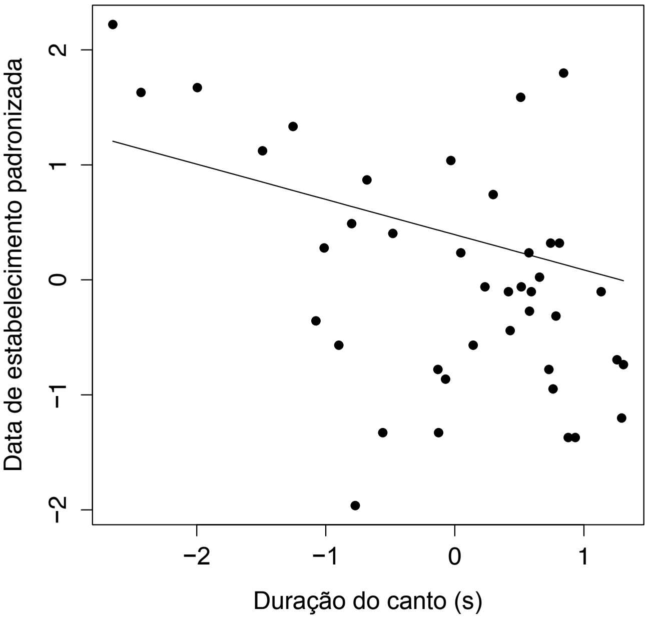

Figura 6. Relação entre a data de estabelecimento (dia 1 = 24 de outubro) e a duração do canto de machos de tiziu. Os eixos representam as variáveis centralizadas em zero e padronizadas pelo desvio padrão.

Fertilizações extra par nas agregações

Encontramos fraco suporte para a hipótese de que a perda de FIP é influenciada pela centralidade ou data de estabelecimento (β ± EP, IC: 0,25 ± 0,35, -0,45, 0,95 e 0,25 ± 0,34, -0,43, 0,92, respectivamente, Tabela 5) (*n* = 24 machos). O sucesso em FEP não relacionou-se com centralidade (β ± EP = -0,41 ± 0,36, IC = -1,12, 0,31), porém foi explicada pela data de estabelecimento dos machos nas agregações (β ± EP = -0,79 ± 0,35, IC = -1,49, -0,09, *n* = 40 machos, Tabela 5, Fig. 7).

Tabela 5. Resultado da seleção de modelos da probabilidade de perda de fertilização intra par (FIP) e sucesso em fertilização extra par (FEP) em relação à centralidade e data de estabelecimento de machos de tizius. K = número de parâmetros, AICc = Critério de Informação de Akaike, ΔAICc = diferença entre AICc do modelo e o AICc mínimo encontrado dentre os modelos e w_i = peso de Akaike.

Modelo/variável preditora	K	AICc	ΔAICc	w_i
Perda de FIP[1]				
Data	4	41,38	0,00	0,767
Centralidade+data	5	44,16	2,77	0,192
Nulo	3	47,89	6,51	0,030
Centralidade	4	49,85	8,47	0,011
Sucesso em FEP[2]				
Data	4	49,47	0,00	0,475
Nulo	3	51,05	1,58	0,216
Centralidade+data	5	51,10	1,62	0,211
Centralidade	4	52,62	3,15	0,099

Variância do efeito aleatório:

[1] agregação/ano < 0,001, ano < 0,001.

[2] agregação/ano < 0,001, ano = 0,10.

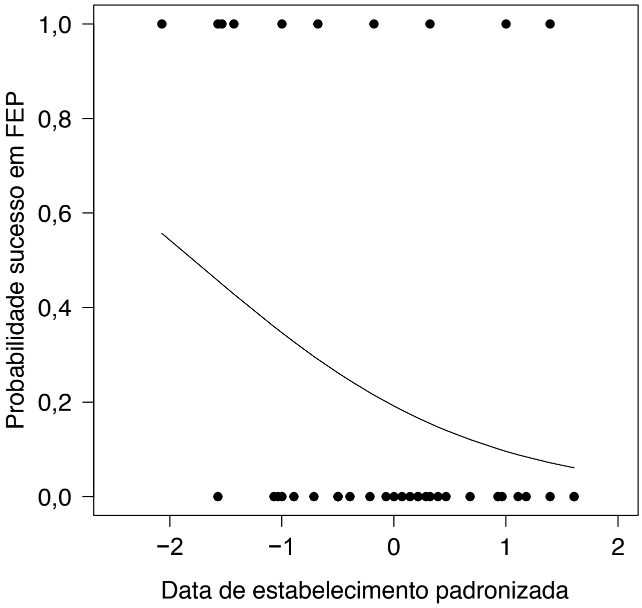

Figura 7. Probabilidade de sucesso em fertilização extra par e data de estabelecimento dos machos de tizius. A curva representa valores preditos pelo modelo e os pontos representam os valores observados. A data de estabelecimento foi centralizada em zero e padronizada pelo desvio padrão.

Tamanho da agregação e sucesso reprodutivo

Não encontramos evidência de que o número de machos em uma agregação pode predizer a proporção de machos pareados com uma fêmea (β ± EP = 0,05 ± 0,03, IC = -0,02, 0,10, Tabela 6). Da mesma forma, a probabilidade de perda de FIP não aumentou com o número de machos na agregação (β ± EP = -0,16 ± 0,08, IC = -0,32, 0,002) (Tabela 6).

Tabela 6. Resultado da seleção de modelos da proporção de machos pareados e do número de ninhos com perda de fertilização intra par (FIP) em relação ao número de machos na agregação. K = número de parâmetros, AICc = Critério de Informação de Akaike, ΔAICc = diferença entre AICc do modelo e o AICc mínimo encontrado dentre os modelos e w_i = peso de Akaike.

Modelo/variável preditora	K	AICc	ΔAICc	w_i
Sucesso em pareamento[1]				
Nulo	2	37,34	0,00	0,61
Número de machos	3	38,26	0,92	0,39
Perda de FIP[2]				
Nulo	2	25,95	0,00	0,67
Número de machos	3	27,40	1,45	0,33

Variâncias do efeito aleatório (ano):

[1] 0,13.

[2] 1,89.

DISCUSSÃO

Demanda conflitante em sinais multimodais

A demanda conflitante entre os componentes motores foi evidente nas exibições de corte dos tizius. Machos que saltaram mais baixo apresentaram relação inversa entre duração do salto e rotação. Além disso, o aumento na proporção de exibições completas limitou a altura do salto de machos em condições corporais inferiores. Em contraposição, machos que atingiram maiores rotações também realizaram exibições completas em maiores taxas. Os parâmetros do canto não influenciaram os parâmetros motores altura e rotação, apesar da demanda energética e mecânica serem importantes na produção e manutenção do canto (Gil e Gahr 2002; Podos et al. 2009).

A limitação na duração do salto em função da rotação do corpo indica que esses dois componentes devem ser mutuamente limitantes. É possível, por exemplo, que a complexidade acrobática em uma exibição demande maior tempo e espaço (representado pela altura) para sua execução. Nossos resultados são também consistentes com uma demanda conflitante entre investimento em altura e proporção de exibições que envolvem o salto. Esse resultado indica que a execução de saltos altos e o tempo investido neste comportamento são energeticamente custosos e qualquer aumento em um dos atributos resulta na redução do outro, especialmente em situações de baixa condição corporal. Assim, os atributos motores altura, rotação bem como a proporção de saltos executados são indicativos de qualidade corporal, sendo que apenas indivíduos em boas condições físicas estão aptos a exibir combinações custosas desses sinais.

A demanda conflitante pode ser explicada por limitações biomecânicas ou energéticas. Restrições biomecânicas resultam de limitações em estruturas corporais, como características musculares, neurológicas ou tamanho de partes corporais, e portanto, podem levar a variações entre indivíduos na habilidade em exibições (e.g. Podos 1997). Alternativamente, limitações energéticas são frequentemente encontradas em espécies cujas exibições comportamentais são metabolicamente custosas (Vehrencamp et al. 1989). Embora o custo metabólico de cada componente das exibições dos tizius assim como a estrutura muscular e neurológica envolvida nesse comportamento ainda seja desconhecida, nossos resultados evidenciam que restrições biomecânicas ou energéticas devem impor limites ao desempenho dos machos dessa espécie.

A relação positiva entre rotação e proporção de exibições completas contradiz a expectativa de uma demanda conflitante na produção de múltiplos sinais. Tal cenário pode ocorrer quando a seleção sexual atua fortemente sobre esses sinais e favorece a maior conspicuidade dessas características pela preferência das fêmeas (Zahavi 1975; Andersson 1994). É possível também que a variabilidade da disponibilidade de recursos adquiridos pelos indivíduos seja alta e, ao mesmo tempo, a variação na alocação desses recursos em cada característica seja baixa (Stearns 1989).

Sistema de acasalamento e preferência das fêmeas

Os níveis de paternidade extra par são elevados em tizius, e estratégias reprodutivas alternativas das fêmeas (parasitismo intraespecífico de ninho e quasi-parasitismo) são comuns. Nossos resultados não corroboram a hipótese de benefício direto (recursos) da busca por múltiplas cópulas pelas fêmeas, já que machos extra par não defenderam territórios com maior disponibilidade de sementes do que os parceiros sociais das fêmeas. Entre possíveis explicações, a quantidade de alimento nos territórios de parceiros sociais pode ser suficiente para a nutrição dos filhotes, ou as fêmeas podem ter acesso ilimitado a territórios vizinhos, independentemente de ter copulado com machos extra par.

Os componentes visuais e acústicos das exibições dos machos tiveram diferentes impactos sobre as decisões de acasalamento das fêmeas. Altura do salto foi importante na escolha de parceiros sociais enquanto que a duração do canto influenciou a busca por parceiros extra par. O primeiro resultado suporta a hipótese de que fêmeas ganham benefícios indiretos na seleção de parceiros sociais ou sexuais, uma vez que a altura do salto é provavelmente uma característica custosa e indicativa de condição física do macho, como demonstrado neste trabalho. A maior parte dos machos sociais (79,2%) produz ao menos um filhote em tentativas reprodutivas com as fêmeas, portanto a escolha por parceiros em melhores condições aumenta a chance de que ao menos parte da prole irá herdar “bons genes” (Zahavi, 1975; Eshel et al. 2000; Kokko et al. 2006). Em contraste, outros componentes das exibições (canto e proporção de exibições completas) foram menos importantes no pareamento social. Esse resultado indica que tais características não sinalizam necessariamente propriedades relevantes das características dos machos nesse contexto de acasalamento.

A evidência de preferência por parceiros sociais com cantos mais curtos na fertilização de seus ovos é, embora fraca, intrigante. Esse resultado contrasta com a expectativa para as aves já estudadas, pois cantos mais longos poderiam revelar a habilidade na coordenação de múltiplas estruturas e atividades fisiológicas ou mecânicas (e.g. respiração, movimento do bico e atividade da siringe, Nowicki et al. 1992) e, portanto, melhor qualidade. É possível, no entanto, que o aumento na duração da vocalização dos tizius não seja custosa sendo que o canto nessa espécie é tipicamente curto, composto por uma única nota (Fandiño-Mariño e Vielliard 2004). Assim, considerando-se que fêmeas devem escolher os melhores parceiros para aumentar direta ou indiretamente seu valor adaptativo, nossos dados sugerem que cantos mais curtos revelam melhor qualidade dos machos. Entretanto, a relação entre duração do canto e qualidade permanece em aberto e deveria ser considerada em estudos futuros.

Nossos resultados não corroboraram a hipótese de benefícios indiretos pela busca de parceiros extra par geneticamente dissimilares ou com menores níveis de endogamia do que os parceiros sociais. Os filhotes intra e extra par também não diferiram em termos de diversidade alélica (heterozigosidade) ou na condição corporal. Esses resultados apontam para a fraca influência da endogamia na busca por múltiplos parceiros sexuais pelas fêmeas de tiziu e que esse comportamento pouco contribui para o aumento da heterozigosidade ou condição corporal dos filhotes (pelo menos no estádio de ninhego). A endogamia é mais provável de ocorrer em espécies cuja dispersão dos filhotes é rara e as oportunidades para escolha de parceiros mais restritas (Cockburn et al. 2003; Foerster et al. 2003; Tarvin et al. 2005). Sugerimos que fêmeas de tizius sofreram poucas limitações na busca por parceiros sexuais mais geneticamente dissimilares, fato que deve ser favorecido pelo comportamento migratório dos tizius e aumento do fluxo gênico entre diferentes populações.

Agregações territoriais

Nossos resultados não corroboram totalmente as previsões dos modelos de lek escondido *hotshot* e preferência das fêmeas. As fracas relações entre as características das exibições dos machos e a posição espacial na agregação sugerem que machos mais atraentes não estão no centro. Além disso, machos em territórios centrais não tiveram menor perda de FIP e maior sucesso em FEP, também contrariando uma das previsões do modelo *hotshot*. Aparentemente, machos *hotshot* em tizius são aqueles que estabelecem território primeiramente, uma vez que estes foram os que apresentaram melhores atributos, como saltos mais altos. Esses machos também tiveram maior sucesso em FEP. Assim, podemos presumir que o estabelecimento de machos pode resultar de um processo hierárquico com aqueles em melhores condições estabelecendo territórios anteriormente a machos em condições inferiores.

Machos mais adiantados executaram saltos mais altos e, portanto, estavam em melhor condição corporal (Aguilar et al. 2008b, presente trabalho) e devem ter atraído mais fêmeas na agregação. Para machos menos atraentes, o estabelecimento em territórios vizinhos aos machos *hotshot* poderia ser uma estratégia adaptativa para aumentar a chance de encontro com potenciais parceiras reprodutivas (e.g. Greene et al. 2000). No entanto, machos *hotshot* produzem cantos longos, característica que foi previamente relacionada ao maior risco de perda de paternidade na prole (presente trabalho). Esta inconsistência indica um conflito no sucesso reprodutivo dos machos, de tal forma que machos adiantados podem ter maiores chances de parear e reproduzir, mas também tendem a perder paternidade na prole. No entanto, ao associar perda de FIP e data estabelecimento dos machos, encontramos fraca evidência para esta hipótese.

O modelo *hotshot* de lek escondido também prevê um desvio do sucesso reprodutivo em favor de machos centrais ou dominantes (Wagner 1998; Fletcher e Miller 2006). No entanto, as fêmeas de tiziu não buscam por cópulas extra par preferencialmente com esses machos, nem procuram múltiplos parceiros sexuais mais frequentemente quando pareadas socialmente com machos em territórios periféricos. Este padrão, que contradiz a proposta de aumento de oportunidade para cópulas extra par em agregações (Wagner 1998), é reforçado pelo fato de que os machos centrais não eram mais atraentes no contexto de acasalamento extra par (i.e. possuem cantos mais longos, presente estudo). Em contraposição, machos adiantados obtiveram maior sucesso em FEP, o que reforça a importância da data de estabelecimento na formação das agregações. Machos adiantados, por apresentarem atributos mais atrativos para as fêmeas (saltos mais altos), devem ter conquistado maior número de fertilizações.

Testamos também três previsões do modelo de preferência da fêmea (ver Fletcher e Miller 2006): 1) machos em agregações teriam maior sucesso em pareamento do que os machos solitários, 2) a proporção de machos pareados aumentaria com o número de indivíduos na agregação e, finalmente, 3) que a proporção de fertilizações extra par seria positivamente associada ao número de indivíduos na agregação. No entanto, nossos dados não suportaram estas previsões. Apenas dois dos 224 territórios monitorados em três anos eram solitários, o que poderia indicar uma forte pressão de seleção contra a reprodução em locais isolados de outros indivíduos. Além disso, mesmo nas menores agregações (dois a quatro machos) a maioria dos machos formou vínculo social com uma fêmea. E, finalmente, a perda de FIP não foi maior em agregações maiores. Estes resultados sugerem que as fêmeas não selecionam machos em função do número de parceiros potenciais extra par nos territórios adjacentes.

Diferenças nas características de machos de tiziu que se estabelecem nas agregações em momentos diferentes, sugerem que machos *hotshot* podem de alguma forma ter estimulado o estabelecimento posterior de machos menos atraentes. Padrões semelhantes foram mostrados em outras espécies socialmente monogâmicas que agregam territórios no período reprodutivo (e.g. Tarof et al. 2004; Cockburn et al. 2009). Em espécies territoriais a atração entre coespecíficos parece beneficiar indivíduos menos experientes que se estabelecem mais tardiamente pela obtenção de "pistas" em relação à qualidade do habitat e o potencial de sucesso reprodutivo (Boulinier e Danchin 1997; Muller et al. 1997).

Conclusões

Poucos estudos com aves procuraram entender a demanda conflitante entre componentes motores e atributos acústicos de exibições de corte, apesar de movimentos aéreos serem comuns nesse grupo (Byers et al. 2010). Nesse trabalho evidenciamos conflitos de alocação entre movimentos acrobáticos e investimentos energéticos em tizius (duração do salto x rotação e altura do salto x proporção de exibições completas). Portanto, é possível que machos de tiziu exibam suas habilidades em voo, e muito provavelmente a qualidade da sua plumagem, durante essas exibições. Sabe-se que a altura do salto e o investimento em exibições completas são importantes na conquista de parceiros sociais pelos machos (Carvalho et al. 2006). Assim, nosso resultado reforça a possibilidade de que as exibições acrobáticas dos tizius devem ser importantes no contexto da seleção sexual e devem estar sob avaliação das fêmeas na escolha por parceiros reprodutivos por serem indicativos de qualidade corporal.

Corroborando essa expectativa, mostramos que as exibições comportamentais dos machos de tizius tiveram efeitos múltiplos na escolha de parceiros sociais e sexuais. Fêmeas preferiram machos que saltam mais alto e, portanto, devem ganhar benefícios indiretos uma vez que este sinal deve estar associado à qualidade genética dos machos (Zahavi 1975). O componente acústico foi importante na busca por fertilizações extra par, favorecendo machos com cantos mais curtos. Sugerimos que cantos com curta duração também devem sinalizar boa qualidade do indivíduo. Porém, não encontramos evidências de benefícios indiretos pelo aumento da compatibilidade genética ou heterozigosidade e condição corporal dos filhotes em função da poliandria. Além disso, também não encontramos evidências de benefícios diretos (alimento) da escolha por machos extra par.

Agregação territorial em tizius é uma questão há muito tempo questionada (Murray 1982; Webber 1985; Almeida e Macedo 2001; Carvalho et al. 2006; Dias et al. 2009). Estudos anteriores já mostraram que a distribuição espacial nesta espécie está relacionada à disponibilidade de recursos e evitação da predação (Dias et al. 2009). No entanto, custos também parecem estar associados, como a redução na sobrevivência de ninhos próximos a coespecíficos (Aguilar et al. 2008a) ou de machos em exibições (Dias et al. 2010). Nesse trabalho sugerimos que os benefícios de acasalamento podem compensar os custos da vida em agregações. Nossos dados suportam parcialmente as predições da hipótese lek escondido, e a possibilidade de que o estabelecimento de machos em agregações ocorra pela escolha das fêmeas. Machos mais adiantados são mais atraentes ou estão em melhores condições (têm saltos mais altos) e têm maior sucesso em FEP do que machos menos adiantados.

Portanto, a questão que permanece em aberto é se os tizius realmente formam lek escondidos. A resposta é ainda contraditória, uma vez que encontramos uma fraca relação entre a posição espacial do macho na agregação e sua qualidade e sucesso em cópulas extra par. No entanto, a chegada antecipada do macho *hotshot* em agregações corrobora uma expectativa importante de um sistema em lek e poderia ter desencadeado a formação da agregação. Assim, não podemos refutar completamente que o tiziu segue um modelo lek escondido.

REFERÊNCIAS

Aguilar TM, Dias RI, Oliveira AC, Macedo RH (2008a) Nest-site selection by Blue-black Grassquits in a Neotropical savanna: do choices influence nest success? Journal of Field Ornithology 79:24–31

Aguilar TM, Maia R, Santos ESA, Macedo RH (2008b) Parasite levels in blue-black grassquits correlate with male displays but not female mate preference. Behavioral Ecology 19:292–301

Akçay E, Roughgarden J (2007) Extra-pair paternity in birds: review of the genetic benefits. Evolutionary Ecology Research 9:855–868

Alberto F (2009) MsatAllele_1.0: An R package to visualize the binning of microsatellite alleles. Journal of Heredity 100:394–397

Almeida JB, Macedo RH (2001) Lek-like mating system of the monogamous blue-black grassquit. Auk 118:404–411

Andersson M (1994) Sexual selection. Princeton University Press, Princeton.

Andersson S, Pryke SR, Ornborg J, Lawes MJ, Andersson M (2002) Multiple receivers, multiple ornaments, and a trade-off between agonistic and epigamic signaling in a widowbird. American Naturalist 160:683–691

Aparicio JM, Ortego J, Cordero PJ (2006) What should we weigh to estimate heterozygosity, alleles or loci? Molecular Ecology 15:4659–4665

Arnqvist G, Kirkpatrick M (2005) The evolution of infidelity in socially monogamous passerines: the strength of direct and indirect selection on extrapair copulation behavior in females. American Naturalist 165:S26–S37

Boulinier T, Danchin E (1997) The use of conspecific reproductive success for breeding patch selection in terrestrial migratory species. Evolutionary Ecology 11:505–517

Burke T, Daviest N, Bruford M, Hatchwell B (1989) Parental care and mating behaviour of polyandrous dunnocks *Prunella modularis* related to paternity by DNA fingerprinting. Nature 338:249–251

Burnham K, Anderson D (2002) Model selection and multimodel inference: a practical information-theoretic approach. Springer-Verlag, New York.

Byers J, Hebets E, Podos J (2010) Female mate choice based upon male motor performance. Animal Behaviour 79:771–778

Candolin U (2003) The use of multiple cues in mate choice. Biological Reviews 78:575–595

Cardoso GC, Atwell JW, Hu Y, Ketterson ED, Price TD (2012) No correlation between three selected trade-offs in birdsong performance and male quality for a species with song repertoires. Ethology 118:584–593

Carvalho CBV, Macedo RH, Graves JA (2006) Breeding strategies of a socially monogamous neotropical passerine: extra-pair fertilizations, behavior, and morphology. Condor 108:579–590

Cockburn A, Dalziell AH, Blackmore CJ, Double MC, Kokko H, Osmond HL, Beck NR, Head ML, Wells K (2009) Superb fairy-wren males aggregate into hidden leks to solicit extragroup fertilizations before dawn. Behavioral Ecology 20:501–510

Cockburn A, Osmond HL, Mulder RA, Green DJ, Michael C, Double MC (2003) Divorce, dispersal and incest avoidance in the cooperatively breeding superb fairy-wren *Malurus cyaneus*. Journal of Animal Ecology 72:189–202

Dias RI, Castilho L, Macedo RH (2010) Experimental evidence that sexual displays are costly for nest survival. Ethology 116:1011–1019

Dias RI, Kuhlmann M, Lourenço LR, Macedo RH (2009) Territorial clustering in the blue-black grassquit: reproductive strategy in response to habitat and food requirements? Condor 111:706–714

Eshel I, Volovik I, Sansone E (2000) On Fisher–Zahavi's handicapped sexy son. Evolutionary Ecology Research 2:509–523

Fandiño-Mariño H, Vielliard JME (2004) Complex communication signals: the case of the Blue-black Grassquit *Volatinia jacarina* (Aves, Emberizidae) song. Part I-A structural analysis. Anais da Academia Brasileira de Ciências 76:325–334

Fletcher RJ, Miller CW (2006) On the evolution of hidden leks and the implications for reproductive and habitat selection behaviours. Animal Behaviour 71:1247–1251

Foerster K, Delhey K, Johnsen A, Lifjeld JT, Kempenaers B (2003) Females increase offspring heterozygosity and fitness through extra-pair matings. Nature 425:714–717

Gil D, Gahr M (2002) The honesty of bird song: multiple constraints for multiple traits. Trends in Ecology & Evolution 17:133–141

Gray EM (1997) Female red-winged blackbirds accrue material benefits from copulating with extra-pair males. Animal Behaviour 53:625–639

Greene E, Lyon BE, Muehter VR, Ratcliffe LM, Oliver SJ, Boag PT (2000) Disruptive sexual selection for plumage coloration in a passerine bird. Nature 407:1000–1003

Griffith SC (2000) A trade-off between reproduction and a condition-dependent sexually selected ornament in the house sparrow *Passer domesticus*. Proceedings of the Royal Society B: Biological Sciences 267:1115–1119

Griffith SC, Owens IPF, Thuman KA (2002) Extra pair paternity in birds: a review of interspecific variation and adaptive function. Molecular Ecology 11:2195–2212

Hardy OJ, Vekemans X (2002) SPAGeDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. Molecular Ecology Notes 2:618–620

Hebets EA, Papaj DR (2004) Complex signal function: developing a framework of testable hypotheses. Behavioral Ecology and Sociobiology 57:197–214

Johnstone R (1996) Multiple displays in animal communication: “backup signals” and “multiple messages”. Philosophical Transactions of the Royal Society of London, Series B 351:329–338

Kalinowski ST, Taper ML, Marshall TC (2007) Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Molecular Ecology 16:1099–1106

Kokko H, Jennions MD, Brooks R (2006) Unifying and testing models of sexual selection. Annual Review of Ecology, Evolution, and Systematics 37:43–66

Lahti DC, Moseley DL, Podos J (2011) A tradeoff between performance and accuracy in bird song learning. Ethology 117:802–811

Mazerolle MJ (2010) AICcmodavg: Model selection and multimodel inference based on (Q)AIC(c). R package version 1.25.

Møller AP, Pomiankowski A (1993) Why have birds got multiple sexual ornaments? Behavioral Ecology and Sociobiology 32:167–176

Muller KL, Stamps J, Krishnan V, Willists NH (1997) The effects of conspecific attraction and habitat quality on habitat selection in territorial birds (*Troglodytes aedon*). American Naturalist 150:650–661

Murray BGJ (1982) Territorial behavior of the blue-black grassquit. Condor 84:119–119

Neff BD, Pitcher TE (2005) Genetic quality and sexual selection: an integrated framework for good genes and compatible genes. Molecular Ecology 14:19–38

Nowicki S, Westneat M, Hoese W (1992) Birdsong: motor function and the evolution of communication. Seminars in Neuroscience 4:385–390

Partan S, Marler P (2005) Issues in the classification of multimodal communication signals. American Naturalist 166:231–245

Patricelli GL, Krakauer AH (2010) Tactical allocation of effort among multiple signals in sage grouse: an experiment with a robotic female. Behavioral Ecology 21:97–106

Podos J (1997) A performance constraint on the evolution of trilled vocalizations in a songbird family (Passeriformes: Emberizidae). Evolution 51:537–551

Podos J, Lahti DC, Moseley DL (2009) Vocal performance and sensorimotor learning in songbirds. In: Naguib M, Janik VM (eds.) Advances in the Study of Behavior. Vol. 40. Academic Press, Burlington, pp. 159–195.

Queller D, Goodnight K (1989) Estimating relatedness using genetic markers. Evolution 43:258–275

R Development CoreTeam (2011) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/.

Rousset F (2008) GENEPOP’007: a complete re-implementation of the GENEPOP software for Windows and Linux. Molecular Ecology Resources 8:103–106

Rubenstein DR (2007) Female extrapair mate choice in a cooperative breeder: trading sex for help and increasing offspring heterozygosity. Proceedings of the Royal Society B: Biological Sciences 274:1895–1903

Schneider CA, Rasband WS, Eliceiri KW (2012) NIH Image to ImageJ: 25 years of image analysis. Nature Methods 9:671–675

Seutin G, White BN, Boag PT (1991) Preservation of avian blood and tissue samples for DNA analyses. Canadian Journal of Zoology 69:82–90

Sheldon BC (1994) Male phenotype, fertility, and the pursuit of extra-pair copulations by female birds. Proceedings of the Royal Society B: Biological Sciences 257:25–30

Sick H (2001) Ornitologia brasileira. Editora Nova Fronteira, Rio de Janeiro.

Tarof SA, Ratcliffe LM, Kasumovic MM, Boag PT (2004) Are least flycatcher (*Empidonax minimus*) clusters hidden leks? Behavioral Ecology 16:207–217

Tarvin KA, Webster MS, Tuttle EM, Pruett-Jones S (2005) Genetic similarity of social mates predicts the level of extrapair paternity in splendid fairy-wrens. Animal Behaviour 70:945–955

van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) Micro-Checker: software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Notes 4:535–538

Varian-Ramos CW, Webster MS (2012) Extrapair copulations reduce inbreeding for female red-backed fairy-wrens, *Malurus melanocephalus*. Animal Behaviour 83:857–864

Vehrencamp S, Bradbury J, Gibson R (1989) The energetic cost of display in male sage grouse. Animal Behaviour 38:885–896

Wagner RH (1998) Hidden leks: sexual selection and the clustering of avian territories. In: Parker P, Burley N (eds.) Avian reproductive tactics: female and male perspectives Ornithological Monographs No 49. American Ornithologists’ Union, Washington, pp. 123–145.

Wagner WE, Beckers OM, Tolle AE, Basolo AL (2012) Tradeoffs limit the evolution of male traits that are attractive to females. Proceedings of the Royal Society B: Biological Sciences 279:2899–2906

Webber T (1985) Songs, displays, and other behavior at a courtship gathering of blue-black grassquits. Condor 87:543–546

Wetton JH, Parkin DT (1991) An association between fertility and cuckoldry in the house sparrow, *Passer domesticus*. Proceedings of the Royal Society B: Biological Sciences 245:227–233

Zahavi A (1975) Mate selection-A selection for a handicap. Journal of Theoretical Biology 53:205–214

CAPÍTULO 1

Trade-offs and constraints within an acrobatic mating display

ABSTRACT

Trade-offs in multimodal signals may be due to energetic, biomechanical or physiological conflicts during the simultaneous production of such signals. Here we predicted and tested for trade-offs in male blue-black grassquits' (*Volatinia jacarina*) courtship displays, which comprises either only vocalizations (incomplete displays) or vocalization with a synchronized "leap" flight display (complete display). We assessed trade-offs i) within modality by relating leap duration, leap height, and the extent of vertical rotation at the leap peak, and ii) across modalities and at varying frequencies by testing whether leap height and rotation are associated with song traits and display rates. We also tested if leap height and rotation angle are correlated with male body condition. During three breeding seasons, we conducted video and audio recordings of 56 males in displays, and for 36 of these males calculated proportion of complete display rates in relation to the overall displays executed during focal observations. We found leap height correlated positively with leap duration, but a trade-off was evidenced by rotation angles negatively associated with leap duration for males leaping lower. A similar constraint was shown by decreased leap heights with increased proportion of complete displays, but only for those in lower body condition. Males that displayed at greater rates showed maximum rotation in a greater percentage in their displays. Our results offer evidence of a trade-off between aerial acrobatic movements and energetic investment in a multimodal courtship display. We suggest that the complex acrobatic display of blue-black grassquit males should be critically important in a sexual selection context, and may be specifically targeted by female choice.

Keywords: sexual selection, multimodal signal, motor display, performance, birds

INTRODUCTION

Communication signals in many species are complex and may involve multiple modalities that stimulate different sensorial channels such as vision, audition and chemoreception (Partan and Marler 2005). Some examples include vibrational and chemical signaling in ants (Hölldobler 1999), gape color and repetitive calling by begging nestlings (Götmark and Ahlström 1997; Glassey and Forbes 2002), vibrational and visual sexual courtship in spiders (Taylor et al. 2006; Elias et al. 2012) and visual and acoustic signal in birds (Patricelli and Krakauer 2010). Previous studies of multimodal signaling focused on the perception of such signals by receivers, and the information different modes may confer about individual quality. The foremost evolutionary hypotheses to explain multiple sensorial signal components emphasize signal function, which hinges on the receivers' ability to detect and interpret signal content (Møller and Pomiankowski 1993; Iwasa and Pomiankowski 1994; Johnstone 1996). In multimodal signals, each component might reveal a distinct and particular aspect of the sender's overall condition (multiple message hypothesis), or might together reveal common aspects of signaler quality (redundant signal or "backup signals" hypothesis, Møller and Pomiankowski 1993; Johnstone 1996; Candolin 2003; see also Hebets and Papaj 2004).

Theory suggests that signals should indicate the quality of signalers, with only high quality individuals being able to express and sustain the most elaborate signal traits (Zahavi 1975). However, the relationship between quality and sexual traits is likely to be complex (e.g. Møller 1989; Griffith 2000; Andersson et al. 2002). Of particular note, a non-elaborate trait might not indicate an individual of low quality per se, but instead an individual who has made specific "choices" regarding the allocation of available resources across multiple sexual traits. This is analogous to differential allocation across life-history traits (Stearns 1989; Höglund and Sheldon 1998; Kokko 2001), for instance when elevated reproductive investment is followed by reductions in survival (Martin 1995; Ghalambor and Martin 2000), or when elaboration of secondary sexual characters reduce future breeding success (Gustafsson et al. 1995). Life-history trade-offs such as these are most likely to occur when resource availability is limited (Höglund and Sheldon 1998). It follows that trade-offs in relative investment across multiple signals are also more likely to occur in individuals that have limited access to scarce resources (Höglund and Sheldon 1998). Variability in how resources are acquired and allocated can thus influence the nature of correlations between multiple signal traits, or the relationship between traits and measures of individual quality (Stearns 1989).

Mechanistic or energetic trade-offs in the production of multiple signals, either within or between sensorial modalities, have been described in several species (Bertram and Warren 2005; Ornelas et al. 2009; Lahti et al. 2011; Cardoso et al. 2012; Wagner et al. 2012). Most studies have attempted to understand the mutual combination of acoustic parameters and plumage characteristics (Badyaev et al. 2002; Ornelas et al. 2009; Germain et al. 2011), plumage and morphometric measurements (Andersson et al. 2002) or constraints within acoustic components (Podos 1996, 1997; Cardoso and Hu 2011). However, few studies have examined possible trade-offs in the synchronized production of motor displays with acoustic components (e.g. Patricelli and Krakauer 2010) and no studies, to our knowledge, have documented trade-offs among motor components within acrobatic displays such as aerial mating displays of many birds. Trade-offs within acrobatic displays seem likely to occur due to energetic, biomechanical or physiological conflicts of the motor components during the simultaneous production of such signals. This contrasts with the production patterns of display components such as breeding plumage and song, for example, which can be produced at different points in time by the organism: in some species molting usually occurs right before the onset of the breeding season (Shutler 2011) whereas song is produced later in the season, thus minimizing possible conflicts in their expression.

Our study focused on male blue-black grassquits (*Volatinia jacarina*), a neotropical passerine that exhibits two types of courtship displays during the breeding season, one involving motor and acoustic components (complete displays, hereafter) and the other involving only vocalizations (incomplete displays, hereafter) (Alderton 1963; Almeida and Macedo 2001; Sick 2001). The first is characterized by conspicuous repeated vertical flights (”leaps”), typically initiating and ending on the same perch, synchronized with a forward and then reverse rotation of the body and the exhibition of white underwing patches during wing flapping. The display is finalized with a short vocalization produced as the bird descends and lands on his perch (Macedo and Podos, unpublished data). Just prior to breeding, males molt into a blue-black iridescent nuptial plumage, which improves male conspicuousness when performing such acrobatics (Sicsu et al. submitted.). The complete display is probably costly energetically because it involves a sustained vigorous effort of up to 19 leaps/min (present study). Leap displays might also have receiver-dependent costs, such as increased exposure to predators (Dias et al. 2010) or retaliation from neighboring males. The incomplete display is only repeated vocalizations, structurally similar to those produced during complete displays but while the male remains perched. Previous studies have shown a potential ability of males to adjust the investment between these two types of display depending on sunlight incidence upon their bodies. More specifically, males tend to produce more complete displays in bright sunlight, and more incomplete displays under intermediate sunlight levels (Sicsu et al. submitted). Previous studies found that certain complete display parameters, such as leap rate, height and duration (Costa and Macedo 2005; Aguilar et al. 2008), as well as plumage characteristics (Doucet 2002; Aguilar et al. 2008), vary with male condition or stage of molt (Maia and Macedo 2010), suggesting a costly production and maintenance of these signals. We might, therefore, expect conflicts of allocation within and between sensorial modalities such as vertical flight and song traits, as well as trade-offs dependent on male body quality (Macedo et al. 2012).

Descriptions of the blue-black grassquits' motor component traits, such as leap height and acrobatic movements, and the consistency of these parameters through the time, have been limited in scope (Alderton 1963; Costa and Macedo 2005; Carvalho et al. 2006). Additionally, constraints on grassquit motor displays, such as body size, locomotor proficiency and acoustic signaling capacity are unknown. Our study addresses these issues following three main objectives. The first was to test for trade-offs among motor traits. Towards this end we document the relative timing among leap duration, leap height, and the extent of vertical rotation at the leap peak ("acrobatics"). If the expression of these parameters is not limited by trade-offs, we would expect to find them to correlate positively, e.g., with the execution of complex acrobatics requiring more time and perhaps greater height. Alternatively, these display parameters may be subject to trade-offs, e.g., with longer leap duration or higher leap height impinging negatively on acrobatic complexity. Potential trade-offs among multiple motor components in acrobatic displays have gone virtually unexplored. Our second main objective was to assess potential trade-offs across display modalities (motor and acoustic) and at varying frequencies, by testing the relationships between two leap parameters (height and rotation) and song parameters and display rate, respectively. In particular we posited that leap height or body rotation angle would negatively correlate with song duration and frequency bandwidth and display rates. Finally, we hypothesized that leap height and rotation angle should be particularly reliable indicators of male quality, given that they should require morphological and physiological adaptations (such as specialized muscles and increased heart rates, e.g. Barske et al. 2011) that possibly limit the performance in displays. Thus, we expected to find these variables positively related with male body condition.

METHODS

We collected data during three breeding seasons (October to February, 2008-2009, 2009-2010 and 2010-2011; years 1, 2 and 3, hereafter) at Fazenda Água Limpa (15°56'S 47°56'W), a property of the Universidade de Brasília, located 28 km from Brasília, DF, Brazil. All data were collected in an area of approximately 6.56 ha, consisting of an abandoned orchard, altered grasslands and shrubby savanna vegetation at the edge of a natural *cerrado* area (typical tropical savanna).

Morphological data

Our sample of 53 males (year 1: *n* = 4; year 2: *n* = 29; year 3: *n* = 20) was captured with mist nets and marked with a combination of three colored plastic bands and one numbered aluminum band supplied by the Brazilian Bird Banding Agency (CEMAVE/ICMBio). Mist netting occurred from 0700h to 1200h three times weekly from October to December in each breeding season, at specific areas where birds occurred at high densities. This period coincides with the arrival of migrant blue-black grassquits, when most individuals are found in flocks and males start to establish their territories. From January to March we set up mist nets within each male's territory to capture unmarked individuals. We measured male body mass with a spring scale (to nearest 0.2 g) and tarsus length with calipers (to nearest 0.1 mm). We calculated a body condition index by dividing body mass by tarsus length, a parameter previously found to reliably indicate male body quality in terms of parasite infection (Costa and Macedo 2005; Aguilar et al. 2008).

Motor and acoustic displays

We recorded 65 video clips (year 1: *n* = 9, year 2: *n* = 29 and year 3: *n* = 27) of 56 males executing complete displays (motor and acoustic components together); seven of these birds were recorded in two different years, and an eighth in three different years. All but three of the 56 males that were filmed had also been captured and measured. We filmed displays at 30 frames per second with a mini-dv Canon XL1 digital camcorder (*n* = 38 birds) or a Casio HD digital camera EX-FH25 (*n* = 27 birds). Recordings were made from 0700 h to 1200 h, in one (*n* = 36), two (*n* = 22) or three (*n* = 7) different days for each male. This sampling allowed us to record and identify 5 to 12 complete displays of high video quality (in focus from a lateral angle) for each male (9.6 ± 1.3 x ± S.D, *n* = 624 samples). Videos from the Canon recorder were digitized using iMovie v. 7.1 (Apple Inc. 2008), and together with the Casio recordings were transferred to a computer in order to measure three parameters: leap height, leap duration, and forward body axis rotation angle (rotation angle, hereafter). To measure these parameters we identified for each video clip, using Windows® Movie Maker v. 5.1 (Microsoft Corporation 2007), the frame corresponding to (i) the very beginning of the leap, i.e., the frame before the bird left the perch or flapped its wings to initiate the leap; (ii) the maximum height of the leap, and (iii) the end of the leap, i.e. the frame in which the bird touched the perch again with both feet. Leap duration was calculated as the time interval (in hundreds of seconds) between measures (i) and (iii). We used the program ImageJ® v. 1.45s (Schneider et al. 2012) to measure leap height, as the distance between the subject's beak when at the peak of the leap, and a horizontal line at the perch. This distance measure was calibrated to the average head height, a distance that was visible in video frames and also measured from a sample of grassquit specimens in hand (mean head height = 13.5 mm). Finally, we measured rotation angle as the angle between two lines drawn using from the bird's longitudinal body axis at the beginning and then at the peak of the leap (Fig. 1). When multiple replicates in the same breeding season were taken from a given male, we used the mean of replicates for statistical analyses.

We conducted focal observations on the motor and acoustic display activity of 36 males (year 1: n = 4; year 2: n = 18; year 3: n =14) from which we also had measures of body condition index, as well as video recordings of leap (see above). Focal observations conducted via annotations or narration into a portable audio recorder, lasted approximately 30 min, from 0700 h to 1000 h, in one (n = 17), two (n = 10) or three (n = 9) different days over a period of two weeks. These observations were conducted so that there was no overlap with the male breeding activities (which was also being monitored), since males reduce display rates when nesting (Alderton 1963, L. Manica & R. Macedo pers. observ.). During each focal observation, we noted the occurrence of each display and whether it was complete or incomplete. From these data we calculated three variables: cumulative duration of all displays, display intensity (sum of complete and incomplete displays divided by cumulative duration), and proportion of complete displays relative to overall displays executed.

Finally, we conducted focal audio recordings of songs in incomplete or complete displays from all males using a digital Marantz PMD 660 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 a Telinga parabola. Recordings were made from 0700 h to 1200 h over one (n = 19), two (n = 15) or three (n = 2) different days for each male. Song recordings were transferred to a computer and, using the software program Audacity® v. 2.0.0 (http://audacity.sourceforge.net), we selected five song samples from each bird, excluding introductory notes that often precede the stereotyped vocalization (Fig. 2). From each sample, in the program Cool Edit Pro® v. 2.1 (Syntrillium Software Corporation 2003) we measured song duration (ms) from oscillograms and spectrograms, minimum and maximum frequencies from power spectrum using a -24 dB amplitude cut-off criterion (as in Podos 1997), which were confirmed in spectrograms (Fig. 2). We then calculated frequency bandwidth as maximum minus minimum frequency for each song.

Figure 1. Illustration of method for measuring leap height and body rotation angle for a leap display, showing the male at the beginning and at the peak of the display leap. Rotation angle was calculated by measuring the angle between the body axes (diagonal yellow lines), and leap height (vertical red line) was calculated as the perpendicular distance between the beak at the peak of the display and the perch (horizontal red line).

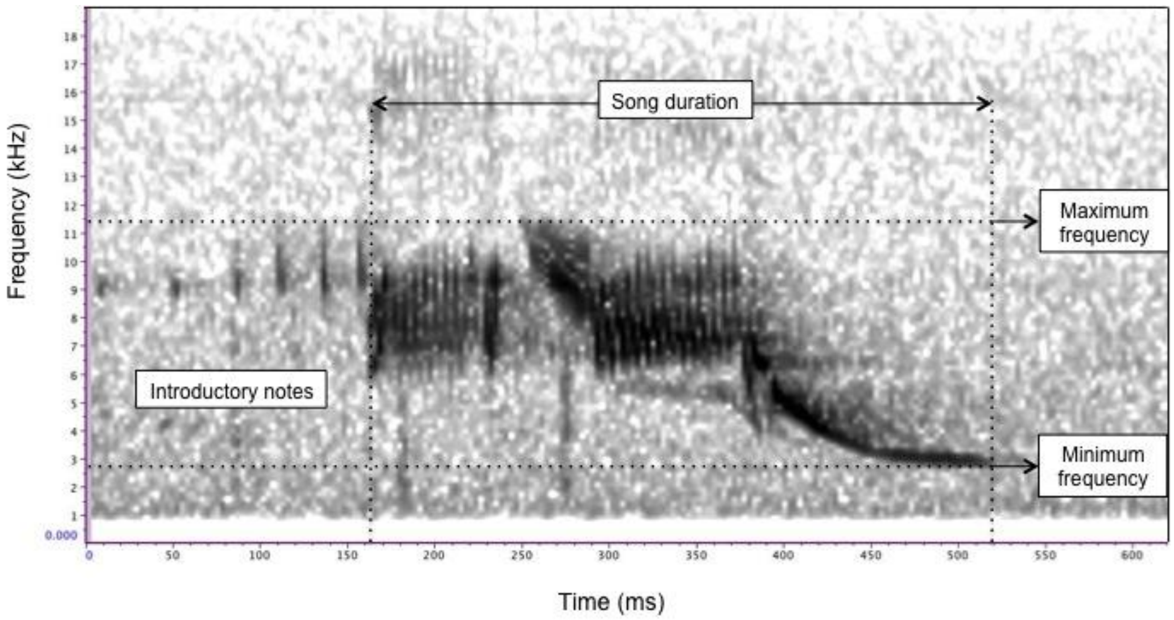

Figure 2. Spectrogram showing the blue-black grassquit vocalization from which song duration and frequency parameters were measured.

Analyses

We first assessed relationships among leap motor parameters. Towards this end we modeled leap duration in relation to leap height and rotation angle for 55 males, using only one replicate for each individual and excluding one male from which we were unable to measure all variables. We used multiple linear regression with leap duration as the response variable and including as predictors leap height, rotation angle and the interaction between leap height and rotation angle. Details on parameters importance in models are explained below. Male consistency in leap duration, height and rotation angle in repeated years was tested using pairwise t-tests for eight individuals monitored in both 2009 and 2010 (2 males) or 2010 and 2011 (6 males).

We associated motor display traits with song characteristics, display rates and body condition using two multiple regression models with leap height and rotation angle as response variables, respectively, and song duration, frequency bandwidth, total display intensity, proportion of complete displays, body condition index and year as predictors. We also included in models the interactions between body condition index and a) proportion of complete displays and b) total display intensity, because previous data exploration indicated possible interactions between these variables.

For all three multiple regression models (leap duration, leap height and rotation angle models), we calculated the second-order Akaike's Information Criteria (AICc) to make inference about importance of predictors in models (Burnham and Anderson 2002) using the R package AICcmodavg (Mazerolle 2010). We fitted a set of candidate models for the leap duration using all possible combinations of leap height, rotation angle and their interaction. We fitted candidate models of leap height and rotation angle models using the following combination of variables: a) all variables, b) each variable alone, c) only variables of a single sensorial modality (acoustic: song duration and frequency bandwidth, motor: total display intensity and proportion of complete displays, and their interactions with body condition index) including year as a covariate or not, and d) a null model. We calculated model probabilities (Akaike's weight), and model-averaged estimates and unconditional standard errors and 95% confidence intervals (CI) of each predictor to make inferences about their importance in best models (Burnham and Anderson 2002).

All variables, except "year", were centered to zero and scaled by their standard deviation. We checked normality of response and predictor variables using Shapiro Wilk tests, homogeneity of variances by inspecting residuals versus fitted plots, and colinearity by calculating the correlation between predictors and variance inflator factor (GVIF, considering a predictor non-colinear if $GVIF < 2$) of each model that included all variables (Quinn and Keough 2002). We excluded one outlier (body condition index > 0.70) in the leap height and rotation angle model and two outliers (song duration < 0.33) in leap height model to reduce heterocedasticity and improve model fitting. These outliers were strongly influencing the importance of parameters in models and were excluded to avoid bias in the results. Rotation angle and body condition index were log-transformed and proportion of complete displays was arc-sin transformed in the leap height and rotation angle models to achieve normalization or approximate to a normal distribution. In all models we used only complete observations, excluding individuals with incomplete data for any of predictor parameter, and when repeated measures occurred in different years for an individual, we used data from the first year. All analyses were performed using R (R Development CoreTeam 2011), with statistical significance set at 0.05. All values are presented as mean ± standard deviation, unless otherwise noted.

RESULTS

All display parameters varied widely: leap duration (0.43 to 0.84 s, 0.60 ± 0.09 s), leap height (9.79 to 35.72 cm, 19.81 ± 5.78 cm) and rotation angle (51.78 to 128.30°, 83.12 ± 17.84°). Best models of leap duration, based on AIC values, included leap height and angle as predictors (Table 1). Leap duration increased with leap height ($\beta \pm SE = 0.76 \pm 0.09$, 95% CI

= 0.57, 0.93, Table 1, Fig. 3), as would be expected. Some of the scatter in this relationship is likely attributable to the fact that many leaps were not made directly upwards but rather off-angle (e.g., Fig. 1), which would reduce the overall leap height for a given duration. Across the entire sample, rotation angles associated negatively with leap duration but showed a weaker effect than leap height ($\beta \pm SE = -0.14 \pm 0.09$, 95% CI = -0.32, 0.04, Table 1), meaning that birds rotated their bodies less extensively when executing longer leaps. Looking more closely, we found that males that rotated their body at smaller angles (50 to 75°) had wide-ranging leap durations, but that birds that expressed more pronounced rotations (100° or above) had uniformly short leap durations (Fig. 4). Thus it appears that birds might be able to maximize leap rotation or leap duration, but not both at the same time. Analysis of the relationship between rotation angle and leap height was complicated by the occurrence of an interaction between these two parameters ($\beta \pm SE = 0.24 \pm 0.11$, 95% CI = 0.03, 0.45, Table 1). The model including the interaction term was approximately four times more important than models including only leap height or both leap height and rotation angle (evidence ratio between Akaike's weights = 4.18 and 4.22, respectively, Table 1). Closer inspection of this relationship reveals that males leaping lower (less than the average height 19.8 cm, left panel in Fig. 4) reduced leap duration when they increased the rotation angle of their bodies, consistent with the combined rotation angle x leap duration data. By contrast, males leaping higher (above the average height 19.8 cm, right panel in Fig. 4) showed a weak positive relationship between leap duration and rotation angle. Leap duration, leap height and rotation angle were similar among years (mean difference: duration = 0.02 s, 95% CI = -0.10, 0.06, $t_7 = -0.46$, $p = 0.27$; height = 2.34 cm, 95% CI = - 4.6, 9.3, $t_7 = 0.80$, $p = 0.23$ and angle = 3.09°, 95% CI = -22.8, 16.7, $t_7 = 0.34$, $p = 0.72$).

Table 1. Model selection table for linear model of leap duration in relation to leap height and rotation angle. K = number of parameters, AICc = second-order Akaike's Information Criteria, w_i = Akaike's weight.

Model terms	K	AICc	ΔAICc	w_i
Height*rotation	5	114.89	0.00	0.677
Height+rotation	4	117.75	2.86	0.162
Height	3	117.77	2.88	0.161
Null	2	159.30	44.41	0.000
Rotation	3	161.38	46.49	0.000

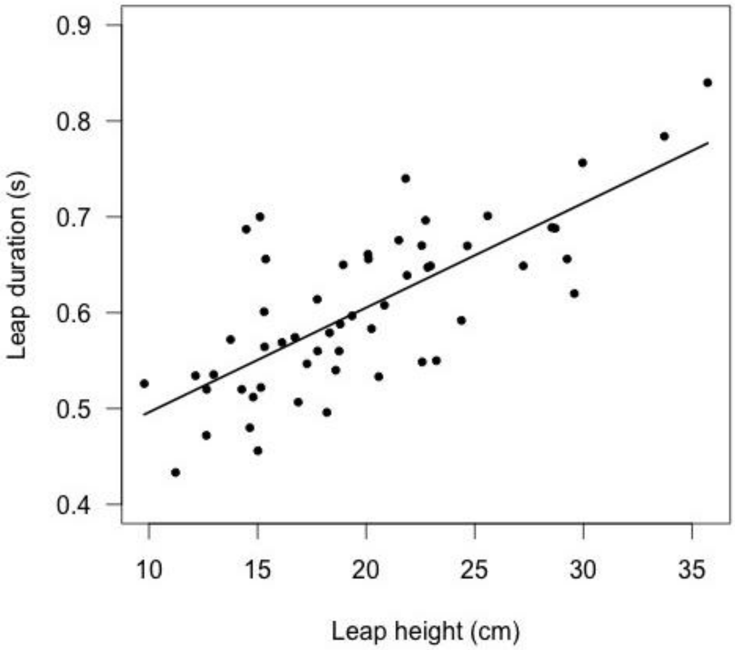

Figure 3. Relation between leap duration and leap height of the blue-black grassquit display.

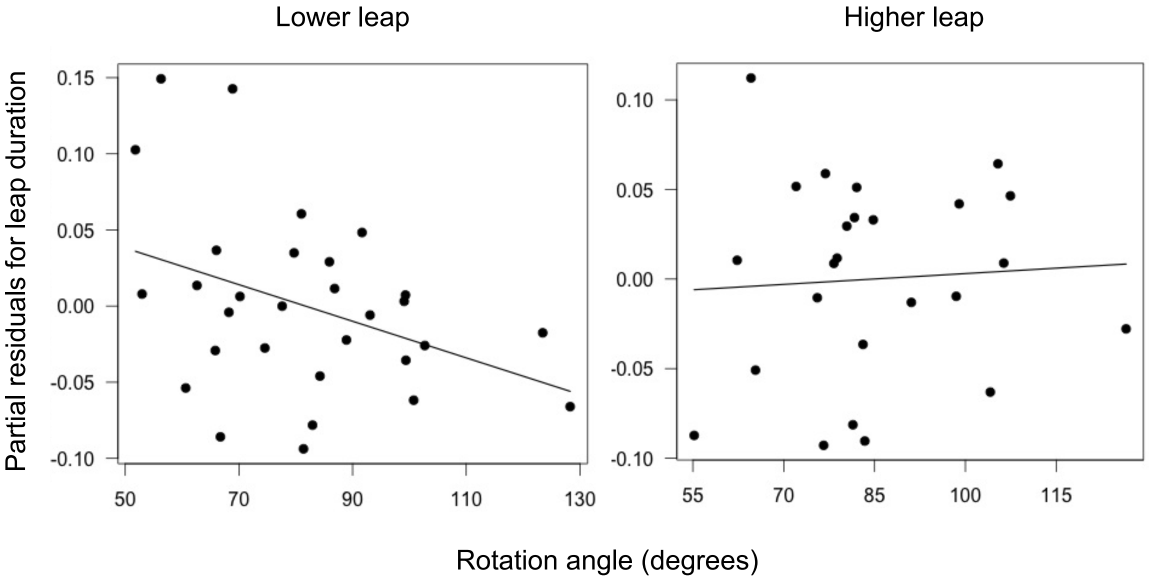

Figure 4. Relation between leap duration (represented by partial residuals after accounting for leap height) and rotation angle in the blue-black grassquit display when males leap lower (≤ 19.8 cm, left panel) and higher (> 19.8 cm, right panel).

The most parsimonious models of leap height included only the motor components of the display interacted with body condition index (Table 2), but the evidence was stronger only for the interaction with the proportion of complete displays (Table 3). The inclusion of total display intensity and body condition index interaction term did not improve model fit (evidence ratio ~ 1.00 in relation to the second best model, Table 2). In particular, males in lower body condition (body condition index < central value of 0.56) decreased leap height as they increased the proportion of complete displays, whereas males in higher body condition (body condition index ≥ central value of 0.56) increased leap height with the proportion of complete displays (Fig. 5). All other variables were not important in model selection of leap height (Tables 2 and 3).

The rotation angle model revealed strong effect of year (model probability = 64.4%), with males in years 2 and 3 showing lower rotation angles than males in year 1. Total display intensity and proportion of complete displays were also important predictors in rotation angle models (Table 2), but the evidence was higher only for the second as shown by model-averaged estimates biased toward positive values (Fig. 6). This result indicates that males that displayed at higher rates also increased rotation angles. In contrast, acoustic display parameters and body condition index showed weak relationships with rotation angle (Tables 2 and 3).

Table 2. Model selection table for linear models of leap height and rotation angle in relation to song duration (sdur) and frequency bandwidth (fband), total display intensity (intensity), proportion of complete displays (p.complete), body condition index (BC), year and interactions. K = number of parameters, AICc = second-order Akaike's Information Criteria, w_i = Akaike's weight.

Model/predictor	K	AICc	ΔAICc	w_i
Leap height				
Intensity*BC + p.complete*BC	4	86.23	0.00	0.280
P.complete*BC	3	86.24	0.01	0.279
Null	2	88.32	2.08	0.099
P.complete	3	89.59	3.36	0.052
BC	3	89.91	3.68	0.044
Fband	3	89.95	3.72	0.044
Sdur	3	90.03	3.80	0.042
Intensity*BC + p.complete*BC + year	6	90.25	4.02	0.038
Year	4	90.66	4.43	0.031
Intensity	3	90.72	4.49	0.030
Intensity*BC	3	90.73	4.50	0.029
Intensity + p.complete	4	92.18	5.95	0.014
Sdur + Fband	4	92.24	6.01	0.014
Intensity + p.complete + year	6	95.17	8.94	0.003
Sdur + Fband + year	6	95.51	9.27	0.003
Sdur + Fband + Intensity*BC + p.complete*BC + year	10	102.82	16.59	0.000
Rotation angle				
Year	4	95.26	0.00	0.644
Intensity + p.complete + year	6	97.86	2.60	0.175
Intensity*BC + p.complete*BC + year	6	99.03	3.77	0.098
Sdur + Fband + year	6	100.63	5.37	0.044
Null	2	103.70	8.44	0.010
P.complete	3	104.79	9.53	0.006
Intensity*BC	3	105.60	10.34	0.004
P.complete*BC	3	105.64	10.37	0.004
Intensity	3	105.79	10.52	0.003
Sdur	3	105.81	10.55	0.003
Fband	3	105.92	10.66	0.003
BC	3	106.10	10.83	0.003
Intensity + p.complete	4	106.62	11.35	0.002
Intensity*BC + p.complete*BC	4	108.04	12.77	0.001
Sdur + Fband	4	108.32	13.06	0.001
Sdur + Fband + Intensity*BC + p.complete*BC + year	11	113.75	18.49	0.000

Table 3. Model averaged estimates and 95% confidence intervals of song duration, frequency bandwidth, total display intensity, proportion of complete displays, body condition index, year and interactions for models of leap height and rotation angle in the blue-black grassquit display.

Model/predictor	Estimate ± SE	95% CI
Leap height		
Song duration	0.13 ± 0.19	-0.24, 0.50
Frequency bandwidth	0.12 ± 0.16	-0.18, 0.43
Total display intensity	0.02 ± 0.17	-0.31, 0.34
Proportion complete displays	-0.17 ± 0.16	-0.48, 0.15
Body condition	0.17 ± 0.19	-0.21, 0.55
Total display intensity*body condition	0.02 ± 0.21	-0.38, 0.42
Proportion complete displays*body condition	0.53 ± 0.24	0.06, 1.00
year		
year 2	0.64 ± 0.49	-0.32, 1.59
year 3	0.64 ± 0.50	-0.34, 1.62
Rotation angle		
Song duration	0.03 ± 0.17	-0.31, 0.37
Frequency bandwidth	-0.08 ± 0.17	-0.42, 0.25
Total display intensity	-0.08 ± 0.18	-0.45, 0.28
Proportion complete displays	0.28 ± 0.18	-0.07, 0.63
Body condition	0.01 ± 0.22	-0.42, 0.44
Total display intensity*body condition	0.16 ± 0.23	-0.61, 0.29
Proportion complete displays*body condition	0.16 ± 0.23	-0.30, 0.62
year		
year 2	-1.63 ± 0.49	-2.59, -0.66
year 3	-1.89 ± 0.51	-2.88, -0.90

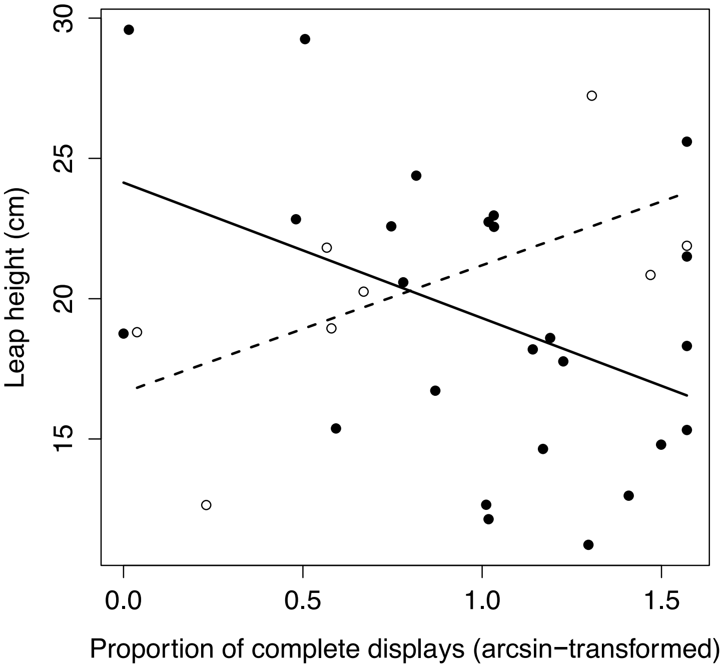

Figure 5. Relation between leap height and proportion of complete displays (arcsin transformed) of male blue-black grassquits with lower body condition indexes (< 0.56, filled circles, solid line) and higher body condition indexes (≥ 0.56, open circles, dashed line).

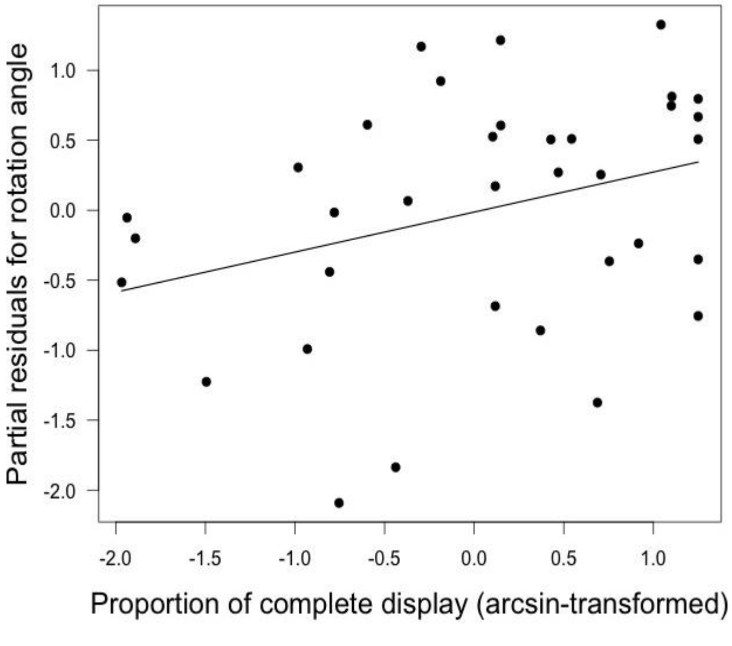

Figure 6. Relation between rotation angle (represented by partial residuals, after controlling for total display intensity and year) and proportion of complete displays of male blue-black grassquits.

DISCUSSION

Grassquit males made trade-off between components of the acrobatic motor display. Lower leaps (≤ 19.8 cm) were associated with an inverse correlation between rotation angle and leap duration. A greater proportion of complete displays constrained leap height for individuals with lower body condition. In contrast, males who achieved maximum rotation also displayed complete exhibitions at higher rates. This finding suggests that execution of acrobatic movements does not necessarily require a reduction of the more expensive type of display (leaps with songs). Interestingly, song parameters were not important in determining leap height and the degree of investment in executing acrobatics, although energetic and mechanical demands are important for production and maintenance of song (Oberweger and Goller 2001; Gil and Gahr 2002; Podos et al. 2009; Gillooly and Ophir 2010). These results suggest the possibility of independent energetic pathways for the production of the acoustic and motor components in the blue-black grassquit display. However, a question that remains unclear is whether the relationship between motor and acoustic traits depends on the type of display (incomplete or complete) in which song is produced, since we did not distinguish this display characteristic in our analyses.

Leap duration was limited by changes in body vertical rotation axis in displays with lower leaps. This pattern reveals that both the duration and rotation components may be mutually restrictive since increased maneuverability in the display could be possible given more time and amplitude in space (represented by leap height) for execution. Changing the body axis while in the air probably requires enhanced ability in body positioning as well as coordination of movements, limiting the execution of any other motor component concomitantly produced and forcing males to rapidly return to the perch. Although not measured in the present study, blue-black grassquit males may also change their horizontal axis during displays, by reversing their bodies at the top of the leap and landing facing the opposite direction (Alderton 1963). In the trade-off scenario found, it is possible that horizontal axis rotation is constrained when changes in body axis are increased, but further investigations are required to clarify this possibility.

Our results are also consistent with a trade-off mechanism between investment in height and proportion of time invested in the leaping display, since a negative relationship between these variables was found for males in lower body condition. This strongly suggests that leaping higher and spending longer periods doing so are both energetically costly, and an increase in one attribute necessarily results in a decrease in the other, especially in situations of restricted nutritional condition. There was a tendency for an opposite (positive) relationship between leap height and proportion of leaping displays for males with higher body condition (Fig. 5), but the wide 95% CI of the estimate unable us to make any strong inference. Nonetheless, taken together, these results indicate that males in better condition may be able to increase both leap height and time spent in this energetically demanding activity.

These patterns suggest that there is a potential conflict between natural and sexual selection relative to the evolution of the display traits of the blue-black grassquit, despite the expectation that sexual selection favors maximum exaggeration of secondary sexual characteristics (Zahavi 1975; Andersson 1994). We found that specific components of the display were reduced when performed in combination with other signals, although they are very likely under strong selection through female choice. In fact, leap height and complete display rates are known to be important in mating success in this species (Carvalho et al. 2006), therefore our results raise new questions about what exactly females may be selecting. Are they able to discriminate among males based on their performance of distinct attributes of the multiple component signals or do they evaluate the unique possible combinations that result from combining components? Furthermore, how informative are the distinctive components of this combination relative to some aspect of male quality?

In several taxa, trade-off patterns can be explained by biomechanical or energetic constraints. Biomechanical constraints result from limits in body structure, such as muscular and neural characteristics or body part sizes (e.g. beak size, wing length in birds), and thus are expected to induce variation among individuals regarding their displaying capacity. For example, males of several emberizid species are constrained when producing fast trills with regard to the frequency bandwidth of songs (Podos 1997; Ballentine et al. 2004; Cardoso et al. 2007), due to a limitation in coordinating syringeal activity and vocal tract movement (Nowicki et al. 1992; Podos 1996). Energetic constraints, alternatively, are frequently found in species with signals that are metabolically costly (Vehrencamp et al. 1989; Barske et al. 2011), as illustrated by the negative relationship between the two vocal components in the courtship of male greater sage grouse (*Centrocercus urophasianus*; Gibson 1996; Patricelli and Krakauer 2010). In such cases, unhealthy individuals or those with limited access to resources are more prone to trade-off traits. Any adjustment in trait allocation may enhance survival while minimizing severe consequences to signal transmission. In general, metabolic costs of courtship displays are probably high, especially for those species with repeated aerial movements. Male golden-collared manakins (*Manacus vitellinus*), for example, have twice the heartbeat rate during complex acrobatic movements of displays (up to 1300 b.p.m) when compared to other flight activities (Barske et al. 2011; Fusani and Schlinger 2012). Although metabolic costs of each component of the blue-black grassquit display and specific structure of muscles and neurons involved in the leaping flight have not been described yet, our results provide evidence that biomechanical or energetic restrictions impose limits to male performance. We suggest the existence of a mechanical constraint in the execution of higher leaps with wider rotation (Fig. 4), possibly because any muscular or neurological deficiency could reduce the male´s balance, impairing acrobatic maneuvers. Moreover, the reduction in leap height with increased complete display rates for individuals in poorer quality illustrates a situation of energetic constraint.

In addition to the constraints hypothesis discussed above, social and environmental characteristics, such as presence of potential mates, predators, light and climatic conditions, could also shape male behavior to maximize the quality of signal information (Roberts et al. 2006). The display behavior in the blue-black grassquit is costly because it attracts predators; nest predation rates are higher in territories occupied by displaying individuals compared with unoccupied territories (Dias et al. 2010). A reduction in leap height, for example, could reduce detectability by predators, but the associated maintenance or increase in the frequency of displays would assure adequate signal transmission and the attraction of potential mates, and hence optimize breeding success. Furthermore, differential allocation in display strategies by males, from a less costly (only songs in incomplete displays) to a more energetically expensive display (leaps with songs in complete displays), apparently is dependent on sunlight incidence upon their bodies, which increases male conspicuity (Siscu et al. submitted). Therefore, we do not discard the possibility that male plasticity in investment among different display components, as shown here, could also reflect social factors such as the presence of other individuals or physical conditions of the environment. Differential display allocation dependent on social conditions has been verified for a few birds, such as the satin bowerbird (*Ptilonorhynchus violaceus*), where males modulate display intensity to avoid frightening females and to increase their mating chances (Patricelli et al. 2002).

Our finding of the vertical rotation of the males' body during flight displays positively related with proportion of complete displays is contrary to what is predicted by a trade-off based mechanism resultant from the combination of multiple signals. However, positive relationships between traits can happen when there is high variability in the amount of resources acquired among individuals, but a low variation in the effort allocated to each trait (Stearns 1989). In our study, males that exhibit higher display rates with a pronounced rotation of their body angle may have good resource availability, and may not need to adjust allocation of effort.

Blue-black grassquits in central Brazil migrate during the non-breeding period (Sick 2001), probably to the Amazon region, but effects of this phenomenon, in addition to ageing consequences on male motor courtship behavior, are poorly understood. We found a low variation in average values of motor components (leap duration, height and rotation angle) for males measured in repeated samples in the three years of the study. Song consistency of males studied in the same area, evaluated through cross-correlation analyses (Dias pers. comm.), shows intermediate similarity scores in different years (0.47 ± 0.08), suggesting a moderate ability of males to maintain repeatable song structure through time. By contrast, longitudinal analyses of males in different years show that nuptial plumage molting initiates earlier and increases in speed through time (Maia 2008). In the present study, despite the small sample size (n = 8), we have documented for the first time that differences in motor display components of males measured in different breeding seasons are not significant and that, although long-distance migrations may impose severe conditions that could affect body quality (Alerstam et al. 2003), males may retain distinctive motor display courtship signatures through time.

Few studies have focused on the trade-offs between motor components performed during courtship displays, as well as how these interact with acoustic traits, although movement and acrobatics are common in birds (Byers et al. 2010). Recent studies using high-speed and traditional video recordings show detailed information about bird displays, emphasizing variations in velocity of flight as well as mechanical songs produced by wing feather specializations (Bostwick and Prum 2003; Clark 2009; Patricelli and Krakauer 2010; Barske et al. 2011). However, the mutual limitations between expression and quality of distinct components of multimodal displays have been largely overlooked in previous studies. Our results offer evidence of a trade-off between aerial acrobatic movements and energetic investment in a multimodal courtship display (leap duration *versus* rotation angle and leap height *versus* proportion of complete displays). We suggest that blue-black grassquit males exhibit their flight abilities, and very probably their plumage qualities, during their courtship displays. A past study found that males that successfully acquire mates spend longer periods of time carrying out displays, and that their leaps are higher (Carvalho et al. 2006). Thus, it should be expected that the highly complex acrobatics executed during the leaps, which combine vertical lifting through wing flapping with rotation of the body, should be critically important in a sexual selection context, and may be specifically targeted by female choice.

REFERENCES

Aguilar TM, Maia R, Santos ESA, Macedo RH (2008) Parasite levels in blue-black grassquits correlate with male displays but not female mate preference. Behavioral Ecology 19:292–301

Alderton C (1963) The breeding behavior of the blue-black grassquit. Condor 65:154–162

Alerstam T, Hedenström A, Åkesson S (2003) Long-distance migration: evolution and determinants. Oikos 103:247–260

Almeida JB, Macedo RH (2001) Lek-like mating system of the monogamous blue-black grassquit. Auk 118:404–411

Andersson M (1994) Sexual selection. Princeton University Press, Princeton.

Andersson S, Pryke SR, Ornborg J, Lawes MJ, Andersson M (2002) Multiple receivers, multiple ornaments, and a trade-off between agonistic and epigamic signaling in a widowbird. American Naturalist 160:683–691

Badyaev A, Hill GE, Weckwort BV (2002) Species divergence in sexually selected traits: increase in song elaboration is related to decrease in plumage ornamentation in finches. Evolution 56:412–419

Ballentine B, Hyman J, Nowicki S (2004) Vocal performance influences female response to male bird song: an experimental test. Behavioral Ecology 15:163–168

Barske J, Schlinger BA, Wikelski M, Fusani L (2011) Female choice for male motor skills. Proceedings of the Royal Society B: Biological Sciences 278:3523–3528

Bertram SM, Warren PS (2005) Trade-offs in signalling components differ with signalling effort. Animal Behaviour 70:477–484

Bostwick KS, Prum RO (2003) High-speed video analysis of wing-snapping in two manakin clades (Pipridae: Aves). Journal of Experimental Biology 206:3693–3706

Burnham K, Anderson D (2002) Model selection and multimodel inference: a practical information-theoretic approach. Springer-Verlag, New York.

Byers J, Hebets E, Podos J (2010) Female mate choice based upon male motor performance. Animal Behaviour 79:771–778

Candolin U (2003) The use of multiple cues in mate choice. Biological Reviews 78:575–595

Cardoso GC, Atwell JW, Hu Y, Ketterson ED, Price TD (2012) No correlation between three selected trade-offs in birdsong performance and male quality for a species with song repertoires. Ethology 118:584–593

Cardoso GC, Atwell JW, Ketterson ED, Price TD (2007) Inferring performance in the songs of dark-eyed juncos (*Junco hyemalis*). Behavioral Ecology 18:1051–1057

Cardoso GC, Hu Y (2011) Birdsong performance and the evolution of simple (rather than elaborate) sexual signals. American Naturalist 178:679–86

Carvalho CBV, Macedo RH, Graves JA (2006) Breeding strategies of a socially monogamous neotropical passerine: extra-pair fertilizations, behavior, and morphology. Condor 108:579–590

Clark CJ (2009) Courtship dives of Anna's hummingbird offer insights into flight performance limits. Proceedings of the Royal Society B: Biological Sciences 276:3047–3052

Costa FJV, Macedo RH (2005) Coccidian oocyst parasitism in the blue-black grassquit: influence on secondary sex ornaments and body condition. Animal Behaviour 70:1401–1409

Dias RI, Castilho L, Macedo RH (2010) Experimental evidence that sexual displays are costly for nest survival. Ethology 116:1011–1019

Doucet SM (2002) Structural plumage coloration, male body size, and condition in the blue-black grassquit. Condor 104:30–38

Elias DO, Maddison WP, Peckmezian C, Girard MB, Mason AC (2012) Orchestrating the score: complex multimodal courtship in the *Habronattus coecatus* group of *Habronattus* jumping spiders (Araneae: Salticidae). Biological Journal of the Linnean Society 105:522–547

Fusani L, Schlinger BA (2012) Proximate and ultimate causes of male courtship behavior in Golden-collared Manakins. Journal of Ornithology 153:119–124

Germain RR, Reudink MW, Marra PP, Boag PT, Ratcliffe LM (2011) Delayed maturation of multiple signals in a migratory songbird. Behavioral Ecology and Sociobiology 66:419–431

Ghalambor C, Martin T (2000) Parental investment strategies in two species of nuthatch vary with stage-specific predation risk and reproductive effort. Animal Behaviour 60:263–267

Gibson RM (1996) Female choice in sage grouse: the roles of attraction and active comparison. Behavioral Ecology and Sociobiology 39:55–59

Gil D, Gahr M (2002) The honesty of bird song: multiple constraints for multiple traits. Trends in Ecology & Evolution 17:133–141

Gillooly JF, Ophir AG (2010) The energetic basis of acoustic communication. Proceedings of the Royal Society B: Biological Sciences 277:1325–1331

Glassey B, Forbes S (2002) Muting individual nestlings reduces parental foraging for the brood. Animal Behaviour 63:779–786

Griffith SC (2000) A trade-off between reproduction and a condition-dependent sexually selected ornament in the house sparrow *Passer domesticus*. Proceedings of the Royal Society B: Biological Sciences 267:1115–1119

Gustafsson L, Qvarnström A, Sheldon BC (1995) Trade-offs between life history traits and a secondary sexual character. Nature 375:311–313

Götmark F, Ahlström M (1997) Parental preference for red mouth of chicks in a songbird. Proceedings of the Royal Society B: Biological Sciences 264:959–962

Hebets EA, Papaj DR (2004) Complex signal function: developing a framework of testable hypotheses. Behavioral Ecology and Sociobiology 57:197–214

Höglund J, Sheldon BC (1998) The cost of reproduction and sexual selection. Oikos 83:478–483

Hölldobler B (1999) Multimodal signals in ant communication. Journal of Comparative Physiology A 184:129–141

Iwasa Y, Pomiankowski A (1994) The evolution of mate preferences for multiple sexual ornaments. Evolution 48:853–867

Johnstone R (1996) Multiple displays in animal communication: "backup signals" and "multiple messages". Philosophical Transactions of the Royal Society of London, Series B 351:329–338

Kokko H (2001) Fisherian and "good genes" benefits of mate choice: how (not) to distinguish between them. Ecology Letters 4:322–326

Lahti DC, Moseley DL, Podos J (2011) A tradeoff between performance and accuracy in bird song learning. Ethology 117:802–811

Macedo RH, Manica L, Dias RI (2012) Conspicuous sexual signals in a socially monogamous passerine: the case of neotropical blue-black grassquits. Journal of Ornithology 153:15–22

Maia R (2008) Coloração estrutural iridescente do tiziu (*Volatinia jacarina*, Aves: Emberizidae): mecanismos de produção, variação e função. Dissertação de Mestrado, Universidade de Brasília, Brasília.

Maia R, Macedo RH (2010) Achieving luster: prenuptial molt pattern predicts iridescent structural coloration in blue-black grassquits. Journal of Ornithology 152:243–252

Martin TE (1995) Avian life history evolution in relation to nest sites, nest predation, and food. Ecological Monographs 65:101–127

Mazerolle MJ (2010) AICcmodavg: Model selection and multimodel inference based on (Q)AIC(c). R package version 1.25.

Møller A (1989) Viability costs of male tail ornaments in a swallow. Nature 339:132–135

Møller AP, Pomiankowski A (1993) Why have birds got multiple sexual ornaments? Behavioral Ecology and Sociobiology 32:167–176

Nowicki S, Westneat M, Hoese W (1992) Birdsong: motor function and the evolution of communication. Seminars in Neuroscience 4:385–390

Oberweger K, Goller F (2001) The metabolic cost of birdsong production. The Journal of Experimental Biology 204:3379–3388

Ornelas JF, González C, Espinosa de los Monteros A (2009) Uncorrelated evolution between vocal and plumage coloration traits in the trogons: a comparative study. Journal of Evolutionary Biology 22:471–484

Partan S, Marler P (2005) Issues in the classification of multimodal communication signals. American Naturalist 166:231–245

Patricelli GL, Krakauer AH (2010) Tactical allocation of effort among multiple signals in sage grouse: an experiment with a robotic female. Behavioral Ecology 21:97–106

Patricelli GL, Uy JAC, Walsh G, Borgia G (2002) Male displays adjusted to female's response. Nature 415:279–280

Podos J (1996) Motor constraints on vocal development in a songbird. Animal Behaviour 51:1061–1070

Podos J (1997) A performance constraint on the evolution of trilled vocalizations in a songbird family (Passeriformes: Emberizidae). Evolution 51:537–551

Podos J, Lahti DC, Moseley DL (2009) Vocal performance and sensorimotor learning in songbirds. In: Naguib M, Janik VM (eds.) Advances in the Study of Behavior. Vol. 40. Academic Press, Burlington, pp. 159–195.

Quinn GP, Keough MJ (2002) Experimental design and data analysis for biologists. Cambridge University Press, Cambridge.

R Development CoreTeam (2011) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/.

Roberts JA, Taylor PW, Uetz GW (2006) Consequences of complex signaling: predator detection of multimodal cues. Behavioral Ecology 18:236–240

Schneider CA, Rasband WS, Eliceiri KW (2012) NIH Image to ImageJ: 25 years of image analysis. Nature Methods 9:671–675

Shutler D (2011) Sexual selection: when to expect trade-offs. Biology Letters 7:101–104

Sick H (2001) Ornitologia brasileira. Editora Nova Fronteira, Rio de Janeiro.

Sicsu P, Manica LT, Maia R, Macedo RH Here comes the sun: display behavior is influenced by sunlight incidence Behavioral Ecology and Sociobiology (submitted)

Stearns S (1989) Trade-offs in life-history evolution. Functional Ecology 3:259–268

Taylor P, Roberts J, Uets G (2006) Compensation for injury? Modified multi-modal courtship of wolf spiders following autotomy of signalling appendages. Ethology, Ecology and Evolution 18:79–89

Vehrencamp S, Bradbury J, Gibson R (1989) The energetic cost of display in male sage grouse. Animal Behaviour 38:885–896

Wagner WE, Beckers OM, Tolle AE, Basolo AL (2012) Tradeoffs limit the evolution of male traits that are attractive to females. Proceedings of the Royal Society B: Biological Sciences 279:2899–2906

Zahavi A (1975) Mate selection-A selection for a handicap. Journal of Theoretical Biology 53:205–214

CAPÍTULO 2

Multiple benefits of social and sexual mating choice in the blue-black grassquit

ABSTRACT

Adaptive models of sexual selection predict that females in socially monogamous species may gain direct (non genetic) or indirect (genetic) benefits from multiple mating. We tested these hypotheses in the socially monogamous and sexually polygamous blue-black grassquit (*Volatinia jacarina*). During breeding seasons, males of this species repeat complex multimodal displays that integrate motor (vertical flights, "leaps") and acoustic (short vocalization) components. We used 15 pairs of microsatellites markers to access genetic paternity and maternity of nestlings in a three-year study. We expected that 1) extrapair males would have territories richer in food resources than social males; 2) leap display height and rate would positively correlate with success in pairing, within-pair or extrapair fertilizations; 3) genetic similarity with females and inbreeding level of extrapair males would be lower when compared to social mates, and 4) extrapair young would be less inbred and in better body condition than their within-pair half-sibs. In addition, we explored female preference for song traits (duration and frequency bandwidth) that could potentially reveal male vocal performance, thus his quality. We found extrapair paternity levels ranging from 8.2% to 34.2% of all nestlings and 11.4% to 47.1% of broods analyzed in the three years of study. Extrapair males were not settled in territories with higher seed density than social males. We found that the motor and acoustic display components had different impacts upon mating decisions of females: higher leaps were targeted for social mate choice whereas shorter songs were targeted for within-pair and, to a lesser extent, extrapair fertilizations. Genetic similarity with female and inbreeding levels did not differ between extrapair and social mates, nor did inbreeding and body condition differ between extrapair young and within-pair young. In sum, our results did not corroborate the direct (resource) benefit hypothesis of extrapair copulations, but we found support for indirect benefits through "good genes" for the offspring by showing that females chose males with better motor performance in their displays, i.e., those that had higher leaps. We also suggest that shorter songs might indicate good male body quality.

Keywords: sexual selection, social monogamy, polyandry, good genes, multimodal signals

INTRODUCTION

Multiple mating by females in socially monogamous species is a puzzling issue in evolutionary biology. Although polygyny can enhance male reproductive success, the adaptive value of sexual polyandry is less clear (Westneat and Stewart 2003; Arnqvist and Kirkpatrick 2005), primarily because female reproductive success should increase not with the number of mates, but rather with the viability of their eggs (Bateman 1948). However, few females in socially monogamous species may be able to pair socially and sexually with the most attractive or preferred males (Wagner 1998), thus extrapair copulations can be a strategy to compensate for a previous unfavorable choice (Gowaty 1996). Thus, contrary to early theoretical assumptions, females may increase their fitness by having multiple sexual partners (insects: Arnqvist and Nilsson 2000; Michalczyk et al. 2011; birds: Foerster et al. 2003; Tarvin et al. 2005; Gerlach et al. 2011; Varian-Ramos and Webster 2012 and mammals: Cohas et al. 2007, review in Jennions and Petrie 2000; Simmons 2005). Direct observations of females passively accepting copulation solicitations from extrapair males (Akçay et al. 2011) or actively seeking extrapair copulations (Kempenaers et al. 1992; Gray 1996; Double and Cockburn 2000) reinforce the likelihood of benefits from polyandry.

Adaptive sexual selection models rely on direct (non-genetic) and indirect (genetic) benefits of female mate choice (reviewed in Griffith et al. 2002; Arnqvist and Kirkpatrick 2005; Akçay and Roughgarden 2007). Females may benefit directly when copulating with multiple males by gaining access to resources ("resource benefits", Gray 1997; Tryjanowski and Hromada 2005), help with parental care (Burke et al. 1989; Otter et al. 1994; Rubenstein 2007) or increasing fertility assurance (Wetton and Parkin 1991; Sheldon 1994). Therefore, multiple mating to obtain direct benefits must be under natural selection as it results in immediate consequences that affect components of female fitness (e.g. survival or fertility).

Alternatively, benefits could be indirect, resulting in additive or non-additive genetic variance to the offspring genome (Griffith et al. 2002; Neff and Pitcher 2005). According to this proposal, by choosing extrapair males of better genetic quality, females may guarantee offspring inheritance of "good genes" (additive benefit) that improve their viability or attractiveness ("sexy sons" or Fisher-Zahavi process, Zahavi 1975; Eshel et al. 2000; Kokko et al. 2006), or "compatible genes" (non-additive benefit) that increase offspring heterozygosity (Neff and Pitcher 2005; Tarvin et al. 2005; Rubenstein 2007; Varian-Ramos and Webster 2012). Females can achieve greater levels of heterozygosity by choosing extrapair males that are less genetically related to them or that have increased levels of heterozygosity (Tregenza and Wedell 2000; Griffith and Immler 2009), resulting in increased offspring fitness (Amos et al. 2001; Foerster et al. 2003).

Male expression of sexual signals (ornaments and displays) is probably the main cue used by females to assess the genetic or non-genetic qualities of potential partners. A wide range of bird species exhibit multimodal signals (e.g. plumage coloration, song) that stimulate multiple sensorial channels that potential reveal male ability to perform energetically expensive displays (Møller and Pomiankowski 1993; Gil and Gahr 2002). For instance, each component of a multimodal signal may reveal a single property of the individual (multiple message hypothesis) or, alternatively, different components may convey a message about the overall quality of the individual (redundant signal or "backup signals" hypothesis, Møller and Pomiankowski 1993; Johnstone 1996). Females use different cues during mate choice (Candolin 2003). In the common yellowthroat (*Geothlypis trichas*), for example, plumage ornaments predict within-pair mating success while song consistency predict extrapair mating success (Taff et al. 2012). However, less is known about the simultaneous production of motor and acoustic components of displays in bird and the association with breeding success (Byers et al. 2010; Patricelli and Krakauer 2010).

Our study focused on sexual selection in the socially monogamous blue-black grassquit (*Volatinia jacarina*), which has a complex multimodal display that integrates motor and acoustic components. Males of this species provide an excellent opportunity to examine the association between display components and fitness for several reasons. They execute two types of courtship displays: repetition of a stereotyped, short and buzzy vocalization while perched (incomplete display, hereafter); and repeated leaps coupled with a forward and reversed rotation in body axis and synchronized with the same vocalization (complete display, hereafter) (Alderton 1963; Almeida and Macedo 2001; Sick 2001). During the breeding season males nuptial plumage is blue-black iridescent with white underwing patches, that are exposed during complete displays (Sick 2001). The complete display rates, body axis rotation, height and duration of the leap (Costa and Macedo 2005; Aguilar et al. 2008, Chapter 1), and plumage characteristics (Doucet 2002; Aguilar et al. 2008) signal male body condition, implying that the production of these ornamental traits is challenging for individuals. In a previous study, extrapair fertilizations were recorded in 50% of broods and 64% of nestlings (11 nests and 20 nestlings sampled), and male pairing and nesting success was associated with leap height and complete display rate (Carvalho et al. 2006). However, the importance of the acoustic and plumage components in mating choice remains unknown.

Our objective was to describe parentage patterns in the blue-black grassquit to test if female preference for male motor and acoustic display traits in social and sexual mating contexts could accrue direct or indirect benefits. First, we expected that females seeking extrapair males would favor those with better territories (i.e. higher food density) than their social mates ("resources benefit" hypothesis). A food-provisioning experimental test showed that males in food-rich territories were more frequently visited by extrapair females and that socially paired females produced larger clutches (Dias and Macedo 2011). This suggests that resource availability is important for female fitness and could drive extrapair mate choice. Next, we tested for indirect benefits, predicting that females would prefer to form social bonds and seek for extrapair copulation with males that signal better physical/health conditions. More specifically, we expected that males executing complete displays with higher leaps and performed at greater frequencies, would have greater success in pairing, within-pair or extrapair fertilizations. In addition, we explored female preference for song traits (duration and frequency bandwidth) that could potentially reveal vocal performance (Gil and Gahr 2002; Podos et al. 2009). Finally, if genetic compatibility between mates drives the polygamous sexual behavior in this species, we expected females to be less genetically similar to extrapair males than to their social mates, and extrapair young (EPY) less inbred, more heterozygous and in better body condition than their within-pair young (WPY) half-sibs.

METHODS

Study area and field procedures

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). All data were collected in an area of approximately 6.56 ha, consisting of an abandoned orchard, altered grasslands and shrubby savanna vegetation at the edge of a natural *cerrado* area (typical tropical savanna).

We captured birds with mist nets from 0700h to 1200h three times weekly from October to December in each breeding season, at specific areas where birds occurred within individuals aggregations. After males established their territories, usually from January to March, we used directional mist netting within territories to increase chances of capturing focal individuals. We captured 106 males and 56 females in the three years. Of these, 22 males and 5 females were captured in two different years, and 5 males and 1 female in three different years. In summary, we captured 39, 50 and 48 males and 17, 29 and 17 females in years 1, 2 and 3, respectively.

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). We collected samples of approximately 60 µl of blood via brachial venipuncture per bird, and stored in Queen´s lysis buffer solution (100 mM Tris – pH 7.5; 100 mM EDTA; 10 mM NaCl; 0.5% SDS, Seutin et al. 1991) and refrigerated at 4ºC.

Nest monitoring

We searched for nests by following individuals carrying nest material and by inspecting all potential nesting sites, especially in areas close to perches of displaying males. We checked active nests to monitor the number of eggs/nestlings every two or three days, or every day near hatching or fledgling times to precisely define nest age. We assigned social parents for each nest by identifying the individuals brooding or feeding the nestlings during approximately 15 min focal observation per nest. We included social parent identity in parentage analyses only when observations of the parents at the nest confirmed social pair.

We measured nestling body mass with a spring scale (to the nearest 0.1 g), and tarsus length with calipers (to the nearest 0.1 mm) after hatching (1 to 3 days) and at the middle-end of the nestling period (4 to 7 days). We a calculated body condition index as the body mass divided by tarsus length. Growth rates were calculated using difference in body mass divided by the difference in tarsus length between the measurements at the two ages (minimum interval between measurements = 3 days). We collected blood samples of approximately 20 µl, via brachial venipuncture, from 281 nestlings (61, 112 and 18 in years 1, 2 and 3, respectively) six days after hatching. The blood was immediately stored in Queen´s lysis buffer solution (Seutin et al. 1991) and refrigerated at 4ºC.

Male acoustic and motor display traits

We conducted observations of the motor and acoustic displays of 55 captured males (year 1: n = 15, year 2: n = 20, and year 3: n = 20). One of these birds was monitored in two different years, and three birds in three different years. Focal observations for each male consisted of approximately 30 min periods, from 0700 h to 1000 h, in one (n = 31), two (n = 13) or three (n = 11) different days over a period of two weeks. We recorded the type of display (complete or incomplete) and calculated the proportion of complete displays relative to overall displays executed. To avoid pseudoreplicates when males were monitored in more than one observation period in the same breeding season, we used the mean of the measurements in further analyses.

We recorded songs of all observed males using 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 occurred from 0700 h to 1200 h over one (*n* = 30), two (*n* = 20), three (*n* = 4) or four (*n* = 1) different days for each male. Five song samples (excluding the introductory notes) from each bird were selected and edited using the software program Audacity® v. 2.0.0 (http://audacity.sourceforge.net). We measured song duration (ms) from oscillograms and spectrograms, minimum and maximum frequencies from power spectrum using a -24 dB amplitude cut-off criterion (Podos 1997) in the software program Cool Edit® v. 2.1 (Syntrillium Software Corporation 2003). We then calculated frequency bandwidth as maximum minus minimum frequency for each song.

We also video-recorded complete displays of 33 individuals (year 2: *n* = 17 and year 3: *n* = 16) from which we also had focal observation data and song recordings. Two individuals were recorded in both years. We used a mini-dv Canon XL1 digital camcorder in year 2, which recordings were digitalized using iMovie v. 7.1 (Apple Inc. 2008), and a Casio digital camera EX-FH25 in year 3. Recordings occurred from 0700 h to 1200 h, in one (*n* =16), two (*n* = 15), three (*n* = 1) and four (*n* = 1) different days for each male. We registered from 5 to 12 complete displays for each male (mean ± standard deviation= 9.6 ± 1.4, *n* = 316 samples). In order to measure leap height (distance between beak at peak of the leap and a horizontal line where the bird perched), we used the program ImageJ® v. 1.45s (Schneider et al. 2012). We calibrated measurements to the average head height, a distance that was visible in video frames and also measured from a sample of grassquit specimens in hand (mean head height = 13.5 mm).

Territory quality in food resources

We estimated seed density in males' territories at the end of the breeding season, when most nests were already inactive. We counted the number of stalks with seeds and stalks showing evidence of previous seed production (i.e. with the presence of inflorescence without seeds) within four 0.5 m x 0.5 m quadrats placed 5 m from the nest in each cardinal direction. Seed density of each territory was estimated as the average of the four quadrats.

Molecular and parentage analysis

We used the Puregene® DNA Isolation Kit protocol to extract DNA from blood samples. Twenty-one pairs of microsatellites were tested for polymorphism (Table 1). We arranged microsatellites in four groups for multiplex Polymerase Reaction Chain's (PCR) using QIAGEN® Multiplex PCR kit, according to the fragment size range of each marker and the dye color used (Table 1). Multiplex PCR reactions of 10 µl contained 5 µl of 2x QIAGEN Multiplex PCR Master Mix, 2 µl of primer mix, 1 µl of 5x Q-Solution and 20 ng of DNA template. Primer mix solutions were prepared with 2 µM of each primer (forward 5'-3' and reverse 5'-3') and with 0.4 to 3.0 µM of fluorescently labeled forward primer WellRED^{TM} Oligos Sigma-Aldrich® (for specific concentrations of each marker see Table 1). We amplified DNA using a thermal cycler under the following conditions: a) HotStar Taq Polymerase activation step at 95°C for 15 minutes, b) 30 cycles of: denaturation at 94°C for 30 seconds, annealing at 54°C for 90 seconds, extension at 72°C for 90 seconds, and c) final extension at 72°C for 10 minutes. Individuals' genotypes were scored using Beckman Coulter CEQ^{TM} sequencer with a 400-bp size standard and deionized formamide and using CEQ 8000^{TM} Genetic Analysis System software. We repeated sequencing analyses for 99 samples to calculate genotyping error as 3%. All molecular analyses were conducted at the Molecular Ecology Lab of the University of St. Andrews, UK.

We used the R package "MsatAllele" (Alberto 2009) to bin microsatellite fragment sizes. Seventeen markers showed polymorphism, ranging from 4 to 35 alleles per locus (13.94 ± 9.20). We tested for Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD) 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 been from separate populations, we looked at HWE, LD and null alleles for each breeding season and conducted parentage analyses separately. From the 17 markers amplified, *Gf05* and *ThP1-014* did not fit the assumptions in any of the three years, so were excluded from final analyses, resulting in 15 microsatellites included in parentage assignments (Table 2). 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 2). The combined probability of excluding an unrelated candidate parent from parentage of a given nestling at all loci was 0.9837, 0.9479 and 0.9413, for the three years respectively, and 0.9991, 0.9950 and 0.9931, respectively, when the genotype of a parent of the opposite parent sex was known (Table 2).

Table 1. Characteristics of 21 microsatellite loci tested in the blue-black grassquit and PCR conditions applied. Ta: annealing temperature.

				Optimal PCR conditions		Multiplex PCR	
Locus	Size range (bp)	Variability[a]	Primer sequence (5'-3')	$MgCl_2$ and primer concentration	Number of cycles and Ta (°C)	dye-labeled primer concentration	Locus group
GF01[1]	142-161	P	F: TAGCATTTCTATGTAGTGTTATTTTAA R: TTTATTTATGTTCATATAAACTGCATG	1.5 mM, 0.50 µM	30, 53°C	1.0 µM	1
GF05[1]	183-246	P	F: AAACACTGGGAGTGAAGTCT R: AACTATTCTGTGATCCTGTTACAC	1.5 mM, 0.25 µM	30, 53°C	1.0 µM	4
GF11[1]	124-170	P	F: GTGCTATCAGCGAGGCATTTC R: AGGAGGATTTGGCTGACTGG	1.5 mM, 0.25 µM	26, 62°C	2.0 µM	2
GF12[1]	157-198	P	F: AATCCTTCTCGTCCCTCTTGG R: TTTGAGTGTGCAGCAGTTGG	1.5 mM, 0.25 µM	26, 55°C	1.0 µM	2
GF14[1]	100-124	P	F: TTTTTACAGAGCTTCTACAATTATAGC R: TCAAAAAATTGCATTAATTCTG	1.5 mM, 0.25 µM	30, 53°C	0.8 µM	4
GF16[1]	107-120	P	F: CCCTTCAGGGCATGAGTGAGG R: ATGTCATGAACTCAACCAACTCC	1.5 mM, 0.25 µM	30, 59°C	0.4 µM	1
TG01-040[2]	not amplified	-	F: TGGCAATGGTGAGAAGTTTG R: AGAATTTGTACAGAGGTAATGCACTG	none	none	-	-
TG01-148[2]	187-203	P	F: TTGCAACACATTCTAATATTGC R: TTTAAAGTACATCAAACAACAAAATC	2.0 mM, 0.5µM	35, 56°C	2.0 µM	1
TG02-088[2]	255-263	P	F: TGTGTGTTGACAGTATTCTCTTGC R: TTTAAACCTAATAAACGTCACACAGTC	2.0 mM, 0.5µM	35, 56°C	1.5 µM	2
TG03-002[2]	119-129	P	F: TCTTGCCTTTTTGGTATGAGTATAG R: TACAAAGCACTGTGGAGCAG	2.0 mM, 0.5µM	35, 56°C	1.0 µM	2
TG03-098[2]	231-242	P	F: TTTGCCTTAATTCTTACCTCATTTG R: TTGCAACCTCTGTGGAAGC	2.0 mM, 0.5µM	35, 56°C	1.5 µM	3
TG04-012[2]	134	M	F: TGAATTTAGATCCTCTGTTCTAGTGTC R: TTACATGTTTACGGTATTTCTCTGG	2.0 mM, 0.5µM	35, 56°C	-	-
TG04-061[2]	185-196	P	F: GACAATGGCTATGAAATAAATTAGGC R: AGAAGGGCATTGAAGCACAC	2.0 mM, 0.5µM	35, 56°C	1.0 µM	1
TG07-022[2]	~450*	-	F: CAGAAGACTGTGTTCCTTTTGTTC R: TTCTAATGTAGTCAGCTTTGGACAC	2.0 mM, 0.5µM	35, 56°C	-	-
TG11-011[2]	211-227	P	F: ACAAACTAAGTACATCTATATCTGAAG R: TAAATACAGGCAACATTGG	2.0 mM, 0.5µM	35, 56°C	2.0 µM	3
TG12-015[2]	278	M	F: ACAACAGTGGCTTTACTGTGTGA R: TACAGCAGCTGCAGCAAAGT	2.0 mM, 0.5µM	35, 56°C	-	-

TG13-017[2]	314-336	P	F: GCTTTGCATCTTGCCTTAAA R: GGTAACTACAACATTCCAACTCCT	2.0 mM, 0.5µM	35, 56°C	2.0 µM	3
TG22-001[2]	244-258	P	F: TTGGATTTCAGAACATGTAGC R: TCTGATGCAAGCAAACAA	2.0 mM, 0.5µM	35, 56°C	3.0 µM	4
ThP1-14[3]	228-285	P	F: GTAAATTTCAGGAGTCCAGGTTGC R: AAGCGCCCAAAATTAGCCAGAA	not tested alone		1.0 µM	4
VJE5[4]	156-235	P	F: CGGCTTCACCTGGATTGTA R: CCTAAAATTGTTGCTGGCTCA	1.5 mM, 0.25 µM	26, 59°C	1.5 µM	1
VJJ13[4]	179-222	P	F: ATGAGAAATCCTGGGGAGGT R: TCCTTCACATTTTACATTTTGTCTTT	1.5 mM, 0.25 µM	26, 59°C	1.0 µM	2

Source species: [1]*Geospiza fortis* (Petren 1998), [2]*Taeniopygia guttata* and *Gallus gallus* (Dawson et al. 2010), [3]*Thryothorus pleurostictus* (Brar et al. 2007), [4]*Volatinia jacarina* (Carvalho et al. 2006).

[a] P = polymorphic (> 1 allele), M = monomorphic (1 allele).

* Not amplified in multiplex PCR due to the larger length of fragments than the 400 bp size standard.

Table 2. Characteristics of 15 microsatellite loci amplified in *Volatinia jacarina* and used in maternity and paternity analyses in Cervus 3.0.3 (Kalinowski et al. 2007) for individuals monitored during three breeding seasons. 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 parent from the opposite sex, and P(HWE): probability of Hardy-Weinberg equilibrium.

		Heterozygosity					
Locus	Number of alleles	H_O	H_E	NE-1P	NE-2P	P(HWE)	Null allele frequency
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

We 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 (LOD scores). This is the likelihood that the candidate parent is the true parent divided by the likelihood that it is not the true parent. Critical values of LOD scores were estimated through simulations in Cervus using the following parameters: i) 10000 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) 3% of genotyping error (see above), and v) minimum typed loci as half of total number of loci (default parameter in Cervus). We ran two simulations with different number of candidate parents: in simulation A we used the number of individuals monitored in each breeding season (all banded birds video/audio recorded or registered in the area as breeders or floaters), and in simulation B, only the number of individuals genotyped. The proportion of candidates genotyped varied according to the number of candidate parents (Table 3). Therefore, we were able to confirm maternity and paternity assignments using more and less conservative approaches, respectively (Table 3).

We confirmed if females incubating or feeding the nestlings were actually biological mothers of the offspring with maternity analysis, and when maternity was confirmed, known 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 I: *Gf14* and year III: *VjE5*) or 8% (year II: *TG03-098*) of null alleles to confirm parentage. Additionally, we assigned maternity and paternity to social parents, even when unassigned by Cervus, when the following conditions were met: social parent was one of the two most-likely mothers/fathers in all simulations, loci mismatches between parent-nestling pair was lower than 2, and when parentage was confirmed for siblings in the same nest.

Table 3. Simulation parameters included in Cervus 3.0.3 (Kalinowski et al. 2007) maternity and paternity analyses to calculate critical LOD scores.

	Maternity analyses		Paternity analyses	
Parameter	A[1]	B[2]	A[1]	B[2]
Candidate parents				
Year 1	20	17	50	39
Year 2	35	29	63	50
Year 3	22	17	71	48
% candidates genotyped				
Year 1	85.0	100	78.0	100
Year 2	82.9	100	79.4	100
Year 3	77.3	100	67.6	100

[1] All individuals monitored in the area

[2] All individuals monitored in the area and genotyped

Genetic similarity and inbreeding

We estimated relatedness between females and social/extrapair males by calculating Queller and Goodnight's (1989) *r* relationship coefficient, which represents the proportion of alleles shared between two individuals weighted by the alleles' frequencies in a population, using the program SPAGeDi 1.3 (Hardy and Vekemans 2002). Analyses were conducted for each year separately, since allele frequencies of the reference populations differed among years. We estimated *r* coefficients for 28 mother-nestling pairs (mean ± standard deviation: 0.49 ± 0.10). There was no significant difference between the observed and expected *r* coefficients (*t* test: $t_{27} = 0.16$, $P = 0.87$). We additionally calculated an average *r* coefficient between each female with all males in the studied group for each year. We estimated heterozygosity weighted by locus (HL, Aparicio et al. 2006) to measure levels of inbreeding and as individual measures of genetic quality. HL is a homozygosity index that weighs the contribution of loci depending on their allelic variability (expected heterozygosity), varying from 0 (lowest heterozygosity) to 1 (greatest homozygosity) (Aparicio et al. 2006). When a female had multiple extrapair partners, we used the average of *r* coefficient and HL values in analyses.

Statistical analyses

We estimated three measures of male breeding success: 1) probability of pairing with a female and having at least one nesting attempt (pairing success), 2) probability of a paired male losing paternity of at least one nestling in his social brood (WPP loss), and 3) probability of any male in the studied group siring an EPY (EPP success).

We used logistic regressions with complementary log-log link function to associate motor and acoustic display variables with pairing success (*n* = 31 males; year 2 = 16 and year 3 = 15), WPP loss (*n* = 32 males; year 1 = 7, year 2 = 12 and year 3 = 13) and EPP success (*n* = 33 males; year 1 = 15 and year 2 = 18). In the pairing success model, we included the predictors leap height, proportion of complete display rates, song duration, frequency bandwidth and year, to control for possible differences among breeding seasons. Probabilities of WPP loss and EPP success were modeled with the same set of variables, but with the exclusion of leap height to increase sample size (increase in number of observations: WPP loss model = 9 and EPP model = 14), and because this predictor was not important in previous model selection procedures. When a male was sampled in multiple years, we used data from the first year or from the year we had the most complete information to avoid pseudoreplication.

We calculated the second-order Akaike's Information Criteria (AICc) to select the best models (Burnham and Anderson 2002) using the R package AICcmodavg (Mazerolle 2010). We fitted a set of candidate models using the following combination of variables: a) all variables, b) each variable alone, c) only variables of a single sensorial modality (acoustic: song duration and frequency bandwidth, motor: proportion of complete display rate and leap height, when present) including year as a covariate or not, and d) a null model. We calculated model probabilities (Akaike's weight), and model-averaged estimates and unconditional standard errors and 95% confidence intervals (Burnham and Anderson 2002) of each predictor to make inferences about their importance in a best model. All predictor variables were centered to zero and scaled by their standard deviation. We applied arc-sin transformations to proportion of complete displays in all models to achieve normalization or approximate a normal distribution.

We compared seed density in territories, motor and acoustic display traits, relatedness with females and HL of social males and extrapair males using paired *t* tests. EPY and WPY body condition indexes and growth rates were compared with a paired *t* test. When we monitored more than one EPY or WPY we used the average for the nest. All statistical analyses were performed in R (R Development CoreTeam 2011), with statistical significance set at 0.05. All values are presented as mean ± standard deviation, unless otherwise noted.

RESULTS

During the three breeding seasons we monitored 174 nests of blue-black grassquits, from which 131 (33, 53 and 45 in years 1, 2 and 3, respectively) we genotyped nestlings for parentage analyses. Field observations allowed us to identify social mothers of 56 nests (14, 23 and 19 in years 1, 2 and 3, respectively) and social fathers of 95 nests (26, 34 and 35 in years 1, 2 and 3, respectively).

Maternity analyses

We tested maternity of 127 nestlings with known social mothers and assigned maternity to 119 (93.7%) mother-nestling pairs. Cervus assigned 106 (83.5%) maternities at 80% confidence level, of which 48 (37.8% of the total) were also assigned at 95% confidence level. Eleven mother-nestling pairs were significant at the 80% level only after including an additional locus (year 1: *Gf14*, year 2: *TG03-098* and year 3: *VjE5*, using null alleles percentage acceptance threshold of 8%, see methods). Thirteen (4.6%) social candidate mothers unassigned by Cervus were between the two most likely parents in all simulations and were hence considered as true mothers. We were able to detect maternity for additional 8 mother-nestling pairs in cases where social females were unknown.

We confirmed social females as biological mothers of most nestlings they were brooding (94.5% of nestlings and 91.1% of broods). Exceptions included five females that were rearing nestlings (7 individuals) from unidentified mothers (Table 4). In one case (year 2), a female was not the genetic mother of any nestling in her nest, while in the other four cases, social females were the genetic mother of at least one nestling in the nest (Table 4). In the above cases, three nestlings (2.4% of all 127 nestlings which maternity was tested) resulted from intraspecific brood parasitism, where the social fathers also did not sire the nestlings, and four nestlings (3.1% of all 127 nestlings which maternity was tested) resulted from quasi-parasitism, where the social males were the biological fathers of the EPY (see paternity analyses below). Among the intraspecific brood parasitism cases, we identified two of the extrapair fathers: one was probably a floater, not registered breeding or displaying in the area, and the other established a territory and nested approximately 50 m from the nest where he sired the EPY.

Paternity analyses

We assigned paternity to 184 (88%) of the 209 nestlings with known social fathers. Among Cervus assignments, 171 (81.8%) father-nestling pairs were assigned at 80% level of confidence, of which 89 (42.6%) were also assigned at 95% level. Seventeen father-nestling pairs were significant at the 80% level only after including an additional locus (similarly as in maternity analyses above). We assigned paternity to 13 father-nestling pairs not assigned by Cervus since candidate fathers were already social fathers and were between the two most likely fathers in all simulations. We detected true fathers for additional 14 nestlings which social fathers were unknown. Finally, from all 127 mother-nestling pairs we assigned in this study (see maternity analyses), we were able to detect 98 (77.2%) fathers of the nestlings, of which 21 were assigned by Cervus only after including females' genotypes (16.5% from all trios).

Extrapair paternity rates varied greatly among years, ranging from 8.2% to 34.2% of all nestlings and 11.4% to 47.1% of broods analyzed. The second breeding season (year 2) had the highest extrapair paternity rate, and the third season (year 3), the lowest rate (Table 4). Complete loss of paternity by social males within a single brood occurred in 13.7% of nests (44.8% of broods with EPY), but in 16.8% of nests monitored (55.2% of broods with EPY) social males sired at least one nestling in the nest. We identified 21 extrapair males, of which 17 were also socially bonded to females and 9 of which sired at least one WPY. From all EPY (44 nestlings), we identified the extrapair fathers of 20 individuals, for which 10 we also identified mothers.

We also found six broods with unknown social fathers containing nestlings (n = 16) sired by different fathers. Because we did not know the social fathers' identity, we were unable to determine if a nestling was extrapair or within-pair and these cases were not included in Table 4 or in further analyses.

Table 4. Extrapair paternity and maternity rates in broods of the blue-black grassquit. EPY: extrapair young; WPY: within-pair young.

				Broods with EPY (%)		
	Nestlings analysed	EPY (%)	Broods analysed	Complete EPY broods	Mixed broods (EPY+WPY)	Total
Maternity analyses						
Year 1	29	1 (3.4)	14	0 (0.0)	1 (7.1)	1 (7.1)
Year 2	52	4 (7.6)	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	127	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	73	25 (34.2)	34	7 (20.6)	10 (29.4)	16 (47.1)
Year 3	85	7 (8.2)	35	2 (5.7)	2 (5.7)	4 (11.4)
Total	209	44 (21.1)	95	13 (13.7)	17 (17.9)	29 (30.5)

Male breeding success

All best models of pairing success included leap height (sum of best models probabilities = 57.8%). Leap height had positive averaged-estimates (β_{height} = 0.70 ± 0.36, 95% CI = -0.002, 1.40) (Table 5, Fig. 1), indicating that males with higher leaps tend to have greater probabilities of pairing with a female and breeding. Proportion of complete display rates was also included in the best models, but with a weak importance ($\beta_{display}$ = -0.42 ± 0.42, 95% CI = -1.25, 0.41). Contrarily, song parameters were included in models with lower support (ΔAIC > 2.9) and showed lower averaged-estimates in models ($\beta_{song\ duration}$ = 0.13 ± 0.28, 95% CI = -0.41, 0.67; $\beta_{freq.band}$ = 0.29 ± 0.29, 95% CI = -0.27, 0.86). Pairing success tended to be greater in the last breeding season when compared to the second season (β_{year3} = 1.40 ± 0.84, 95% CI = -0.24, 3.05).

Probability of losing paternity in the social brood was explained by male song characteristics; models including song duration and frequency bandwidth had 53.3% of support as best model, against 14.7% of the null model (Table 5). Song duration had the highest effect size ($\beta_{song\ duration}$ = 0.66 ± 0.41, 95% CI = -0.15, 1.47, Fig. 2), although parameter estimate uncertainty was high (CI crossing zero). Best models including song duration were ~ 2.40 times more important than the null model (Table 5). In contrast, frequency bandwidth had a weaker effect ($\beta_{freq.band.}$ = -0.49 ± 0.35, 95% CI = -1.17, 0.19) and the addition of this parameter did not improve model fit (evidence ratio between models “sdur+fband” and “sdur” in Table 5 is ~ 1). These results indicated that males having EPY within their social broods had longer songs, but were unlikely to differ in song frequency bandwidth from males that did not lose paternity. We found no support for proportion of complete displays as important predictor ($\beta_{display}$ = -0.05 ± 0.39, 95% CI = -0.82, 0.71). There was a slight variation in the probabilities of WPP loss across years, with probabilities being lower in the third breeding season (year 1 as reference level; β_{year2} = -0.08 ± 0.77, 95% CI = -1.58, 1.43; β_{year3} = -1.97 ± 1.19, 95% CI = -4.30, 0.37).

We found weak support for male characteristics in explaining EPP success (Table 5), but year (β_{year2} = 1.26 ± 0.82, 95% CI = -0.33, 2.86) showed a slightly large effect than other variables ($\beta_{display}$ = 0.15 ± 0.38, 95% CI = -0.55, 0.85; $\beta_{song\ duration}$ = -0.26 ± 0.38, 95% CI = -1.01, 0.49; $\beta_{freq.band.}$ = 0.03 ± 0.36, 95% CI = -0.68, 0.74). However, the evidence for year was just 1.33 times higher than for the null model.

Table 5. Model selection for male probability of pairing with a female (pairing success), losing paternity in the social brood (WPP loss) and of siring extrapair young (EPP success). K = number of parameters, AICc = second-order Akaike's Information Criteria, w_i = Akaike's weight.

Models[1]	K	AICc	ΔAICc	w_i
Pairing success				
Display+height+year	4	32.88	0.00	0.291
Height	2	32.91	0.03	0.287
Null	1	35.26	2.38	0.089
Display+height	3	35.33	2.46	0.085
Fband	2	35.77	2.89	0.068
Year	2	36.08	3.21	0.059
Sdur	2	36.91	4.03	0.039
Display	2	37.13	4.25	0.035
Sdur+Fband	3	38.19	5.32	0.020
Display+height+sdur+fband+year	6	38.59	5.72	0.017
Sdur+fband+year	4	39.38	6.51	0.011
WPP loss				
Sdur	2	39.75	0.00	0.181
Year	3	39.77	0.02	0.179
Sdur+fband	3	39.84	0.10	0.173
Null	1	40.16	0.41	0.148
Fband	2	40.39	0.64	0.132
Sdur+fband+year	5	40.78	1.04	0.108
Display	2	42.18	2.43	0.054
Display+sdur+fband+year	6	43.63	3.88	0.026
EPP success				
year	2	40.24	0.00	0.358
Null	1	40.80	0.56	0.270
Sdur	2	42.72	2.48	0.103
Display	2	42.93	2.69	0.093
Fband	2	43.06	2.83	0.087
Sdur+fband+year	4	44.38	4.14	0.045
Sdur+fband	3	45.14	4.91	0.031
Display+sdur+fband+year	5	46.81	6.57	0.013

[1] Response variables: 0 or 1, explanatory variables: display = proportion of complete display relative to total displays executed; height = leap height (cm); sdur: song duration, fband: frequency bandwidth and year = 2008-2009, 2009-2010 and 2010-2011 breeding seasons.

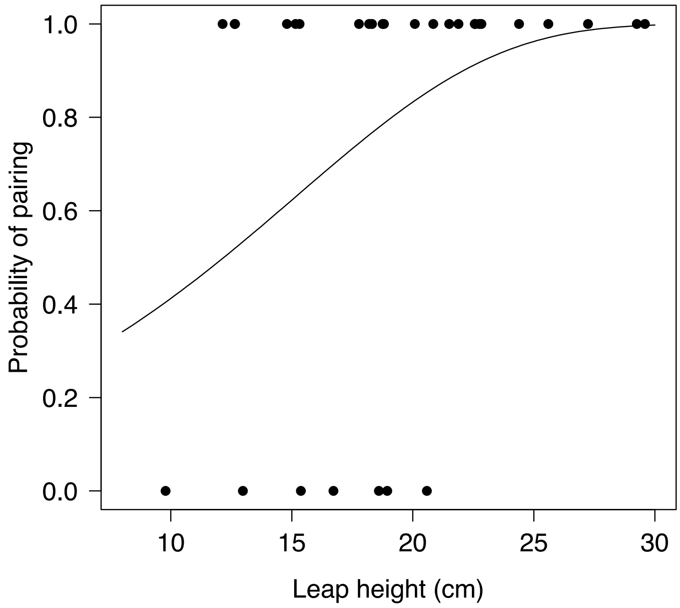

Figure 1. Probability of blue-black grassquit males pairing with a female and having at least one nesting attempt in relation to leap height during complete displays. Line represents the fitted values of a GLM with binomial distribution and points represents observed values.

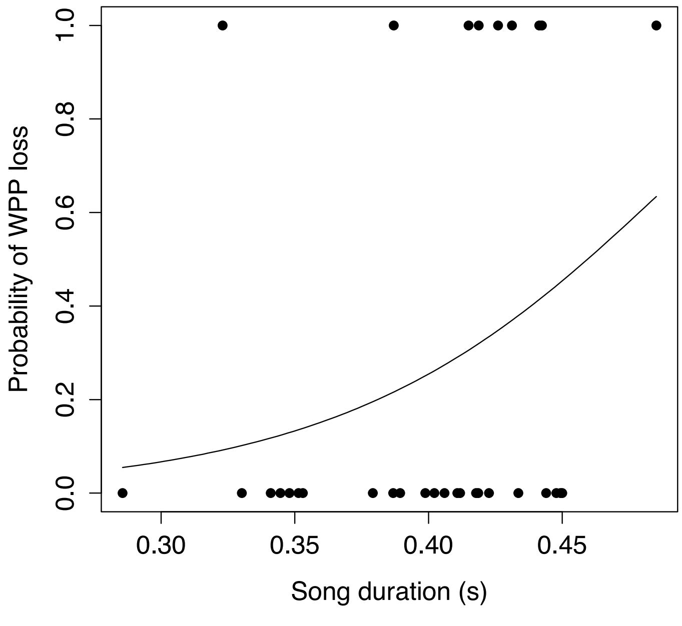

Figure 2. Probability of blue-black grassquit male paternity loss in his social brood in relation to song duration. Line represents the fitted values of a GLM with binomial distribution and points represents observed values.

Contrasts between extrapair *versus* social pair males

We found no evidence of higher seed density in extrapair males' territories when compared to social males' territories (Table 6). Extrapair males did not differ from social males in song parameters (Fig. 3), leap height and proportion of complete displays (Table 6).

Table 6. Pairwise comparisons of social and extrapair male quality traits where: *n* = number of male pairs, fband = frequency bandwidth, *r* = relatedness with female, HL = inbreeding level.

Trait	Social Mean (SD)	Extrapair Mean (SD)	*n*	*t*	P
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
Fband (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	4	0.12	0.91
% complete displays	0.65 ± 0.41	0.88 ± 0.13	3	1.35	0.31
Genetic parameters					
r	0.01 ± 0.23	0.07 ± 0.21	9	0.62	0.55
HL	0.33 ± 0.16	0.31 ± 0.17	9	0.22	0.83

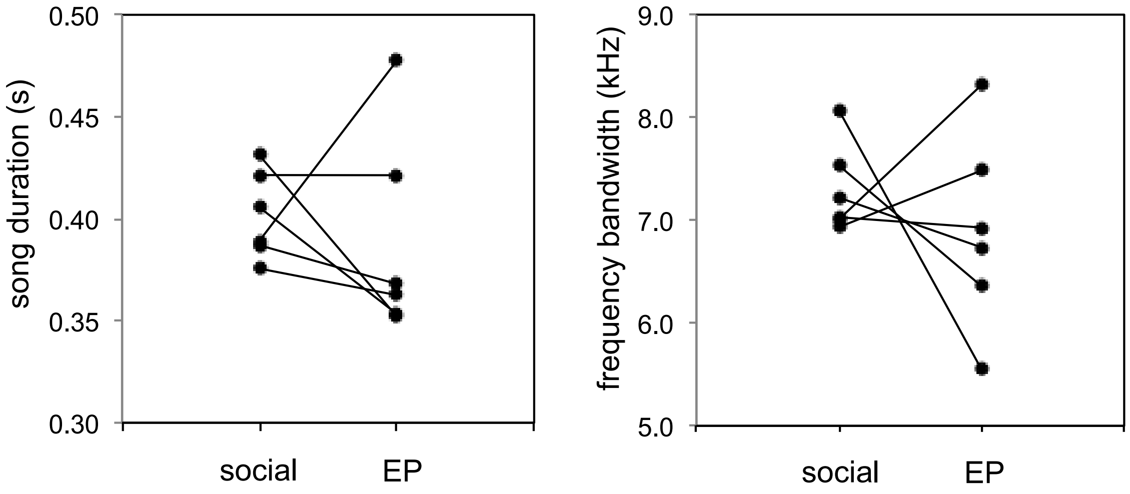

Figure 3. Paired comparisons for song traits of blue-black grassquit social males that lost paternity in their broods and the extrapair males (EP). Among these traits, song duration was an important predictor of WPP loss by social males.

Genetic similarity and heterozygosity

In broods with EPY (r = 0.0006 ± 0.20) females were not more genetically similar to their social mates compared with those from broods with only WPY (r = 0.02 ± 0.20) (t_{47} = 0.39, P = 0.70). Females and their social mates were not more related than females and their extrapair males (Table 6). In three cases we found that extrapair males were strongly related to females with whom they mated (r = 0.55, 0.42 and 0.43). Relatedness between females and social males that lost paternity and between females and extrapair partner pairs was similar to that of females and all males in the population ($r_{\text{social male}}$ = 0.0005 ± 0.20, $r_{\text{population}}$ = -0.01 ± 0.08, W = 81, P = 0.85, n = 17; $r_{\text{EP male}}$ = 0.07 ± 0.23, $r_{\text{population}}$ = 0.005 ± 0.10, W = 32, P = 0.30, n = 9). Extrapair males were not less inbred than were social males (Table 6), nor were mothers of broods with only WPY (HL = 0.36 ± 0.12) less inbred when compared with mothers of broods with EPY (HL = 0.37 ± 0.15) (t_{46} = -0.34, P = 0.73).

Nestling characteristics

EPY was similar to WPY in HL (EPY: 0.35 ± 0.11 and WPY: 0.32 ± 0.17, t_{16} = -0.56, P = 0.58). Nestlings sired by extrapair fathers did not differ in body condition relative to their within-pair half-sibs (EPY: 0.34 ± 0.08 g/mm and WPY: 0.31 ± 0.05 g/mm, t_{12} = 0.19, P = 0.50), nor did they have greater growth rates (EPY: 0.48 ± 0.16 g/mm and WPY: 0.52 ± 0.12 g/mm, t_4 = 0.73, P = 0.50).

DISCUSSION

Extrapair paternity rates are high in the blue-black grassquit, and also alternative female breeding strategies (intraspecific brood parasitism and quasi-parasitism) are common. Our results did not support the direct (resource) benefit hypothesis of extrapair copulations, but did support indirect benefits of female choice. Motor and acoustic display components have different impacts on mating decisions of females. Leap height was important for females in their choice of social partners whereas song duration was related with male chance of loosing paternity. Below, we discuss these results considering the reliability of leap height and song duration as indicators of male quality, where we suggest that females may obtain "good genes" for their offspring by favoring males that exhibit these traits. Nevertheless, our results did not support indirect benefits through female preference for extrapair males with attributes signaling better physical/health conditions, more genetically compatible or heterozygous. In addition, allele diversity and body condition did not differ between EPY and WPY.

Female preference for male traits

We predicted that females would prefer extrapair males in territories with higher food resources than that of their social partner. Female fertility in blue-black grassquits appears to be dependent upon seed abundance (Dias and Macedo 2011), and thus polyandrous behavior could rely on direct benefits for female fitness. However, our results rejected this hypothesis, since seed density was not higher in extrapair male territories. Among possible explanations, food availability in social partner territories may suffice for nestling nutrition, or females may have unlimited access to neighboring territories regardless of having copulated with extrapair males.

Next we postulated that females would prefer social or extrapair males exhibiting cues indicative of better health or physical conditions. We considered rates of leaping, higher leaps (Chapter 1) and possibly acoustic component as potential targets of female choice, as has been shown for several species (review in Byers et al. 2010). Our data revealed that males with higher leaps were indeed more successful in forming social bonds with females, also confirming previous findings (Carvalho et al. 2006). In contrast, other components of displays (song parameters and proportion of complete displays) were less important in a social mating context. The first result indicates indirect benefits to females when selecting preferred social partners as most of them (79.2% of paired males) sired at least one nestling in their broods. Female preference for social males performing higher leaps increases the chance that their offspring will inherit superior genes (Zahavi 1975; Eshel et al. 2000; Kokko et al. 2006). In addition such choosiness could also have direct implications for female fitness, since high quality social partners may be able to provide suitable assistance by defending the nest against predators or feeding the nestlings (Almeida and Macedo 2001). However, to test this hypothesis it would be necessary to compare male phenotypic traits with ability in territory defense and parental effort. The reduced importance of song parameters and proportion of complete displays in pairing success means that such traits do not necessarily signal relevant male properties for social bond formation.

The acoustic component of the display was important in the extrapair mating context indicating preference for social males with shorter songs, however the evidence was not strong. Our pairwise comparison of song duration between social and extrapair males confirms this pattern: the former sang shorter songs than the second (Fig. 3) (although our sample size was too small to find statistical significance in this suggested difference). These patterns can suggest different possibilities of mate choice. First, mate preference for song traits could have evolved to maximize directly or indirectly female fitness, thus our data might indicate that shorter songs reveal high quality of the male in blue-black grassquits. Females "forced" to pair with males in worse condition could compensate for a previous "bad" choice by copulating with multiple males and thus increase chances of transferring good genes to their offspring (Gowaty 1996). This result contrasts of what might be expected for birds in general. For example, longer songs may reveal an individuals' ability to coordinate multiple physiological tasks, such as breathing, beak movement and syringe activity (Nowicki et al. 1992). However, studies reporting the effects of song length in mating choice are mostly based on species with complex repertoires comprising numerous syllables or trills (Hasselquist et al. 1996; Kempenaers et al. 1997; Reid et al. 2004). Differently, the blue-black grassquit has a single note song (Fandiño-Mariño and Vielliard 2004) and possibly any increase in length is not necessarily challenging for males. A possible explanation is that females prefer a shorter song because it signals males' aggressive intents (e.g. Nelson and Poesel 2012), and thus more eagerness to protect the territory or the nest. However, experimental tests relating song length and dispute contexts are needed to confirm such a possibility (Searcy and Beecher 2009).

It is still possible that song length correlates negatively with other characteristic that might indicate quality, for example the plumage condition in the blue-black grassquit (Doucet 2002; Aguilar et al. 2008) that could be targeted by female choice. This situation would typically reflect a trade-off in the production of multiple signals, which predicts that energetic, biomechanical or physiological constraints could "force" individuals to invest differently in each signal component (e.g. Patricelli and Krakauer 2010, Chapter 1). Although we found no trade-offs between acoustic and motor components of the display (Chapter 1), it is still possible that song traits are negatively correlated with plumage characteristics, as shown for other species (Badyaev et al. 2002; Ornelas et al. 2009). Lastly, a non-exclusive possibility is that multiple mating was costly and depended upon female condition, such as body quality, age and previous experiences (Cotton et al. 2006). Recent data have shown that females in lower quality are unlikely to mate with males performing high quality songs (Holveck and Riebel 2010), and that only experienced females seek for multiple mating when indirect benefits are promising (Whittingham and Dunn 2010). We suggest further studies should take into account female condition as potential predictors driving extrapair copulation behavior in this species.

Genetic similarity and heterozygosity

We also tested for another hypothetical genetic benefit of the extrapair behavior in the blue black-grassquit: the genetic similarity between mates and the genetic diversity of breeders. In contrast to earlier findings for 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 genetically diverse males. Social and extrapair males did not differ in relatedness to females, nor when compared with the averaged relatedness of all males in the populations. Moreover, extrapair and social males did not differ in genetic diversity in microsatellites. Additionally, the broods of females with and without EPY did not differ in terms of genetic diversity or body quality. These results suggest that potential inbreeding may not be 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 nestling period). As in the house wren (*Troglodytes aedon*, Forsman et al. 2008) and song sparrows (*Melospiza melodia*, Hill et al. 2010). 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). We suspect, therefore, that females in our studied group were not constrained when seeking for less genetically similar males. Alternatively, when inbreeding occurs, offspring fitness may not necessarily be reduced, and no selection pressure would favor copulations between less genetically similar partners.

Conclusions

In sum, in this study we show that the multimodal display of the blue-black grassquit had multiple effects in social and sexual mating choice, providing evidence for the “multiple message hypothesis” (Møller and Pomiankowski 1993), because females may be using different cues to evaluate particular properties of a mate. We found no support for direct benefits due to increased resource availability, but we corroborated females might accrue indirect benefits through “good genes” for the offspring (Zahavi 1975). We showed that females chose social mates with better motor performance in their complete displays, i.e., those that had higher leaps. We proposed that direct benefits could also be associated with social mate choice, since males in better condition tend also to be good fathers (Kirkpatrick and Ryan 1991; Andersson 1994), although this possibility needs further investigation. The acoustic component was an important predictor of extrapair fertilizations, and the preference favored males with shorter songs. We found no support for indirect benefits through increased genetic compatibility or offspring heterozygosity and body condition due to females’ sexually polyandrous behavior. Although multimodal signals are widespread in several taxa (Byers et al. 2010), our study is one of the few testing multiple effects of aerial and acoustic displays in mate choice in birds (see O'Loghlen and Rothstein 2010a; b; Barske et al. 2011).

REFERENCES

Aguilar TM, Maia R, Santos ESA, Macedo RH (2008) Parasite levels in blue-black grassquits correlate with male displays but not female mate preference. Behavioral Ecology 19:292–301

Akçay E, Roughgarden J (2007) Extra-pair paternity in birds: review of the genetic benefits. Evolutionary Ecology Research 9:855–868

Akçay C, Searcy W, Campbell S, Reed V, Templeton C, Hardwick K, Beecher M (2011) Who initiates extrapair mating in song sparrows? Behavioral Ecology 23:44–50

Alberto F (2009) MsatAllele_1.0: An R package to visualize the binning of microsatellite alleles. Journal of Heredity 100:394–397

Alderton C (1963) The breeding behavior of the blue-black grassquit. Condor 65:154–162

Almeida JB, Macedo RH (2001) Lek-like mating system of the monogamous blue-black grassquit. Auk 118:404–411

Amos W, Wilmer JW, Fullard K, Burg TM, Croxall JP, Bloch D, Coulson T (2001) The influence of parental relatedness on reproductive success. Proceedings of the Royal Society B: Biological Sciences 268:2021–2027

Andersson M (1994) Sexual selection. Princeton University Press, Princeton.

Aparicio JM, Ortego J, Cordero PJ (2006) What should we weigh to estimate heterozygosity, alleles or loci? Molecular Ecology 15:4659–4665

Arnqvist G, Kirkpatrick M (2005) The evolution of infidelity in socially monogamous passerines: the strength of direct and indirect selection on extrapair copulation behavior in females. American Naturalist 165:S26–S37

Arnqvist G, Nilsson T (2000) The evolution of polyandry: multiple mating and female fitness in insects. Animal Behaviour 60:145–164

Badyaev A V, Hill GE, Weckwort B V (2002) Species divergence in sexually selected traits: increase in song elaboration is related to decrease in plumage ornamentation in finches. Evolution 56:412–419

Barske J, Schlinger BA, Wikelski M, Fusani L (2011) Female choice for male motor skills. Proceedings of the Royal Society B: Biological Sciences 278:3523–3528

Bateman AJ (1948) Intra-sexual selection in *Drosophila*. Heredity 2:349–368

Brar RK, Schoenle LA, Stenzler LM, Hall ML, Vehrencamp SL, Lovette IJ (2007) Eleven microsatellite loci isolated from the banded wren (*Thryothorus pleurostictus*). Molecular Ecology Notes 7:69–71

Burke T, Daviest N, Bruford M, Hatchwell B (1989) Parental care and mating behaviour of polyandrous dunnocks *Prunella modularis* related to paternity by DNA fingerprinting. Nature 338:249–251

Burnham K, Anderson D (2002) Model selection and multimodel inference: a practical information-theoretic approach. Springer-Verlag, New York.

Byers J, Hebets E, Podos J (2010) Female mate choice based upon male motor performance. Animal Behaviour 79:771–778

Candolin U (2003) The use of multiple cues in mate choice. Biological Reviews 78:575–595

Carvalho CBV, Macedo RH, Graves JA (2006) Breeding strategies of a socially monogamous neotropical passerine: extra-pair fertilizations, behavior, and morphology. Condor 108:579–590

Cockburn A, Osmond HL, Mulder RA, Green DJ, Michael C, Double MC (2003) Divorce, dispersal and incest avoidance in the cooperatively breeding superb fairy-wren *Malurus cyaneus*. Journal of Animal Ecology 72:189–202

Cohas A, Bonenfant C, Gaillard J-M, Allainé D (2007) Are extra-pair young better than within-pair young? A comparison of survival and dominance in alpine marmot. Journal of Animal Ecology 76:771–781

Costa FJV, Macedo RH (2005) Coccidian oocyst parasitism in the blue-black grassquit: influence on secondary sex ornaments and body condition. Animal Behaviour 70:1401–1409

Cotton S, Small J, Pomiankowski A (2006) Sexual selection and condition-dependent mate preferences. Current Biology 16:R755–R765

Dawson DA, Horsburgh GJ, Küpper C, Stewart IRK, Ball AD, Durrant KL, Hansson B, Bacon I, Bird S, Klein A, Krupa AP, Lee J-W, Martín-Gálvez D, Simeoni M, Smith G, Spurgin LG, Burke T (2010) New methods to identify conserved microsatellite loci and develop primer sets of high cross-species utility - as demonstrated for birds. Molecular Ecology Resources 10:475–494

Dias RI, Macedo RH (2011) Nest predation versus resources in a Neotropical passerine: constraints of the food limitation hypothesis. Ornis Fennica 88:30–39

Double M, Cockburn A (2000) Pre-dawn infidelity: females control extra-pair mating in superb fairy-wrens. Proceedings of the Royal Society B: Biological Sciences 267:465–470

Doucet SM (2002) Structural plumage coloration, male body size, and condition in the blue-black grassquit. Condor 104:30–38

Eshel I, Volovik I, Sansone E (2000) On Fisher–Zahavi’s handicapped sexy son. Evolutionary Ecology Research 2:509–523

Fandiño-Mariño H, Vielliard JME (2004) Complex communication signals: the case of the Blue-black Grassquit *Volatinia jacarina* (Aves, Emberizidae) song. Part I-A structural analysis. Anais da Academia Brasileira de Ciências 76:325–334

Foerster K, Delhey K, Johnsen A, Lifjeld JT, Kempenaers B (2003) Females increase offspring heterozygosity and fitness through extra-pair matings. Nature 425:714–717

Forsman AM, Vogel LA, Sakaluk SK, Johnson BG, Masters BS, Johnson LS, Thompson CF (2008) Female house wrens (*Troglodytes aedon*) increase the size, but not immunocompetence, of their offspring through extra-pair mating. Molecular Ecology 17:3697–3706

Gerlach NM, McGlothlin JW, Parker PG, Ketterson ED (2011) Promiscuous mating produces offspring with higher lifetime fitness. Proceedings of the Royal Society B: Biological Sciences 279:860–866

Gil D, Gahr M (2002) The honesty of bird song: multiple constraints for multiple traits. Trends in Ecology & Evolution 17:133–141

Gowaty PA (1996) Battles of the sexes and origins of monogamy. In: Black JM (ed.) Partnerships in birds: the study of monogamy. Oxford University Press, Oxford, pp. 21–52.

Gray EM (1996) Female control of offspring paternity in a western population of red-winged blackbirds (*Agelaius phoeniceus*). Behavioral Ecology and Sociobiology 38:267–278

Gray EM (1997) Female red-winged blackbirds accrue material benefits from copulating with extra-pair males. Animal Behaviour 53:625–639

Griffith SC, Immler S (2009) Female infidelity and genetic compatibility in birds: the role of the genetically loaded raffle in understanding the function of extrapair paternity. Journal of Avian Biology 40:97–101

Griffith SC, Owens IPF, Thuman KA (2002) Extra pair paternity in birds: a review of interspecific variation and adaptive function. Molecular Ecology 11:2195–2212

Hardy OJ, Vekemans X (2002) SPAGeDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. Molecular Ecology Notes 2:618–620

Hasselquist D, Bensch S, Von Schantz T (1996) Correlation between male song repertoire, extra-pair paternity and offspring survival in the great reed warbler. Nature 381:229–232

Hill CE, Akçay Ç, Campbell SE, Beecher MD (2010) Extrapair paternity, song, and genetic quality in song sparrows. Behavioral Ecology 22:73–81

Holveck M-J, Riebel K (2010) Low-quality females prefer low-quality males when choosing a mate. Proceedings of the Royal Society B: Biological Sciences 277:153–160

Jennions MD, Petrie M (2000) Why do females mate multiply? A review of the genetic benefits. Biological Reviews 75:21–64

Johnstone R (1996) Multiple displays in animal communication: “backup signals” and “multiple messages”. Philosophical Transactions of the Royal Society of London, Series B 351:329–338

Kalinowski ST, Taper ML, Marshall TC (2007) Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Molecular Ecology 16:1099–1106

Kempenaers B, Verheyen G, Van den Broeck M, Burke T, Van Broeckhoven C, Dhondt AA (1992) Extra-pair paternity results from female preference for high-quality males in the blue tit. Nature 357:494–496

Kempenaers B, Verheyen GR, Dhondt AA (1997) Extrapair paternity in the blue tit (*Parus caeruleus*): female choice, male characteristics, and offspring quality. Behavioral Ecology 8:481–492

Kirkpatrick M, Ryan MJ (1991) The evolution of mating preferences and the paradox of the lek. Nature 350:33–38

Kokko H, Jennions MD, Brooks R (2006) Unifying and testing models of sexual selection. Annual Review of Ecology, Evolution, and Systematics 37:43–66

Mazerolle MJ (2010) AICcmodavg: Model selection and multimodel inference based on (Q)AIC(c). R package version 1.25.

Michalczyk Ł, Millard AL, Martin OY, Lumley AJ, Emerson BC, Chapman T, Gage MJG (2011) Inbreeding promotes female promiscuity. Science 333:1739–1742

Møller AP, Pomiankowski A (1993) Why have birds got multiple sexual ornaments? Behavioral Ecology and Sociobiology 32:167–176

Neff BD, Pitcher TE (2005) Genetic quality and sexual selection: an integrated framework for good genes and compatible genes. Molecular Ecology 14:19–38

Nelson DA, Poesel A (2012) Responses to variation in song length by male white-crowned sparrows. Ethology 118:24–32

Nowicki S, Westneat M, Hoese W (1992) Birdsong: motor function and the evolution of communication. Seminars in Neuroscience 4:385–390

Ornelas JF, González C, Espinosa de los Monteros A (2009) Uncorrelated evolution between vocal and plumage coloration traits in the trogons: a comparative study. Journal of Evolutionary Biology 22:471–484

Otter K, Ratcliffe LM, Boag PT (1994) Extra-pair paternity in the black-capped chickadee. Condor 96:218–222

O’Loghlen AL, Rothstein SI (2010a) Multimodal signalling in a songbird: male audiovisual displays vary significantly by social context in brown-headed cowbirds. Animal Behaviour 79:1285–1292

O’Loghlen AL, Rothstein SI (2010b) It’s not just the song: male visual displays enhance female sexual responses to song in brown-headed cowbirds. Condor 112:615–621

Patricelli GL, Krakauer AH (2010) Tactical allocation of effort among multiple signals in sage grouse: an experiment with a robotic female. Behavioral Ecology 21:97–106

Petren K (1998) Microsatellite primers from *Geospiza fortis* and cross-species amplification in Darwin’s Finches. Molecular Ecology 7:1771–1778

Podos J (1997) A performance constraint on the evolution of trilled vocalizations in a songbird family (Passeriformes: Emberizidae). Evolution 51:537–551

Podos J, Lahti DC, Moseley DL (2009) Vocal performance and sensorimotor learning in songbirds. In: Naguib M, Janik VM (eds.) Advances in the Study of Behavior. Vol. 40. Academic Press, Burlington, pp. 159–195.

Queller D, Goodnight K (1989) Estimating relatedness using genetic markers. Evolution 43:258–275

R Development CoreTeam (2011) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/.

Reid JM, Arcese P, Cassidy ALEV, Hiebert SM, Smith JNM, Stoddard PK, Marr AB, Keller LF (2004) Song repertoire size predicts initial mating success in male song sparrows, *Melospiza melodia*. Animal Behaviour 68:1055–1063

Rousset F (2008) GENEPOP’007: a complete re-implementation of the GENEPOP software for Windows and Linux. Molecular Ecology Resources 8:103–106

Rubenstein DR (2007) Female extrapair mate choice in a cooperative breeder: trading sex for help and increasing offspring heterozygosity. Proceedings of the Royal Society B: Biological Sciences 274:1895–1903

Schneider CA, Rasband WS, Eliceiri KW (2012) NIH Image to ImageJ: 25 years of image analysis. Nature Methods 9:671–675

Searcy WA, Beecher MD (2009) Song as an aggressive signal in songbirds. Animal Behaviour 78:1281–1292

Seutin G, White BN, Boag PT (1991) Preservation of avian blood and tissue samples for DNA analyses. Canadian Journal of Zoology 69:82–90

Sheldon BC (1994) Male phenotype, fertility, and the pursuit of extra-pair copulations by female birds. Proceedings of the Royal Society B: Biological Sciences 257:25–30

Sick H (2001) Ornitologia brasileira. Editora Nova Fronteira, Rio de Janeiro.

Simmons LW (2005) The evolution of polyandry: sperm competition, sperm selection, and offspring viability. Annual Review of Ecology, Evolution, and Systematics 36:125–146

Taff CC, Steinberger D, Clark C, Belinsky K, Sacks H, Freeman-Gallant CR, Dunn PO, Whittingham LA (2012) Multimodal sexual selection in a warbler: plumage and song are related to different fitness components. Animal Behaviour:1–9

Tarvin KA, Webster MS, Tuttle EM, Pruett-Jones S (2005) Genetic similarity of social mates predicts the level of extrapair paternity in splendid fairy-wrens. Animal Behaviour 70:945–955

Tregenza T, Wedell N (2000) Genetic compatibility, mate choice and patterns of parentage: invited review. Molecular Ecology 9:1013–1027

Tryjanowski P, Hromada M (2005) Do males of the great grey shrike, *Lanius excubitor*, trade food for extrapair copulations? Animal Behaviour 69:529–533 van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) Micro-Checker: software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Notes 4:535–538

Varian-Ramos CW, Webster MS (2012) Extrapair copulations reduce inbreeding for female red-backed fairy-wrens, *Malurus melanocephalus*. Animal Behaviour 83:857–864

Wagner RH (1998) Hidden leks: sexual selection and the clustering of avian territories. In: Parker P, Burley N (eds.) Avian reproductive tactics: female and male perspectives Ornithological Monographs No 49. American Ornithologists' Union, Washington, pp. 123–145.

Westneat DF, Stewart IRK (2003) Extra-pair paternity in birds: causes, correlates, and conflict. Annual Review of Ecology, Evolution, and Systematics 34:365–396

Wetton JH, Parkin DT (1991) An association between fertility and cuckoldry in the house sparrow, *Passer domesticus*. Proceedings of the Royal Society B: Biological Sciences 245:227–233

Whittingham LA, Dunn PO (2010) Fitness benefits of polyandry for experienced females. Molecular Ecology 19:2328–2335

Zahavi A (1975) Mate selection-A selection for a handicap. Journal of Theoretical Biology 53:205–214

CAPÍTULO 3

Leaping higher, arriving earlier: advantages in a clustered breeding system

ABSTRACT

Extrapair copulations in socially monogamous species can be a mechanism related to territorial clustering, analogous to lekking species in which males aggregate into arenas to display and attract females. We tested two evolution models of the “hidden-lek” hypothesis (female preference and hotshot) in the Neotropical blue-black grassquit (*Volatinia jacarina*). Males of this species defend small and aggregated territories within which they perform courtship displays that consist of repeated vertical flights (“leaps”) and a synchronized, short buzzy vocalization. Displays can be complete, including both visual (leaps) and vocal components, and incomplete, composed solely by vocalizations while the male is perched. We predicted that 1) males establishing earlier or at central territories within a cluster should display more attractive or high quality traits (higher leap displays, higher proportion of complete displays or shorter songs), 2) would have lower paternity loss and higher chances of extrapair fertilizations; 3) males in clusters would have higher pairing success; 4) the proportion of paired males and the proportion of extrapair fertilizations among broods would increase with cluster size. We found earlier males had higher leaps and songs with longer duration, however there was not relationship between these traits and male spatial position. Earlier males were also more likely to obtain extrapair fertilizations, but we found no support for predictions 3 and 4. Our data partially validate the predictions of the hotshot model of hidden lek hypothesis, and the possibility that male settlement in a cluster could occur according to female mate choice. This result is reinforced by earlier males’ increased success in extrapair fertilizations. In conclusion, the blue-black grassquit does not absolutely represent a hidden lek model, specially because of the weak relationship between spatial position of territories within clusters and extrapair copulations or male quality. However, the early arrival of “hotshot” males in clusters corroborates an important expectation of a lek-like mating system and could have triggered clustering, thus we cannot refute completely that the blue-black grassquit is a hidden lek model.

Keywords: social monogamy, extrapair copulation, hidden lek, multimodal signal, *Volatinia jacarina*

INTRODUCTION

Extrapair copulations in socially monogamous species can be a mechanism related to territorial clustering. Aggregations of breeding individuals are common in several species and traditional hypotheses rely on explanations based on resource distribution (Kiester and Slatkin 1974), predation avoidance (Hamilton 1971; Perry and Andersen 2003), competitive exclusion of heterospecifics (Orians and Willson 1964) and conspecific attraction (Stamps 1988). Based on observations of colonial birds, Wagner (1993, 1998) alternatively suggested that aggregations in socially monogamous species should result from increased opportunities for mating, with females visiting dense aggregation of males to seek extrapair copulations. Such “hidden-lek” systems should resemble traditional leks, wherein males of polygamous species aggregate into arenas to display and attract females for copulations (Höglund and Alatalo 1995). Similarly as expected for leks (Beehler and Foster 1988), reproductive skew should be biased toward one or several males that obtain most of fertilizations. The important difference between the traditional versus the hidden lek systems is the fact that males in the latter are socially monogamous, invest in parental care and establish larger and multi-purpose territories (e.g. for foraging and nesting) (Wagner 1998), while in traditional leks male participation in the breeding effort is limited to copulation (Höglund and Alatalo 1995).

Four hidden lek evolution models were proposed, derived from traditional lek models, and can be distinguished by patterns of habitat selection and sexual interaction among extrapair partners (synthesized by Fletcher and Miller 2006). In the female preference model (Bradbury 1981), females should settle preferentially in larger aggregations because of enhanced opportunities for extrapair encounters and facilitated phenotypic comparisons of potential mates (Wagner 1998). Thus, this hypothesis predicts that pairing success and extrapair fertilizations should increase with aggregation size (e.g. Tarof et al. 2004). The hotshot model (Beehler and Foster 1988) proposes that females prefer dominant or more attractive males, which consequently have increased pairing success. Such preference results in subordinates or less-preferred males aggregating near hotshot males to enhance their own pairing and breeding opportunities (e.g. Greene et al. 2000). This model predicts that dominant males should be spatially centralized (e.g. Tarof et al. 2004) and more successful in obtaining extrapair fertilizations (Wagner 1998). The hotspot model hypothesizes that clusters should occur in areas of high female activities, occurring frequently in populations that either explore rare resources and with well-established travel routes or in which female home ranges overlap (Bradbury et al. 1986). Finally, the kin selection model also predicts female preference for aggregations, but posits that subordinate males settle around a dominant and closely-related male, benefiting through increased inclusive fitness (Kokko and Lindström 1996).

Five criteria were proposed as essentially establishing the occurrence of hidden leks: 1) pair bond formation between males and females, 2) aggregation of territories, 3) females being receptive for extrapair copulations, 4) no parental care from extrapair males, and 5) no monopolization of resources by extrapair males (Fletcher and Miller 2006). Given these parameters, the hidden lek hypothesis has been proposed for several non-colonial socially monogamous birds with gregarious behavior, such as the Neotropical dusky bush-tanager (*Chlorospingus semifuscus*, Bohórquez and Stiles 2002), the hooded warbler (*Wilsonia citrine*, Melles et al. 2009), the superb fairy-wren (*Malurus cyaneus*, Cockburn et al. 2009), and the least flycatcher (*Empidonax minimus*, Tarof and Ratcliffe 2004; Tarof et al. 2004). To this list of possible candidates we add the Neotropical blue-black grassquit (*Volatinia jacarina*, Almeida and Macedo 2001), which is our focus in the present study. Although the hidden lek hypothesis seems appropriate to explain mating patterns in several species, there are very few empirical tests of predictions (e.g. Hoi and Hoi-Leitner 1997; Tarof et al. 2004; Cockburn et al. 2009).

Here we studied territorial clustering in the Neotropical blue-black grassquit, a seasonal pattern that occurs during the breeding season after birds arrive in central Brazil from migration. Males defend small (13-72 m) and aggregated territories (Almeida and Macedo 2001; Sick 2001) within which they perform courtship displays that consist of repeated vertical flights ("leaps") and a synchronized, short buzzy vocalization at high rates (up to 19 leaps per minute) for prolonged periods (Sick 2001, Chapter 1). Displays can be complete, including both visual (leaps) and vocal components, and incomplete, composed solely by vocalizations while the male is perched. Grassquits are socially monogamous but sexually polygamous (Carvalho et al. 2006, Chapter 2), and the social pair cooperate in parental care (Almeida and Macedo 2001). The mating system in this species is often suggested to resemble a lek (Murray 1982; Webber 1985; Almeida and Macedo 2001; Tarof et al. 2004; Dias et al. 2009) and could perhaps be better described through the hidden lek model (Almeida and Macedo 2001), however this possibility remains untested.

Territorial clustering was previously investigated in relation to habitat and food availability, and it was shown that birds occur at higher densities and nest in areas covered mostly by savanna grassland (Almeida and Macedo 2001; Aguilar et al. 2008a). Furthermore, that clusters tend to form in areas with complex vegetation and increased food availability (Dias et al. 2009). Despite the importance of habitat quality in determining the spatial distribution of blue-black grassquits during their breeding season, sexual selection may also play a role in determining clusters, given that this species seems to fit all the criteria proposed by Fletcher and Miller (2006). High rates of extrapair fertilizations (Carvalho et al. 2006, Chapter 2) indicate that proximity to conspecifics could be influenced by the possibility of gaining extrapair copulations. Furthermore, the proportion of fertile females is greater in clusters than in solitary territories (Dias et al. 2009), which may favor the establishment of a polygamous mating system constrained by a short breeding season (December to April in central Brazil). Previous evidences also indicate genetic benefits associated with sexual polyandry in this species, as shown by increased pairing and fertilization success of males in better condition (i.e. with higher leaps and shorter songs) that could transfer good genes to their offspring (Chapter 2). This latter finding suggests that females could benefit by maximizing the chances of encountering such males.

We tested predictions of the hidden lek hypothesis in the blue-black grassquit relative to the hotshot and female preference models. We expected that if hotshot males influence clustering, then males establishing earlier or at central territories within a cluster should display more attractive or high quality traits. We assumed males performing higher leap displays, higher proportion of complete displays and singing shorter songs were preferred by females or in better quality (Carvalho et al. 2006, Chapter 1, Chapter 2). We also correlated frequency bandwidth with centrality and establishment date to test if they could potentially indicate male quality. In addition we expected central or earlier males to have lower paternity loss than surrounding males and higher chances of extrapair fertilizations. This temporal and spatial distribution pattern could be driven by the settlement of lower quality (and less preferred) males around hotshot males to increase chances for mating. When testing for the female preference model, we predicted that males in clusters would have higher pairing success than solitary males, and that the proportion of paired males would increase with cluster size. Additionally, if clustering increases opportunities for extrapair copulations as postulated by the female preference model, we also expected a positive relationship between cluster size and the proportion of extrapair fertilizations among broods.

METHODS

Study area and field procedures

We studied blue-black grassquits at Fazenda Água Limpa (15°56'S 47°56'W), located 28 km from Brasília, DF, Brazil, from October to February during three breeding seasons (2008-

2009, 2009-2010 and 2010-2011; years 1, 2 and 3, hereafter). All data were collected in an area of approximately 6.56 ha, consisting of an abandoned orchard, altered grasslands and shrubby savanna vegetation at the edge of a natural *cerrado* area (typical tropical savanna).

Our sample consisted of 108 males and 63 females (year 1: 38 and 20; year 2: 37 and 28, and year 3: 33 and 15, respectively) captured in the study site. We captured birds using mist nets and marked them with unique combinations of three colored plastic bands and one numbered aluminum band supplied by the Brazilian Bird Banding Agency (CEMAVE/ICMBio). Mist netting occurred from 0700h to 1200h three times weekly from October to December in each year in specific areas within bird aggregations. After males established their territories, usually from January to March, we used directional mist netting within territories to increase chances of capturing focal individuals. From all birds, with the exception of 17 males and 13 females, we collected samples of approximately 60 µl of blood via brachial venipuncture, which were stored in Queen´s lysis buffer solution (100 mM Tris – pH 7.5; 100 mM EDTA; 10 mM NaCl; 0.5% SDS, Seutin et al. 1991) and refrigerated at 4ºC.

Nest monitoring

We sought nests in all potential nesting sites near male display perches or using reproductive behavioral cues of adults, such as nest building and nestling feeding. We assigned social parents for each nest by identifying the individuals brooding or feeding the nestlings during approximately 15 minutes of focal observations per nest. We monitored 176 nests (year 1: n = 54; year 2: n = 74; year 3: n = 48), including multiple breeding attempts of 17 males and six females in different years. A total 101 nests belonged to captured males and 50 to captured females. In 57 nests we knew identity of both male and female. We obtained blood samples of approximately 20 µl via brachial venipuncture from nestlings at six days after hatching, immediately stored in Queen´s lysis buffer solution and refrigerated at 4ºC. We collected blood from 179 nestlings (43, 65 and 71 in years 1, 2 and 3, respectively) from 79 nests (21, 30 and 28 in years 1, 2 and 3, respectively), from which we had also genetic material of the social father and, in 44 nests, of the social mother.

Males' spatial and temporal distribution

We searched for males executing displays at least three times weekly in the whole study area. We considered that a male established a territory if he was regularly recorded in the same area for a minimum of two weeks or if he had at least one breeding attempt in that site. We additionally considered the presence of nests belonging to unknown social males breeding at the same time as known males to account for the establishment of non-monitored individuals and to precisely estimate cluster sizes (see below). Territory establishment date was defined as the first day we recorded a territorial male in the area executing displays (day 1 = Oct 24). We identified 224 territories (year 1: n = 63; year 2: n = 88; year 3: n = 73), including territories of all captured males and of 75 non-captured males but whose nests were found (Table 1). Among captured males, 48 did not acquire mates and thus were not associated with a nesting attempt (Table 1).

Table 1. Number of territorial males registered in the study area in all breeding seasons and their breeding status: paired, males that had at least one nesting attempt, and unpaired, males that did not have a nesting attempt. Monitored males are those with known identity and not monitored males, those which presence was inferred by the register of their nest. Year 1: 2008-2009, year 2: 2009-2010 and year 3: 2010-2011.

Male status	Breeding season			
	Year 1	Year 2	Year 3	Total
Paired				
Monitored	32	37	32	101
Not monitored (nest only)	22	37	16	75
Total paired	54	74	48	176
Unpaired	9	14	25	48

We defined clusters as two or more territories separated from other territories with a vegetation structure that did not allow visual contact among individuals or by areas unoccupied with conspecifics with a minimum distance of 20 m (mean ± standard deviation = 45.0 ± 15.5 m). Solitary males were those whose territories were isolated, separated by at least 50 m from other conspecifics´ territories, which prevented any visual and audio contact with neighbors. We assessed the spatial position of territories by taking Universal Transverse Mercator coordinates (*datum* WGS84) at the nest or at places where unpaired males were more frequently found conducting displays, using a Global Positioning System (GPS). These points were considered as centrally located within a territory. We defined territories using Google Earth® version 7.0.2 and used AutoCad® 2013 to outline the minimum polygon convex using outermost points of a cluster and to measure the linear distance of a male territory from the polygon geometric center (male centrality hereafter).

Of the 224 territories recorded, only two were isolated (one in year 1 another in year 3) while the others were distributed in 32 clusters (10 in year 1, 11 in year 2 and 11 in year 3) (Fig. 1, Table 2). In four clusters we were unable to estimate the exact number of males (clusters F and H year 1, and F and G year 2, Fig. 1, Table 2), so they were excluded from further analyses. The number of individuals in clusters averaged 6.9 ± 4.6 (range 2 – 19 males), the mean cluster size was 2326.3 ± 2372.9 m^2 (range 94.5 – 8021.9 m^2) and the distance of territories from the cluster center averaged 26.9 ± 9.7 m (range 1.8 – 72.3 m) (Table 2).

Table 2. Characteristics of 32 blue-black grassquit clusters. Establishment date and nest initiation date were estimated as continuous days with day 1 = Oct 24, centrality represents the distance of territories (spatial position of the nest or males' display main spot) to the geometric center of the cluster. Year 1: 2008-2009, year 2: 2009-2010 and year 3: 2010-2011.

Year/cluster	Males (*n*)	Nests (*n*)	Establishment* range (max-min)	Area (m^2)	Centrality (m) range (average)
Year 1					
A	19	16	73 - 126 (53)	8021.9	10 - 61.3 (42.4)
B	6	5	110 (0)	858.5	8.6 - 64.0 (33.9)
C	6	6	75 - 110 (35)	1415.5	10.5 - 56.2 (32.8)
D	6	5	111 - 118 (7)	654.0	5.5 - 28.6 (16.9)
E	5	5	77 - 130 (53)	968.5	14.9 - 26.1 (21.0)
F[1]	7	7	-	626.0	9.9 - 58.0 (30.3)
G	3	3	73 (0)	153.3	8.6 - 22.9 (15.9)
H[1]	3	3	82 (0)	94.5	3.8 - 22.2 (15.3)
I	5	1	90 - 110 (20)	425.5	7.7 - 34.7 (19.9)
J	2	2	117 (0)	-	
Year 2					
A	15	11	13 - 97 (84)	7120.2	21.3 - 57.2 (42.1)
B	3	2	43 - 62 (19)	265.5	8.8 - 16.2 (13.0)
C	11	9	45 - 70 (25)	4628.5	4.8 - 59.5 (36.2)
D	6	5	46 (0)	747.5	10.5 - 22.6 (17.7)
E	4	3	4 - 68 (64)	265.0	7.0 - 27.2 (16.0)
F[1]	5	5	-	1304.6	11.4 - 37.9 (25.5)
G[1]	8	8	-	1390.0	9.3 - 32.5 (22.7)
H	8	7	27 - 80 (53)	1616.0	4.1 - 41.6 (25.4)
I	17	16	27 - 77 (50)	5394.5	6.1 - 61.0 (36.2)
J	9	7	51 - 71 (20)	4377.5	3.2 - 67.7 (35.0)
K	2	1	17 (0)	-	
Year 3					
A	9	7	42 - 91 (49)	5123.0	1.8 - 53.8 (37.9)

B	12	8	43 - 105 (62)	2775.5	9.2 - 46.9 (29)
C	6	2	50 - 98 (48)	2310.0	11.6 - 42.1 (29.1)
D	2	1	65 (0)	-	
E	2	1	69 (0)	-	
F	5	4	43 - 91 (48)	892.5	7.1 - 28.3 (18.1)
G	4	4	-	430.1	13 - 29.3 (19.1)
H	5	2	49 - 81 (32)	475.5	3.7 - 21.7 (14.9)
I	16	11	37 - 95 (58)	5849.4	10.2 - 72.3 (40.2)
J	9	6	37 - 84 (47)	4627.1	20.6 - 68.9 (40.4)
K	2	1	108 (0)	-	-

* Not estimated for all males in the cluster.

[1] Exact number of individuals and nests unknown.

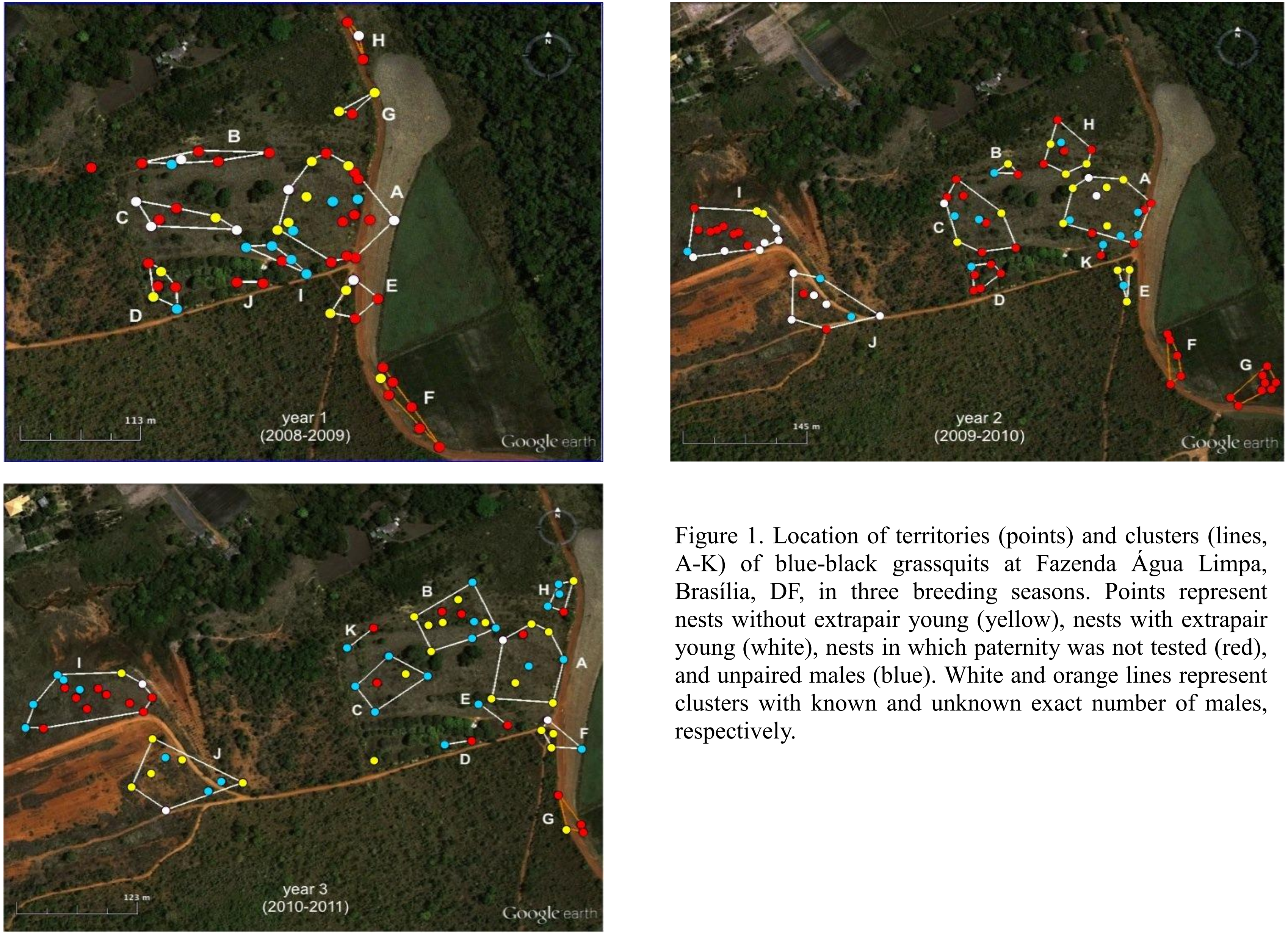

Figure 1. Location of territories (points) and clusters (lines, A-K) of blue-black grassquits at Fazenda Água Limpa, Brasília, DF, in three breeding seasons. Points represent nests without extrapair young (yellow), nests with extrapair young (white), nests in which paternity was not tested (red), and unpaired males (blue). White and orange lines represent clusters with known and unknown exact number of males, respectively.

Motor and acoustic displays

We analyzed songs of 58 territorial males (year 1: *n* = 12, year 2: *n* = 23 and year 3: *n* = 23), which were also captured and banded. Recordings were conducted using 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 a Telinga parabola. We recorded songs from 0700 h to 1200 h over one (*n* = 37), two (*n* = 18), three (*n* = 2) or four (*n* = 1) different days for each male. Song files were transferred to a computer and, using the software program Audacity® v. 2.0.0 (http://audacity.sourceforge.net), we selected five song samples from each bird, excluding introductory notes that often precede the stereotyped vocalization. From each sample, in the software program Cool Edit® v. 2.1 (Syntrillium Software Corporation 2003) we measured song duration (ms) from oscillograms and spectrograms, minimum and maximum frequencies from power spectrum using a -24 dB amplitude cut-off criterion (as in Podos 1997). We then calculated frequency bandwidth as maximum minus minimum frequency for each song.

Next we measured leap height of 41 males (year 1: *n* = 3, year 2: *n* = 21 and year 3: *n* = 17) whose songs had also been recorded. We filmed males complete displays (leaps synchronized with songs) with a mini-dv Canon XL1 digital camcorder in years 1 and 2 and a Casio digital camera EX-FH25 in year 3. Recordings were made from 0700 h to 1200 h, in one (*n* = 22), two (*n* = 15), three (*n* = 3) or four (*n* = 1) different days for each male. We used high video quality (in focus from a lateral angle) to sample 5 to 12 complete displays for each male (mean ± standard deviation = 9.5 ± 1.5, *n* = 389 samples). Videos from the Canon recorder were digitized using iMovie v. 7.1 (Apple Inc. 2008), and together with the Casio recordings were transferred to a computer. Using Windows® Movie Maker v. 5.1 (Microsoft Corporation 2007) we extracted frames corresponding to the very beginning of the leap, i.e., the frame before the bird left the perch or flapped its wings to initiate the leap, and frames for the maximum height of the leap. In order to measure leap height, we used program Image J® v. 1.45s (Schneider et al. 2012) to calculate the distance between the subject's beak when at the peak of the leap, and a horizontal line at the perch. This distance measurement was calibrated to the average head height, which was visible in video frames and also measured from a sample of grassquit specimens in hand (mean head height = 13.5 mm).

Finally we analyzed complete display rates during focal observations of 43 males (year 1: *n* = 10; year 2: *n* = 17; year 3: *n* =16) from which we also had song recordings. Twenty-two of these birds were also filmed and had their leap height measured. Focal observation periods lasted approximately 30 min, from 0700 h to 1000 h, in one (*n* = 22), two (*n* = 11) or three (*n*

= 10) different days over a period of two weeks. All focal observations occurred during the pre-nesting period to avoid biases caused by possible male reduction in display investment (Alderton 1963, L. Manica & R. Macedo pers. observ.). We recorded the occurrence of each display and whether it was complete (leaps synchronized with songs) or incomplete (song only) to calculate the proportion of complete displays relative to overall displays executed.

Song parameters, leap height and complete displays rates of each male presented here include only one sample for each male in the three years of study, and when we had multiple replicates in the same breeding season we used the average values in analyses. We did not include data from males settled in clusters with less than three individuals sampled.

Molecular and parentage analyses

Parentage assignment was conducted as described in detail in Chapter 2. Briefly, we amplified 15 microsatellite markers with fluorescently labeled primers arranged in four groups in a multiplex Polymerase Reaction Chain (PCR). Individuals' genotypes were scored using Beckman Coulter CEQ^{TM} sequencer and CEQ 8000^{TM} Genetic Analysis System software. Fragment sizes binning was conducted using the R package MsatAllele (Alberto 2009) in R software (R Development Core Team 2011). We tested for Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD) using Genepop 4.1.1 (Rousset 2008) and tested for the presence of null alleles using Micro-Checker software (van Oosterhout et al. 2004). Microsatellites showed polymorphism, ranging from 4 to 29 alleles per locus and the combined probability of exclusion was 0.9837, 0.9479 and 0.9413, for years 1, 2 and 3, respectively, and 0.9991, 0.9950 and 0.9931, respectively, when the genotype of a parent of the opposite parent sex was known (Chapter 2).

We 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 (LOD scores). We first ran maternity analyses to confirm if females incubating or feeding the nestlings were actually biological mothers of the offspring, and when maternity was confirmed, known mothers were included in paternity analyses. We registered if males lost paternity of at least one social nestling (within pair paternity loss: WPP loss) and if he sired at least one extrapair young (extrapair paternity success: EPP success) to estimate male breeding success in each season. When repeated measures for a male occurred in different years, we used data from the first year or the one with the most complete information.

Statistical analysis

We linked display traits (song duration and frequency bandwidth, proportion of complete displays and leap height) with male territorial centrality and establishment date using linear mixed models (LMM) including a nested hierarchical random effect “cluster nested within year”, to control for non-independence of males sampled in the same cluster and year. To overcome the low sample size of males with both proportion of complete display and leap height measured (n = 22 from six clusters), we initially assessed the importance of these predictors in models including all variables. Next, we excluded either proportion of complete displays or leap height according to their importance in the models (see criteria below) and fitted a final model with a larger sample size (n = 41 males from 13 clusters and n = 43 males from 11 clusters, respectively).

We associated male WPP loss and EPP success with centrality and establishment date in clusters. In these two models we used data from clusters for which we had assigned paternity for at least three broods and which contained at least one male with WPP loss or one male that sired extrapair young. We also included the nested hierarchical random effect “cluster nested within year”, to control for non-independence of males sampled in the same cluster and year. To evaluate the effect of cluster size on pairing success and the risk of paternity loss, we associated the proportion of successfully paired males and the proportion of nests with WPP loss (considering those with tested paternity) with the number of males in a cluster in two different generalized linear mixed models (GLMM), adding year as random effect. To fit these models, we used GLMM with binomial distribution and complementary log-log link function. All models were fitted using the “lmer” function of the “lme4” R package (Bates et al. 2011) in R software (R Development Core Team 2011). We centered all continuous variables to zero and scaled by their standard deviation and applied arc-sin transformations in proportion of complete displays to achieve normalization or approximate to a normal distribution.

To assess the relationship among variables, we fitted a candidate set of models and used the second-order Akaike’s Information Criteria (AICc) to select those with best support to explain the data (Burnham and Anderson 2002). We also calculated model probabilities (Akaike’s weight), model-averaged estimates and respective unconditional standard errors and 95% confidence interval of each predictor to allow inferences about their importance in a best model (Burnham and Anderson 2002). Centrality and establishment date candidate models were fitted by combining: a) all variables, b) each variable alone, c) only acoustic parameters (song duration and frequency bandwidth), d) only motor component (proportion of complete displays or leap height), and e) only intercept (null model). When assessing the relationship of WPP loss or EPP success with centrality and establishment date we used all possible combinations of the predictors and a null model as candidate models. To evaluate the importance of cluster size, we compared a null model with models using proportion of paired males and proportion of nests with WPP loss, and which included cluster size as the predictor. Model selection was conducted using the R package AICcmodavg (Mazerolle 2010) with R software (R Development Core Team 2011).

RESULTS

Territory establishment and male traits

Male centrality was weakly related with proportion of complete displays when we included all predictors in model selection (Table 1, Appendix), thus we excluded the variable proportion of complete displays from final models. We also found weak support for leap height and acoustic components as good predictors (Table 3). The null model was the best-fit (probability of 45.1%) and all model-averaged estimates of predictors were relatively low and with 95% CI bounding or centered on zero ($\beta_{\text{song duration}} = -0.13 \pm 0.15$, 95% CI = -0.17, 0.42; $\beta_{\text{freq. band.}} = 0.08 \pm 0.15$, 95% CI = -0.21, 0.38; $\beta_{\text{height}} = 0.13 \pm 0.15$, 95% CI = -0.17, 0.43). These results indicate that males in central territories do not differ from peripheral males in display traits.

Both leap height and proportion of complete displays were important predictors in the establishment date model selection when we included all predictors (Table 1, Appendix). Therefore, we conducted two different subsequent model selections to evaluate the importance of each predictor with a larger sample size. In one model we excluded leap height and in the other, we excluded proportion of complete displays, while keeping song duration and frequency bandwidth in all models. Proportion of complete displays was included in the best model of establishment date (Table 3) and had model-averaged estimates biased toward positive values ($\beta_{\text{display}} = 0.28 \pm 0.12$, 95% CI = 0.03, 0.52). However the evidence ratio of the best model against the second-best model, which includes only song duration as predictor, was approximately one. These results indicate that the proportion of complete displays predictor did not improve model fit, thus the relationship with establishment date is weak. Contrarily, leap height was important in model selection as this predictor was included in the best model, which was almost three times more likely than the second and the third best models (evidence ratio = 3.28 and 3.36, respectively, Table 3). Establishment date reduced as leap height increased (β_{height} = -0.28 ± 0.12, 95% CI = -0.52, -0.09). This result indicates that males that settled later in the study area had slightly lower leaps (Fig. 2). In addition, males that established territories later in the breeding season also tended to have shorter songs ($\beta_{song\ duration}$ = -0.33 ± 0.12, 95% CI = -0.58, -0.09, Fig. 3), as all models with lowest AIC included this predictor. Although frequency bandwidth was also among best models, the support was weak as shown by the evidence ratio of approximately one in relation to the second best model (which includes only "sdur" as variable, Table 3) and the large uncertainty of parameter estimate ($\beta_{freq.\ band.}$ = -0.15 ± 0.13, 95% CI = -0.40, 0.10).

Table 3. Model selection tables for LMMs of male centrality and establishment date in relation to display parameters. Proportion of complete displays was excluded from centrality final model selection since it was not important in exploratory analysis (see Appendix). Two different model selection of establishment date were conducted to increase sample size: first, excluding leap height and second, excluding proportion of complete displays (*n* = 43 and 41 males, respectively). K = number of parameters, AICc = second-order Akaike's Information Criteria, w_i = Akaike's weight.

Models[1]	K	AICc	ΔAICc	w_i
Centrality[2]				
Null	4	123.58	0.00	0.451
Height	5	125.50	1.92	0.173
Sdur	5	125.57	1.99	0.166
Fband	5	125.93	2.35	0.139
Sdur+fband	6	127.80	4.22	0.055
Sdur+fband+height	7	130.25	6.67	0.016
Establishment date[2]				
Leap height excluded				
Sdur+fband+display	7	112.24	0.00	0.344
Sdur	5	112.28	0.05	0.336
Sdur+fband	6	114.09	1.86	0.136
Display	5	114.57	2.33	0.107
Fband	5	116.61	4.37	0.039
Null	4	116.62	4.39	0.038
Display excluded				
Sdur+fband+height	7	109.52	0.00	0.541
Sdur	5	111.90	2.38	0.165
Sdur+fband	6	111.95	2.43	0.161
Height	5	113.10	3.58	0.090
Fband	5	115.08	5.56	0.034
Null	4	117.56	8.04	0.010

Year and cluster included as a nested hierarchical random effects

[1] Explanatory variables: sdur = song duration; fband = frequency bandwidth; display = proportion of complete display rates relative to total displays executed; height = leap height (cm).

[2] Random effects variance:

Centrality model: cluster nested within year = 0.16, year < 0.001.

Establishment date model, display excluded: cluster nested within year < 0.001, year = 0.80.

Establishment date model, height excluded: cluster nested within year < 0.001, year = 0.63

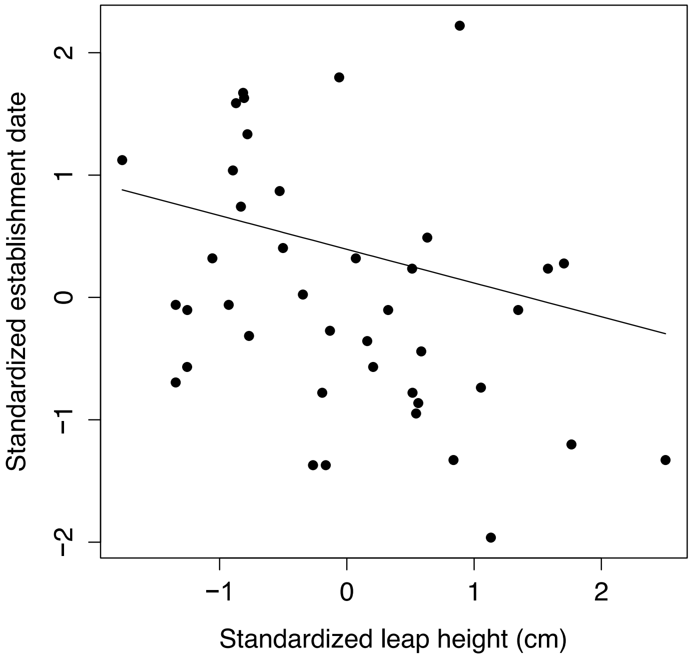

Figure 2. Relation between territory establishment date (day 1 = Oct 24) and leap height of male blue-black grassquits. Axes are standardized to mean zero and scaled by their standard deviation.

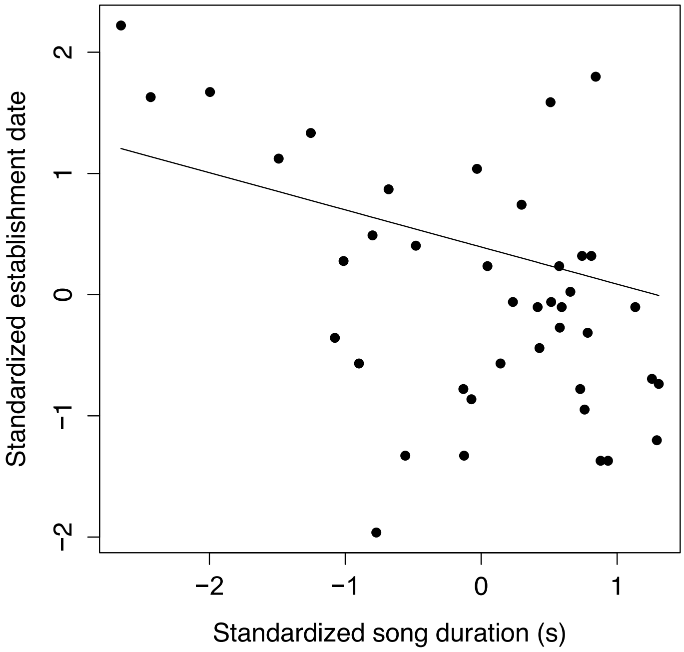

Figure 3. Relation between territory establishment date (day 1 = Oct 24) and song duration of male blue-black grassquits. Axes are standardized to mean zero and scaled by their standard deviation.

Extrapair fertilizations in clusters

We registered WPP loss for 24 males in 26 nests (eight in year 1, 14 in year 2 and four in year 3) settled in 13 clusters (Fig. 1). In fourteen (53.8%) of these nests social males sired at least one within-pair young, and 12 (46.1%) males reared only extrapair young. We associated probability of WPP loss with centrality and establishment date for 24 males, nine of which had WPP losses. We found weak support for the hypothesis that males' distance from the cluster center influenced the risk of losing paternity ($\beta_{centrality} = 0.25 \pm 0.35$, 95% CI = -0.45, 0.95, sum of model probabilities = 20.3%, Table 4). The single best model included establishment date as predictor of WPP loss (Table 4), although the effect was weak ($\beta_{date} = 0.25 \pm 0.34$, 95% CI = -0.43, 0.92).

We identified 19 extrapair fathers of nestlings from 15 nests (four nests had nestlings sired by two extrapair males). Six of these males settled in territories in the same cluster where they sired extrapair young, five in adjacent clusters, and six in clusters more than 350 m distance. We were unable to determine the territory location of two extrapair males. Thirteen extrapair males were paired with a social female and had at least one nesting attempt, but three out of eleven extrapair males for which we had data on WPP success also lost paternity in their social broods. We evaluated the influence of centrality and establishment date on EPP success for 40 males in nine clusters, 10 of whom sired extrapair young. Centrality had a weak importance in the models (sum of model probabilities 30.9%, $\beta_{centrality} = -0.41 \pm 0.36$, 95% CI = -1.12, 0.31, Table 4), indicating that the spatial position of a male in a cluster does not influence his chance of siring an extrapair young. Alternatively, we found support for the hypothesis that males establishing territories later in the season have lower probabilities of siring an extrapair young, as shown by the highest importance of the model including this variable (68.6%) and its effect size ($\beta_{date} = -0.79 \pm 0.35$, 95% CI = -1.49, -0.09) (Table 4, Fig. 4).

Table 4. Model selection tables for GLMM of males' probabilities of within pair paternity loss and extrapair paternity success in relation to centrality and establishment date. K = number of parameters, AICc = second-order Akaike's Information Criteria, w_i = Akaike's weight.

Models	K	AICc	ΔAICc	w_i
WPP loss[1]				
Date	4	41.38	0.00	0.767
Centrality+date	5	44.16	2.77	0.192
Null	3	47.89	6.51	0.030
Centrality	4	49.85	8.47	0.011
EPP success[2]				
Date	4	49.47	0.00	0.475
Null	3	51.05	1.58	0.216
Centrality+date	5	51.10	1.62	0.211
Centrality	4	52.62	3.15	0.099

Year and cluster included as a nested hierarchical random effects

[1] Random effects variance: cluster nested within year < 0.001, year < 0.001.

[2] Random effects variance: cluster nested within year < 0.001, year = 0.10.

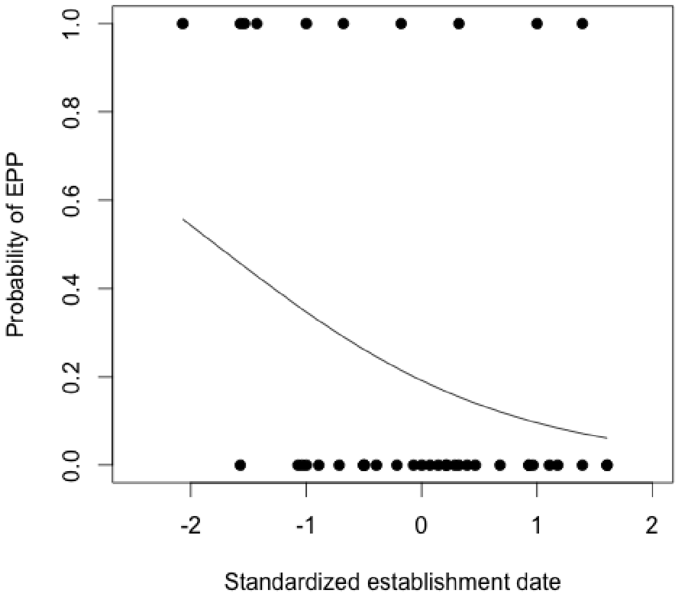

Figure 4. Probability of extrapair paternity success of male blue-black grassquits in relation to territory establishment date. Line represents the fitted values of a GLMM with binomial distribution and points represent observed values. Establishment date is standardized to mean zero and scaled by its standard deviation.

Cluster size and breeding success

We found only two males in solitary territories and both were paired (Fig. 1). Therefore, due to small sample size we were unable to compare differences of pairing success between solitary and clustered males. However, when considering clustered males, we found that in larger clusters most males were successfully paired and had a nesting attempt, whereas the proportion of paired males in smaller clusters varied widely, ranging from 0.2 to 1.0 (Fig. 5; see maps in Fig. 1). However, we found no statistical evidence for cluster size as a good predictor of proportion of paired males in a cluster ($\beta_{size} = 0.05 \pm 0.03$, 95% CI = -0.02, 0.10, Table 5), indicating that males settled in smaller clusters were not at a disadvantage in attracting a female and breeding than males in larger clusters. Similarly, the probability of WPP loss did not increase with number of males in a cluster, and in fact, there was a slight tendency for a negative relationship with larger clusters exhibiting lower chances of WPP losses ($\beta_{cluster\ size} = -0.16 \pm 0.08$, 95% CI = -0.32, 0.002) (Table 5).

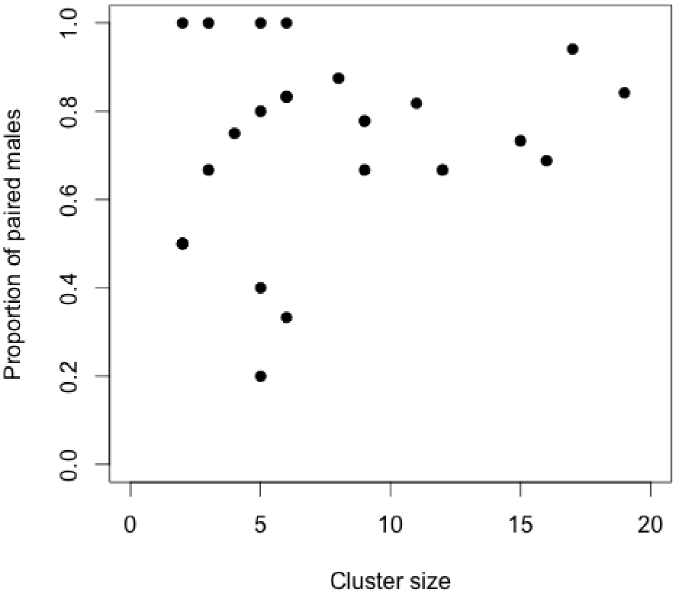

Figure 5. Proportion of paired males in relation to the number of males in blue-black grassquit clusters.

Table 5. Model selection table for GLMM of proportion of paired males and proportion of nests with WPP loss in relation to cluster size. K = number of parameters, AICc = second-order Akaike's Information Criteria, w_i = Akaike's weight.

Models	K	AICc	ΔAICc	w_i
Pairing success[1]				
null	2	37.34	0.00	0.61
cluster size	3	38.26	0.92	0.39
WPP loss[2]				
null	2	25.95	0.00	0.67
cluster size	3	27.40	1.45	0.33

Year included as random effect.

[1] Random effect variance 0.13.

[2] Random effect variance 1.89.

DISCUSSION

Our data do not neatly fulfill the predictions generated by two models proposed to explain the hidden lek: the hotshot and the female preference models. Overall, our study suggests that clustering in blue-black grassquits could be driven either by female preference for more attractive males or for other less attractive males that settle near these desirable individuals, the basic conceptual framework of the hotshot model. We tested two predictions of the hotshot model: (1) males that establish their territories in central positions of clusters or settle earlier should exhibit more exuberant traits; and (2) they should also be more attractive to females (i.e., have lower paternity loss in their own nests or higher rates of EPP). Our results only partially corroborate these expectations. We found that the spatial location of males in aggregations is not as indicative of male attributes or reproductive success as is the temporal aspect of territory acquisition. The weak relationships between male display traits and centrality position suggest that hotshot males are not in the center. Additionally lower WPP loss and greater EPP probabilities were not skewed toward males in central territories, also contradicting one of the hotshot model predictions. In the blue-black grassquit mating system, it appears that hotshot males are those that establish territories earlier, as these were the ones that had better attributes, including courtship displays containing higher leaps. These males also had higher probabilities of siring extrapair young. Thus, we can presume that male settlement may derive from a hierarchical process with those in better condition defining their territories earlier, relative to lower quality males.

The second model we examined with our data is the female preference model, with the expectation that males in clusters would do better in terms of pairing success than would solitary males. This tendency was also expected to apply to cluster size, with males in larger clusters faring better than those in smaller ones. We did not find evidence of female avoidance of social partners in smaller clusters nor increased extrapair fertilizations in larger clusters; consequently, we have inadequate support for the female preference model.

The question of what constitutes a high quality male is an elusive one, perhaps because the strongest evidence of quality in most field studies of mating systems is the choice exercised by females for certain males and their attributes. Such males purportedly have intrinsic genetic value that would be inherited by the offspring of the females. Thus, we could argue that male traits that are preferred by females are indicative of higher quality. However, many male traits may not be correlated with general genetic quality. And an additional obstacle for most empirical studies is that to assess the benefits of female choice the ideal data set should include the number of grandchildren produced through alternative mating strategies of both males and females (see Hunt et al. 2004). Despite such limitations, it is still useful to examine the suite of male attributes, assess their relative production costs, determine their potential in attracting females and, consequently, associate these attributes with male reproductive success.

Individuals settling earlier in the field site executed higher leaps, thus apparently were in better condition (Aguilar et al. 2008b, Chapter 1), and this evidently increased their chances of mating (Carvalho et al. 2006, Chapter 2) and probably attracted more females to the cluster. From a less-attractive male perspective, settling closer to neighbors bearing such attributes could be an adaptive strategy as the chance of potential mates visiting the cluster increases (e.g. Greene et al. 2000). However, we found a contrasting quality pattern in earlier male traits: songs were longer, and this trait was previously found to be associated with higher susceptibility of paternity loss in the social brood (Chapter 2). This inconsistency posits that male breeding success might be under a trade-off, such that earlier males may have greater chances of forming a pair bond and breeding, but also tend to rear extrapair offspring. However, when associating WPP loss and establishment date of males, we found weak evidence supporting this hypothesis (see Table 4 and text). Such contrasting results indicate that the relationship between WPP loss and song duration is not necessarily strong, and reinforce the idea that earlier males could potentially be hotshot males despite their song length characteristic.

The hotshot model of hidden lek evolution also predicts a reproductive skew toward males settled in clusters or central/dominant males (Wagner 1998; Fletcher and Miller 2006). However, females blue-black grassquits did not pursue extrapair copulations with these males, nor did they seek for multiple sexual partners when socially paired with peripheral males. This pattern, which contradicts the proposal of increased opportunities for extrapair copulations in clusters (Wagner 1998), is reinforced by several other evidences. First, 64.7% of extrapair males with known territory position (*n* =17 males) were settled in different clusters from where they sired an extrapair young. Second, we were unable to assign paternity for extrapair young from 11 nests, suggesting that other extrapair males were probably settled at even farther distances (presuming we genotyped the majority of territorial males in the field area). Finally, as argued previously, central males were not more attractive to females as extrapair mating choices in this species.

The increased extrapair fertilization by earlier males is another reproductive behavior that strengthens the importance of territory establishment timing. Earlier males were apparently signaling good body quality through the height of their courtship leaps, and successfully attracted multiple partners as shown by their higher rates of siring extrapair young. However, a non-exclusive possibility is that these males had a lengthy period of time spent in courtship exhibitions relative to later males and hence had more opportunities to encounter an extrapair partner. Moreover, it is equally probable that earlier males invested more in extrapair copulations because they were unpaired and suffered less pressure from social partners or from parental investment, both of which could prevent copulations with additional females (Trivers 1972). This proposition is plausible given the fact that 31.6% (6 out of 19) of extrapair males were unpaired and did not produce within-pair young. Finally, extrapair fertilizations could also have resulted as an alternative breeding effort of paired males that were unsuccessful in fertilizations in previous nesting attempts. Among socially paired males with genotyped social nestlings (11 out of 19) and that also produced extrapair young, nine sired at least one within-pair young but three also lost paternity to another male.

We tested three predictions of the female preference model (see Fletcher and Miller 2006). These included the expectation that males in clusters would have a higher pairing success than solitary males, that the proportion of paired males would increase with cluster size and finally, that cluster size would be positively associated with proportion of extrapair fertilizations. Our data do not conform to any of these predictions. Very few males were in solitary territories (only two of 224 territories monitored in three years), which could indicate a strong selection pressure against solitary breeding. We found weak evidence of cluster size influencing breeding success. Even in several small clusters most of the males were paired with a female, such that all clusters with two to four individuals had at least 50% of successfully paired males (67.7 ± 22.0%). Also contrary to the female preference model expectation, WPP loss was not more elevated in larger clusters. These results suggest that females are not selecting males based on the number of potential extrapair partners in their immediate surroundings or that proximity of potential sexual partners favors multiple mating.

Differences in the phenotypes of blue-black grasquit males settling at different moments suggest that attractive males could somehow have stimulated the subsequent arrival and settlement of less preferred males. Similar patterns have been shown in other socially monogamous clustering species. In the least flycatcher, for example, earlier males were heavier and in better condition, but also settled in central territories and had greater pairing success (Tarof et al. 2004). Yet, extrapair fertilizations were not biased toward these central males (Tarof et al. 2004). In the superb fairy-wren subordinates also tend to aggregate near a dominant male and take advantage of the situation by eventually singing from the more prominent dominant's singing perch and also by copulating with the female (Cockburn et al. 2009). In territorial species conspecific attraction seems to benefit naïve individuals that arrive later, thus obtaining cues regarding habitat quality and potential for successfully breeding (Boulinier and Danchin 1997; Muller et al. 1997). In these situations aggregation possibly occurs as a mechanism that improves individual fitness, and particularly in these species, as well as in blue-black grassquits, aggregation seems to occur at least partially due to breeding strategies (Wagner 1998; Tarof et al. 2004; Cockburn et al. 2009).

Clustering of small territories in the blue-black grassquit has long intrigued researchers (Murray 1982; Webber 1985; Almeida and Macedo 2001; Carvalho et al. 2006; Dias et al. 2009). Several behavioral and mating characteristics, such as the occurrence of extrapair fertilizations and males displaying in aggregations, indicated this species had strong convergences with lek mating systems. Although few studies attempted to understand spatial aggregation in this species, some patterns emerged: resource availability is important and nest predation avoidance may influence breeders´ distribution within a cluster, given that central nests have higher survival chance (Dias et al. 2009). However, costs might also be associated, as the proximity of conspecifics decreases survival rate of nearby nests (Aguilar et al. 2008a), and the presence of a displaying male closer to a nest attracts more predators (Dias et al. 2010). We now suggest that mating benefits can compensate the costs of breeding in clusters by maximizing the breeders' reproductive output. Our data partially validate the predictions of the hidden lek hypothesis, and the possibility that male settlement in a cluster could occur according to female mate choice, with earlier males being more attractive or of better quality (with higher leaps) than later males. This result is reinforced by earlier males' increased success in extrapair fertilizations. So are blue-black grassquit clusters hidden leks? The answer is that this species does not absolutely represent a hidden lek model, specially because of the weak relationship between spatial position of territories within clusters and extrapair copulations or male quality. It is possible that spatial distribution is more important for strategies such as predation avoidance or resources exploitation (Dias et al. 2010) than for mating opportunities. However, the early arrival of "hotshot" males in clusters corroborates an important expectation of a lek-like mating system and could have triggered clustering, thus we cannot refute completely that the blue-black grassquit is a hidden lek model.

REFERENCES

Aguilar TM, Dias RI, Oliveira AC, Macedo RH (2008a) Nest-site selection by Blue-black Grassquits in a Neotropical savanna: do choices influence nest success? Journal of Field Ornithology 79:24–31

Aguilar TM, Maia R, Santos ESA, Macedo RH (2008b) Parasite levels in blue-black grassquits correlate with male displays but not female mate preference. Behavioral Ecology 19:292–301

Alberto F (2009) MsatAllele_1.0: An R package to visualize the binning of microsatellite alleles. Journal of Heredity 100:394–397

Alderton C (1963) The breeding behavior of the blue-black grassquit. Condor 65:154–162

Almeida JB, Macedo RH (2001) Lek-like mating system of the monogamous blue-black grassquit. Auk 118:404–411

Bates D, Maechler M, Bolker B (2011) lme4: Linear mixed-effects models using S4 classes. R package version 0.999375-42.

Beehler B, Foster M (1988) Hotshots, hotspots, and female preference in the organization of lek mating systems. American Naturalist 131:203–219

Bohórquez CI, Stiles FG (2002) The paradoxical social system of the Dusky Bush-Tanager (*Chlorospingus semifuscus*): lekking in a nine-primaried oscine? Journal of Field Ornithology 73:281–291

Boulinier T, Danchin E (1997) The use of conspecific reproductive success for breeding patch selection in terrestrial migratory species. Evolutionary Ecology 11:505–517

Bradbury J (1981) The evolution of leks. In: Alexander RD, Tinkle D (eds.) Natural selection and social behavior. Chiron Press, New York, pp. 138–169.

Bradbury RB, Gibson R, Tsai IM (1986) Hotspots and the dispersion of leks. Animal Behavior 34:1694–1709

Burnham K, Anderson D (2002) Model selection and multimodel inference: a practical information-theoretic approach. Springer-Verlag, New York.

Carvalho CBV, Macedo RH, Graves JA (2006) Breeding strategies of a socially monogamous neotropical passerine: extra-pair fertilizations, behavior, and morphology. Condor 108:579–590

Cockburn A, Dalziell AH, Blackmore CJ, Double MC, Kokko H, Osmond HL, Beck NR, Head ML, Wells K (2009) Superb fairy-wren males aggregate into hidden leks to solicit extragroup fertilizations before dawn. Behavioral Ecology 20:501–510

Dias RI, Castilho L, Macedo RH (2010) Experimental evidence that sexual displays are costly for nest survival. Ethology 116:1011–1019

Dias RI, Kuhlmann M, Lourenço LR, Macedo RH (2009) Territorial clustering in the blue-black grassquit: reproductive strategy in response to habitat and food requirements? Condor 111:706–714

Fletcher RJ, Miller CW (2006) On the evolution of hidden leks and the implications for reproductive and habitat selection behaviours. Animal Behaviour 71:1247–1251

Greene E, Lyon BE, Muehter VR, Ratcliffe LM, Oliver SJ, Boag PT (2000) Disruptive sexual selection for plumage coloration in a passerine bird. Nature 407:1000–1003

Hamilton WD (1971) Geometry for the selfish herd. Journal of Theoretical Biology 31:295–311

Hoi H, Hoi-Leitner M (1997) An alternative route to coloniality in the bearded tit: females pursue extra-pair fertilizations. Behavioral Ecology 8:113–119

Hunt J, Bussière LF, Jennions MD, Brooks R (2004) What is genetic quality? Trends in Ecology & Evolution 19:329–333

Höglund J, Alatalo R V (1995) Leks. Princeton University Press, Princeton.

Kalinowski ST, Taper ML, Marshall TC (2007) Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Molecular Ecology 16:1099–1106

Kiester AR, Slatkin M (1974) A strategy of movement and resource utilization. Theoretical Population Biology 6:1–20

Kokko H, Lindström J (1996) Kin selection and the evolution of leks: whose success do young males maximize? Proceedings of the Royal Society B: Biological Sciences 263:919–923

Mazerolle MJ (2010) AICcmodavg: Model selection and multimodel inference based on (Q)AIC(c). R package version 1.25.

Melles SJ, Badzinski D, Fortin M-J, Csillag F, Lindsay K (2009) Disentangling habitat and social drivers of nesting patterns in songbirds. Landscape Ecology 24:519–531

Muller KL, Stamps J, Krishnan V, Willists NH (1997) The effects of conspecific attraction and habitat quality on habitat selection in territorial birds (*Troglodytes aedon*). American Naturalist 150:650–661

Murray BGJ (1982) Territorial behavior of the blue-black grassquit. Condor 84:119–119

Orians G, Willson M (1964) Interspecific territories of birds. Ecology 45:736–745

Perry EF, Andersen DE (2003) Advantages of clustered nesting for least flycatchers in north-central Minnesota. Condor 105:756–770

Podos J (1997) A performance constraint on the evolution of trilled vocalizations in a songbird family (Passeriformes: Emberizidae). Evolution 51:537–551

R Development CoreTeam (2011) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/.

Rousset F (2008) GENEPOP'007: a complete re-implementation of the GENEPOP software for Windows and Linux. Molecular Ecology Resources 8:103–106

Schneider CA, Rasband WS, Eliceiri KW (2012) NIH Image to ImageJ: 25 years of image analysis. Nature Methods 9:671–675

Seutin G, White BN, Boag PT (1991) Preservation of avian blood and tissue samples for DNA analyses. Canadian Journal of Zoology 69:82–90

Sick H (2001) Ornitologia brasileira. Editora Nova Fronteira, Rio de Janeiro.

Stamps J (1988) Conspecific attraction and aggregation in territorial species. American Naturalist 131:329–347

Tarof SA, Ratcliffe LM (2004) Habitat characteristics and nest predation do not explain clustered breeding in Least Flycatchers (*Empidonax minimus*). Auk 121:877–893

Tarof SA, Ratcliffe LM, Kasumovic MM, Boag PT (2004) Are least flycatcher (*Empidonax minimus*) clusters hidden leks? Behavioral Ecology 16:207–217

Trivers R (1972) Parental investment and sexual selection. In: Campbell BG (ed.) Sexual selection and the descent of man, 1871-1971. Aldine Press, Chicago, pp. 136–179.

van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) Micro-Checker: software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Notes 4:535–538

Wagner RH (1993) The pursuit of extra-pair copulations by female birds: a new hypothesis of colony formation. Journal of Theoretical Biology 163:333–346

Wagner RH (1998) Hidden leks: sexual selection and the clustering of avian territories. In: Parker P, Burley N (eds.) Avian reproductive tactics: female and male perspectives Ornithological Monographs No 49. American Ornithologists' Union, Washington, pp. 123–145.

Webber T (1985) Songs, displays, and other behavior at a courtship gathering of blue-black grassquits. Condor 87:543–546

APÊNDICE

Table 1. Model selection result for LMM of male centrality and establishment date in relation to display parameters of 22 males with complete data for song duration, frequency bandwidth, leap height and proportion of complete displays. K = number of parameters, AICc = second-order Akaike's Information Criteria, w_i = Akaike's weight.

Models	K	AICc	ΔAICc	w_i
Centrality				
Height	5	70.63	0.00	0.385
Null	4	71.49	0.86	0.251
Fband	5	72.77	2.13	0.133
Height+display	6	73.13	2.49	0.111
Display	5	74.82	4.19	0.047
Sdur	5	74.88	4.25	0.046
Sdur+fband	6	76.13	5.50	0.025
Sdur+fband+height +display	8	79.86	9.23	0.004
Establishment date				
Display	5	69.63	0.00	0.266
Height+display	6	70.53	0.90	0.169
Sdur	5	70.63	1.00	0.161
Height	5	70.73	1.11	0.153
Null	4	71.76	2.14	0.091
Fband	5	72.45	2.82	0.065
Sdur+fband+height+display	8	72.58	2.96	0.061
Sdur+fband	6	73.66	4.04	0.035

Year and cluster included as a nested hierarchical random effects

[1]Explanatory variables: sdur = song duration; fband = frequency bandwidth; display = proportion of complete display rates relative to total displays executed; height = leap height (cm).

[2] Random effects variance:

Centrality and establishment date models: cluster nested within year < 0.001, year < 0.001.

CONSIDERAÇÕES FINAIS

A poliandria sexual em sistemas sociais monogâmicos é um assunto extensamente debatido na literatura. Embora o papel da fêmea na escolha dos parceiros sexuais e na evolução de ornamentos e exibições elaboradas em machos seja bem conhecido, os benefícios de múltiplos acasalamentos para as fêmeas ainda são questionados (Andersson 1994; Andersson e Simmons 2006). Nosso trabalho contribui nesse sentido, por mostrar que em tizius o acasalamento não é aleatório e que existe uma tendência de que machos em melhores condições sejam mais bem sucedidos na formação de pares sociais e sexuais com as fêmeas. Tais resultados sugerem que as fêmeas devem garantir a herança de "bons genes" (ou seja, genes que conferem condição corporal ou atratividade) para a prole. Nós iniciamos esse trabalho pela exploração dos atributos dos machos como indicadores de qualidade (Capítulo 1) e depois prosseguimos com o teste de benefícios adaptativos da escolha de parceiros sociais e sexuais das fêmeas (Capítulo 2). Finalmente, nós especulamos a importância da distribuição espacial e temporal no sistema de acasalamento social e sexual desta espécie (Capítulo 3).

No primeiro capítulo, nós avaliamos o desempenho motor e acústico de machos de tiziu durante exibições completas e testamos possíveis limitações na produção simultânea de múltiplos sinais. Registramos demanda conflitante entre atributos motores (duração, altura do salto e rotação do corpo), embora aparentemente não tenha ocorrido limitação na sincronização de atributos motores e acústicos. Primeiramente, nós mostramos que a duração do salto não está correlacionada com o ângulo de rotação do corpo quando machos saltam relativamente mais alto, porém tende a reduzir quando há uma combinação entre maiores rotações e saltos mais baixos. Em sequência, nós mostramos uma relação negativa entre altura do salto e o investimento em exibições completas relativo ao total de exibições realizadas, porém apenas entre machos cujos índices de condição corporal eram menores. A exibição completa é presumidamente mais custosa do que a exibição incompleta pois envolve a produção adicional do componente motor, portanto esse resultado sugere que a exibição repetitiva do voo vertical deve ser custosa para os indivíduos.

Os resultados do primeiro capítulo nos permitiram concluir que machos de tiziu devem enfrentar restrições energéticas, fisiológicas ou biomecânicas durante a execução de exibições comportamentais, de forma que o componente motor deve ser custoso ou um "desafio" para os indivíduos. Embora estudos detalhados da morfologia, fisiologia e custo energético dos saltos sejam desconhecidos para os tizius, estudos com outras espécies mostram que tais características dos organismos devem influenciar o desempenho em atividades motoras (e.g. piprídeos, Barske et al. 2011; Fusani e Schlinger, 2012). Nossos dados também corroboram resultados anteriores (Costa e Macedo 2005; Aguilar et al. 2008a) indicando que os atributos dos machos devem ser importantes na sinalização de suas propriedades (vigor e habilidade) tanto no contexto competitivo quanto na escolha de parceiros reprodutivos. Nos capítulos subsequentes nós utilizamos duas dessas medidas (altura do salto e proporção de exibições completas) como medida de qualidade dos machos para testar hipóteses relacionadas à seleção de parceiros pelas fêmeas. Além destas variáveis, testamos também se características do canto (banda de frequência e duração) são potencialmente avaliadas pelas fêmeas, embora não sejam aparentemente limitadas pela execução do salto.

Estudos sobre demanda conflitante de parâmetros motores em exibições de corte como apresentados aqui são particularmente raros na literatura. Mais especificamente, poucos trabalhos na ornitologia abordaram até hoje combinações de sinais motores e acústicos como indicadores de qualidade (Patricelli & Krakauer, 2010). Até onde conhecemos, não existem estudos que testam conflitos de alocação entre múltiplos atributos de movimentos aéreos, acrobáticos ou que envolvem, por exemplo, a produção mecânica do som pela fricção das penas (Clark 2009; Barske et al. 2011). A abordagem mais utilizada no teste de demandas conflitantes é a comparação de características da plumagem com canto e características morfológicas (Andersson et al. 2002; Badyaev et al. 2002), e o desempenho na produção de cantos compostos por trinados e largas variações na banda de frequência (Podos 1997). Embora movimentos aéreos complexos sejam difundidos entre as aves, esse componente tem sido pouco explorado também no âmbito da seleção sexual (alguns exemplos em Byers et al. 2010). Devemos considerar que a escassez de dados deve-se em parte às dificuldades de manipulação e de controle de variáveis que influenciam o comportamento das aves (e.g. condições climáticas, presença de coespecíficos nas proximidades, etc), principalmente em estudos de vida livre. Em outros grupos taxonômicos, como grilos e aranhas que reconhecidamente produzem sinais múltiplos conflitantes (Elias et al. 2012; Wagner et al. 2012), a manutenção em cativeiro é relativamente mais simples e pouco deve afetar o empenho dos indivíduos na exibição dos sinais. No caso das aves, embora muitas espécies sejam facilmente mantidas em aviários, nem sempre os indivíduos comportam-se como em um ambiente natural. Os machos de tiziu, por exemplo, executam poucas exibições completas quando em gaiolas dificultando a manipulação de variáveis e o desenvolvimento de experimentos adequados. Portanto, o nosso estudo destaca-se principalmente por avaliar características de difícil mensuração em campo, como acrobacias em voo, e por detectar padrões de conflito de alocação em múltiplos sinais apesar da provável influência de outros fatores, como presença de coespecíficos ou predadores nas proximidades.

Os tizius são socialmente monogâmicos (Almeida e Macedo 2001), porém sabe-se que a poligamia genética ocorre com frequência (Carvalho et al. 2006). De fato, os resultados do segundo capítulo corroboram esse padrão, inclusive confirmando altas taxas de paternidade extra par e poucos registros de parasitismo intraespecífico e quasi-parasitismo (estratégias reprodutivas alternativas das fêmeas, Yom-Tov 2001; Griffith et al. 2004). Como nosso objetivo principal nesse trabalho concentrou-se nos mecanismos de escolha das fêmeas por parceiros sexuais, os registros de parasitismo intraespecífico e quasi-parasitismo serão melhor explorados em trabalho futuros. Dentre algumas das possíveis explicações, podemos antecipar que a baixa ocorrência dessas estratégias talvez indique uma fraca função adaptativa desse comportamento em tizius. Este argumento pode ser fundamentado pelos altos custos associados ao cuidado parental nessa espécie (Almeida e Macedo 2001; Dias e Macedo 2011), que podem ter favorecido estratégias de evitação do parasitismo pelos pais sociais (Lyon e Eadie 2008). Da mesma forma, a grande variação interanual nos níveis de fertilização extra par encontrada no nosso estudo exige explicações que ainda não puderam ser respondidas. Nós supomos que esse padrão possa ocorrer em decorrência de flutuações ambientais, tais como variação na temperatura e nos níveis de precipitação, disponibilidade ou distribuição de recursos, que possam ter afetado a condição corporal dos indivíduos e, consequentemente a atratividade e a escolha de parceiros sexuais (Cornwallis e Uller 2010; Botero e Rubenstein 2012). Entretanto, essas hipóteses são apenas especulativas e merecem ser melhor exploradas em estudos subsequentes.

No segundo capítulo testamos hipóteses de benefícios adaptativos diretos e indiretos obtidos pelas fêmeas ao selecionarem parceiros sociais e sexuais com melhores atributos. Não encontramos evidências de que fêmeas beneficiam-se diretamente na busca por múltiplas cópulas, uma vez que machos extra par não defendiam territórios com maior disponibilidade de alimento do que seus parceiros sociais. A escolha por um parceiro extra par, portanto, não deve ocorrer com base em informações da qualidade do seu território, porém isso também não exclui a possibilidade de que fêmeas forrageiem nos territórios de machos extra par. Embora não tenhamos acompanhado o deslocamento das fêmeas entre diferentes territórios, um estudo anterior mostrou que um território com maior disponibilidade de alimento recebe maior taxa de visitação das fêmeas (Dias e Macedo 2011) sugerindo que a quantidade de recursos deve influenciar o comportamento de fêmeas. Monitoramentos futuros da movimentação de fêmeas poderiam esclarecer a importância da área de forrageamento, já que é uma das poucas contribuições diretas que um macho extra par pode fornecer na monogamia social.

Encontramos relações distintas entre cada componente das exibições (motor e acústico) e o sucesso reprodutivo do macho. Machos que saltam mais alto tiveram maior chance de pareamento com uma fêmea e machos que emitiram cantos mais curtos tiveram menor chance na perda de paternidade na prole. Adicionalmente, em comparações pareadas da duração do canto entre pais de uma mesma ninhada, quatro dentre seis machos sociais produziram cantos mais longos do que machos extra par para os quais perderam paternidade. Esse resultado indica que fêmeas preferem cantos mais curtos, ou favorecem a fertilização por machos com essas características, e portanto esse atributo deve indicar qualidade ou atratividade de um macho. Seria interessante se trabalhos subsequentes avaliassem se a duração do canto indica outra propriedade do macho não mensurada nesse estudo, como agressividade ou a habilidade em produção simultânea de outros parâmetros acústicos que sejam custosos e que potencialmente indiquem qualidade. Com base nesses resultados, nós concluímos que a evolução da altura do salto e duração do canto em tizius, assim como outras variáveis que estejam geneticamente correlacionadas à estas, deva ocorrer em função de benefícios indiretos da escolha de parceiros, ou seja, benefícios na qualidade genética da prole. Filhotes de machos que saltam mais alto e produzem canto mais curto devem ser mais saudáveis (por exemplo, por serem mais resistentes a doenças que afetam a produção dos sinais sexuais, Hamilton e Zuk 1982) ou devem expressar o mesmo fenótipo quando adultos e ter maior sucesso reprodutivo.

Nossos resultados, entretanto, não corroboraram a evolução da poliandria em tizius em função de benefícios indiretos pelo aumento da compatibilidade genética entre parceiros reprodutivos (no caso, fêmeas e machos extra par), pela busca de parceiros extra par mais heterozigóticos, nem pelo aumento dos níveis de heterozigosidade ou da condição corporal da prole. Esses resultados mostram claramente que a qualidade genética, medida pela relação de parentesco entre pares sexuais e diversidade alélica em microssatélites, não é importante no sistema de acasalamento em tizius. Efeitos indiretos desse tipo são mais comuns em espécies filopátricas cujos indivíduos aparentados tendem a reproduzir-se em territórios próximos (Cockburn et al. 2003; Foerster et al. 2003; Tarvin et al. 2005). É provável que o comportamento migratório nos tizius dilua esse efeito da filopatria, embora nós tenhamos registrado o retorno de alguns indivíduos à área de estudo em anos subsequentes.

Finalmente, no último capítulo nós testamos a hipótese de que o sistema de acasalamento dos tizius assemelha-se a um sistema de lek, nos quais os machos se agregam em pequenos territórios para se exibir e conquistar fêmeas (Höglund e Alatalo 1995). Mais especificamente, nós testamos as predições de dois modelos evolutivos de "lek escondido" (preferência das fêmeas e machos "hotshot", i.e. atraentes) propostos para explicar a formação de agregações em espécies socialmente monogâmicas em função da busca por cópulas extra par (Wagner 1998). Machos que estabelecem o território mais cedo na estação reprodutiva estão aparentemente em melhor condição (possuem saltos mais altos) e tem maior chance de sucesso em cópulas extra par. Entretanto não encontramos padrões que revelem que o tamanho da agregação influencie a chance de pareamento nem na ocorrência de cópulas extra par. Em resumo, nossos dados corroboram parcialmente o modelo de machos atraentes, pois indicam que o estabelecimento territorial de machos que exibem características preferidas por fêmeas precede o estabelecimento de outros machos. Assim, a presença de "hotshots" pode ter estimulado a visitação de um maior número de fêmeas no seu território e, consequentemente, nos territórios adjacentes, oferecendo vantagem aos machos em pior qualidade ou menos atraentes ao se estabelecerem nesses locais.

O padrão agregado dos territórios dos tizius e a conspicuidade das exibições dos machos são duas características marcantes nessa espécie e que já foram apontados diversas vezes como potenciais facilitadores/promotores da poligamia. Uma alternativa que não foi explorada neste trabalho é o teste do modelo evolutivo de "lek escondido" pela seleção de parentesco (revisão em Fletcher e Miller 2006). É possível que machos se agreguem ao redor de machos "dominantes" ou em melhor qualidade com os quais são proximamente aparentados e para os quais perdem paternidade, pois devem ser mais atrativos para as fêmeas. Dessa forma, machos "subordinados" aumentam seu valor adaptativo tanto pela maior chance de pareamento quanto pelo componente de aptidão inclusiva, ou seja, pelo sucesso reprodutivo de seu parente próximo. Trabalhos anteriores já buscaram entender a distribuição espacial dos tizius em função de características da vegetação (Almeida e Macedo 2001; Aguilar et al. 2008b; Dias et al. 2009) e também do comportamento reprodutivo (Dias et al. 2009). Segundo esses estudos, a distribuição espacial de indivíduos e de ninhos depende da complexidade da vegetação e disponibilidade de alimento, e a agregação pode reduzir o risco da predação de ninhos que estão no centro. Porém, até então o padrão genético de acasalamento era pouco conhecido e nenhum estudo havia testado as relações entre ocorrência de cópulas extra par e distribuição espacial e temporal dos indivíduos.

Uma abordagem ainda não explorada, mas que merece maior atenção refere-se à qualidade das fêmeas. Nós não apresentamos aqui dados relativos à características morfológicas e fisiológicas das fêmeas, entretanto esse será o foco de estudos subsequentes oriundos do nosso trabalho. A maioria dos estudos de seleção sexual procuram explicações com base em características morfológicas e comportamentais dos machos, e tal fato é facilmente justificável devido à maior expressão desses caracteres neste sexo. Entretanto, recentemente um foco maior tem sido direcionado às fêmeas (e.g. Holveck e Riebel 2010). Embora em algumas espécies as fêmeas sejam menos conspícuas, produzam um menor repertório de comportamentos extravagantes ou não produzam nenhum sinal sexual (como ocorre, aparentemente, em fêmeas de tizius), a qualidade corporal pode ser um importante indicativo de habilidade na escolha de parceiros e no sucesso reprodutivo. É possível que fêmeas não estejam fisicamente aptas a procurarem por múltiplos parceiros ou, ainda, que não sejam bem sucedidas em disputas com outras fêmeas por um macho de melhor qualidade (ou de maior preferência). Sendo assim, é importante avaliar esse componente para melhor compreender como relacionam-se a preferência e a escolha de parceiros sexuais.

Por fim, nosso trabalho contribui com dados referentes a um dos componentes do valor adaptativo dos indivíduos que atualmente é reconhecidamente importante em aves socialmente monogâmicas: as fertilizações extra par. Porém, existem ainda questões sobre seleção sexual em tizius que podem ser melhor exploradas. Os dados coletados durante esse trabalho fazem parte de um projeto de estudo maior que busca compreender de forma ampla o comportamento reprodutivo de passeriformes socialmente monogâmicos, e que utiliza o tiziu como modelo experimental. Em trabalhos futuros pretendemos avaliar ainda a influência de outros componentes visuais das exibições dos machos, a plumagem iridescente e as manchas brancas subaxilares, sobre o sucesso reprodutivo. Estudos têm apontado que tais atributos são sinalizadores de qualidade (Doucet 2002; Santos et al. 2009) e que suas expressões são influenciadas pelo contexto social, como a presença de machos ou fêmeas no mesmo ambiente (Maia et al. 2012). Porém, sua importância no contexto sexual é ainda desconhecida.

REFERÊNCIAS

Aguilar TM, Maia R, Santos ESA, Macedo RH (2008a) Parasite levels in blue-black grassquits correlate with male displays but not female mate preference. Behavioral Ecology 19:292–301

Aguilar TM, Dias RI, Oliveira AC, Macedo RH (2008b) Nest-site selection by Blue-black Grassquits in a Neotropical savanna: do choices influence nest success? Journal of Field Ornithology 79:24–31

Almeida JB, Macedo RH (2001) Lek-like mating system of the monogamous blue-black grassquit. Auk 118:404–411

Andersson M (1994) Sexual selection. Princeton University Press, Princeton.

Andersson S, Pryke SR, Ornborg J, Lawes MJ, Andersson M (2002) Multiple receivers, multiple ornaments, and a trade-off between agonistic and epigamic signaling in a widowbird. American Naturalist 160:683–691

Andersson M, Simmons LW (2006) Sexual selection and mate choice. Trends in Ecology & Evolution 21:296–302

Badyaev AV, Hill GE, Weckwort BV (2002) Species divergence in sexually selected traits: increase in song elaboration is related to decrease in plumage ornamentation in finches. Evolution 56:412–419

Barske J, Schlinger BA, Wikelski M, Fusani L (2011) Female choice for male motor skills. Proceedings of the Royal Society B: Biological Sciences 278:3523–3528

Botero CA, Rubenstein DR (2012) Fluctuating environments, sexual selection and the evolution of flexible mate choice in birds. PloS ONE 7:e32311

Byers J, Hebets E, Podos J (2010) Female mate choice based upon male motor performance. Animal Behaviour 79:771–778

Carvalho CBV, Macedo RH, Graves JA (2006) Breeding strategies of a socially monogamous neotropical passerine: extra-pair fertilizations, behavior, and morphology. Condor 108:579–590

Clark CJ (2009) Courtship dives of Anna's hummingbird offer insights into flight performance limits. Proceedings of the Royal Society B: Biological Sciences 276:3047–3052

Cockburn A, Osmond HL, Mulder RA, Green DJ, Michael C, Double MC (2003) Divorce, dispersal and incest avoidance in the cooperatively breeding superb fairy-wren *Malurus cyaneus*. Journal of Animal Ecology 72:189–202

Cornwallis CK, Uller T (2010) Towards an evolutionary ecology of sexual traits. Trends in Ecology & Evolution 25:145–152

Costa FJV, Macedo RH (2005) Coccidian oocyst parasitism in the blue-black grassquit: influence on secondary sex ornaments and body condition. Animal Behaviour 70:1401–1409

Dias RI, Kuhlmann M, Lourenço LR, Macedo RH (2009) Territorial clustering in the blue-black grassquit: reproductive strategy in response to habitat and food requirements? Condor 111:706–714

Dias RI, Macedo RH (2011) Nest predation versus resources in a Neotropical passerine: constraints of the food limitation hypothesis. Ornis Fennica 88:30–39

Doucet SM (2002) Structural plumage coloration, male body size, and condition in the blue-black grassquit. Condor 104:30–38

Elias DO, Maddison WP, Peckmezian C, Girard MB, Mason AC (2012) Orchestrating the score: complex multimodal courtship in the *Habronattus coecatus* group of *Habronattus* jumping spiders (Araneae: Salticidae). Biological Journal of the Linnean Society 105:522–547

Fletcher RJ, Miller CW (2006) On the evolution of hidden leks and the implications for reproductive and habitat selection behaviours. Animal Behaviour 71:1247–1251

Foerster K, Delhey K, Johnsen A, Lifjeld JT, Kempenaers B (2003) Females increase offspring heterozygosity and fitness through extra-pair matings. Nature 425:714–717

Fusani L, Schlinger BA (2012) Proximate and ultimate causes of male courtship behavior in Golden-collared Manakins. Journal of Ornithology 153:119–124

Griffith SC, Lyon BE, Montgomerie R (2004) Quasi-parasitism in birds. Behavioral Ecology and Sociobiology 56:191–200

Hamilton WD, Zuk M (1982) Heritable true fitness and bright birds: a role for parasites? Science 218:384–387

Holveck M-J, Riebel K (2010) Low-quality females prefer low-quality males when choosing a mate. Proceedings of the Royal Society B: Biological Sciences 277:153–160

Höglund J, Alatalo R V (1995) Leks. Princeton University Press, Princeton.

Lyon BE, Eadie JM (2008) Conspecific brood parasitism in birds: a life-history perspective. Annual Review of Ecology, Evolution, and Systematics 39:343–363

Maia R, Brasileiro L, Lacava R V, Macedo RH (2012) Social environment affects acquisition and color of structural nuptial plumage in a sexually dimorphic tropical passerine. PloS one 7:e47501

Patricelli GL, Krakauer AH (2010) Tactical allocation of effort among multiple signals in sage grouse: an experiment with a robotic female. Behavioral Ecology 21:97–106

Podos J (1997) A performance constraint on the evolution of trilled vocalizations in a songbird family (Passeriformes: Emberizidae). Evolution 51:537–551

Santos ESA, Maia R, Macedo RH (2009) Condition-dependent resource value affects male-male competition in the blue-black grassquit. Behavioral Ecology 20:553–559

Tarvin KA, Webster MS, Tuttle EM, Pruett-Jones S (2005) Genetic similarity of social mates predicts the level of extrapair paternity in splendid fairy-wrens. Animal Behaviour 70:945–955

Wagner RH (1998) Hidden leks: sexual selection and the clustering of avian territories. In: Parker P, Burley N (eds.) Avian reproductive tactics: female and male perspectives Ornithological Monographs No 49. American Ornithologists' Union, Washington, pp. 123–145.

Wagner WE, Beckers OM, Tolle AE, Basolo AL (2012) Tradeoffs limit the evolution of male traits that are attractive to females. Proceedings of the Royal Society B: Biological Sciences 279:2899–2906

Yom-Tov Y (2001) An updated list and some comments on the occurrence of intraspecific nest parasitism in birds. Ibis 143:133–143