Universidade de Brasília
Instituto de Ciências Biológicas
Programa de Pós-Graduação em Ecologia

Canto em dueto e sistema de acasalamento do João-de-barro (*Furnarius rufus*)

PEDRO DINIZ ALVES

Orientadora: Regina H. F. Macedo

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RESUMO

A seleção sexual é um dos principais mecanismos de evolução do canto em aves. No entanto, evidências apoiando essa ideia são amplamente baseadas em estudos com canto em machos, mesmo considerando que fêmeas cantem em mais de 70% das espécies de passeriformes, e parceiros reprodutores coordenem seus cantos em duetos em mais 18% das espécies de aves. A função adaptativa do dueto ainda não é bem compreendida e mais de oito hipóteses já foram propostas. O dueto pode surgir através de cooperação ou conflito entre parceiros reprodutores, e pode ser direcionado ao parceiro ou a indivíduos externos ao par reprodutor. Neste estudo, investiguei a função adaptativa dos cantos de fêmeas e machos em uma espécie que canta em dueto, analisando como a expressão desses cantos varia em diferentes contextos de territorialidade, socialidade e reprodução. O modelo de estudo foi o João-de-barro (*Furnarius rufus*; Aves: Furnariidae), uma espécie socialmente monogâmica, territorial durante todo o ano, aparentemente monocromática e com cantos sexo-específicos. Meus objetivos foram: 1) testar se as funções dos cantos em dueto variam com o sexo, papel no dueto (início ou resposta de canto) e nível de organização do dueto (individual ou casal), avaliando a relação entre expressão do canto, sazonalidade reprodutiva e ocorrência de interações territoriais; 2) investigar a resposta de parceiros reprodutores em grupos com ou sem filhotes jovens ao *playback* de solo de fêmea, solo de macho e dueto, testando indiretamente as funções desses tipos de canto; 3) descrever o sistema de acasalamento genético do João-de-barro e testar se o canto nessa espécie se correlaciona com a qualidade territorial e sucesso reprodutivo; 4) testar se o nível de coordenação do dueto sinaliza a qualidade ou motivação do casal em competir por territórios, por meio de um experimento de *playback* de cantos com níveis variados de coordenação temporal; e 5) testar se existe dimorfismo sexual e pareamento seletivo em tamanho do corpo ou coloração da plumagem, o que indicaria um papel da seleção sexual nessa outra modalidade sensorial. Os principais resultados do estudo foram: 1) parceiros reprodutores coordenaram a maioria dos seus cantos em duetos (61%), e machos iniciaram mais cantos do que fêmeas; a função do canto variou em função da interação entre sexo, papel no dueto e nível de organização do dueto, mas, em geral, foi relacionada à defesa de território e do vínculo social do casal; 2) parceiros foram coordenados e equivalentes na resposta aos playbacks de coespecíficos; playbacks de solos foram mais ameaçadores do que playbacks de duetos para casais sem jovens, enquanto playbacks de duetos ameaçaram mais casais com jovens do que playbacks de solos, indicando que a defesa do vínculo social é importante para casais sem jovens e a defesa de territórios é importante para casais com jovens; 3) a taxa de paternidade extrapar foi baixa (<4% dos ninhegos) e o sucesso reprodutivo foi alto (100% dos casais produziram pelo menos um juvenil); o investimento em canto pela fêmea e a duração do dueto se correlacionaram com a qualidade dos territórios, mas não com o sucesso reprodutivo do casal; 4) o nível de coordenação do dueto não indicou a qualidade ou motivação do casal na defesa territorial, visto que parceiros responderam de forma coordenada e equivalente a todos os playbacks coespecíficos; 5) foi encontrada evidência de monocromatismo sexual e pequeno (~4%) dimorfismo em tamanho, além de ausência de pareamento seletivo. Em conclusão, este estudo demonstra que parceiros reprodutores de João-de-barro coordenam seus cantos em dueto de forma cooperativa e por múltiplas razões, principalmente para defenderem território e o próprio vínculo social do casal.

Palavras-chave: canto de fêmeas, defesa de territórios, guarda de parceiro, monogamia, paternidade extrapar, seleção social, seleção sexual, dimorfismo sexual, Furnariidae, Aves.

ABSTRACT

Sexual selection is one of the main forces driving the evolution of bird song. However, evidence supporting this idea comes mainly from studies conducted on male song, despite the fact that female song occurs in 71% of bird species and mated partners coordinate their songs in more than 18% of bird species. The function of duetting is poorly understood and more than eight hypotheses have been proposed to explain duet function. Duets may arise from cooperation or conflict between breeding partners and the duet participation may be directed at either partners or strangers. Here, I investigated the adaptive function of female and male songs in a duetting species, and associated song expression to different contexts of territoriality, sociality and reproduction. The study species was the Rufous Hornero (*Furnarius rufus*; Aves: Furnariidae), a Neotropical, socially monogamous, year-round territorial and apparently monochromatic bird species. Duets are composed by sex-specific song types in this species. My objectives were to: 1) verify whether song function in duets varies with sex, singing role (song initiation or song answering), and level of duet organization (individual or pair), examining the relation among song expression, breeding seasonality and occurrence of territorial interactions; 2) investigate the response of mated pairs with and without juveniles to the playback of female solo, male solo and duet, testing for functions of these song types; 3) describe the genetic mating system of the Rufous Horneros and test for fitness consequences (territorial quality and reproductive success) of song expression; 4) test the coalition quality hypothesis to explain the function of the degree of duet coordination in this species, through a playback experiment with duet stimuli varying in the degree of phrase coordination; and 5) test for sexual dimorphism and assortative mating relative to body size and plumage coloration, which could indicate a role of sexual selection in this sensory modality. Our main results were: 1) partners coordinated most of their songs into duets (61%), and males initiated more songs than females; song function depended on an interaction of sex, singing role and level of duet organization, but, in general, song function was related to territory defense and mutual mate guarding; 2) partners were coordinated and equivalent in playback responses; playback of solos were more threatening than playback of duets to pairs without juveniles, while the playback of duets threatened more pairs with juveniles than did playback of solos, indicating that mutual mate guarding is important to pairs without juveniles, and territory defense is important to pairs with juveniles; 3) extra-pair paternity was low (<4% of the nestlings) and breeding success was high (100% of the pairs produced at least one fledgling); female song rate and duet duration were correlated with territory quality, but not to breeding success; 4) the degree of duet coordination did not signal coalition quality or motivation of breeding partners to fight for territorial resources, because partners responded with equal aggressiveness and coordination all conspecific playbacks; 5) I found evidence of sexual monochromatism, slight (~4%) sexual dimorphism in body size and lack of assortative mating. In conclusion, this study showed that Rufous Hornero's breeding partners coordinate their songs into duets in a cooperative way and for multiple purposes, especially for territory defense and guarding the social pair bond.

Keywords: female song, territory defense, mate guarding, monogamy, extra-pair paternity, social selection, sexual selection, sexual dimorphism, Furnariidae, Aves.

INTRODUÇÃO GERAL

REFERENCIAL TEÓRICO

Evolução do canto em aves

Em 1871, Charles Darwin propôs a seleção sexual como um mecanismo para explicar a evolução de armas e ornamentos na natureza (Darwin, 1871). A seleção sexual abrange dois mecanismos: a seleção intrasexual, ou competição intrasexual, e a seleção intersexual, ou escolha de parceiro (Andersson, 1994). No desenvolvimento de sua teoria, Darwin foi muito influenciado por padrões de coloração e canto dos pássaros (ordem Passeriformes), em que frequentemente se pressupõe que machos exibam plumagens mais brilhantes e coloridas, e vocalizações mais elaboradas e complexas, do que fêmeas (Darwin, 1871; Catchpole, 1987; Owens e Hartley, 1998; Dunn *et al*., 2001). Incontáveis estudos empíricos confirmaram o papel da seleção sexual na evolução do canto em aves (revisões em Gil e Gahr 2002, Catchpole e Slater 2008).

Apesar da aparente ubiquidade do papel da seleção sexual na evolução do canto em aves (Andersson, 1994), a ampla maioria dos estudos empíricos apoiando essa ideia foi realizada em machos, em espécies sexualmente dimórficas ou em espécie em que apenas os machos cantam (Catchpole, 1987; Kroodsma e Byers, 1991; Langmore, 1998; Catchpole e Slater, 2008). Essa pode ser a razão da cristalização de dois pressupostos em estudos com seleção sexual e canto em aves. O primeiro pressuposto é de que o canto ocorre somente em machos é o caráter ancestral e o padrão mais comum entre as aves modernas (Searcy e Andersson, 1986; Kroodsma e Byers, 1991; Gil e Gahr, 2002). O próprio Darwin sugeria a predominância do canto em machos e o papel da escolha da fêmea na sua evolução: "... female birds, by selecting, during thousands of generations, the most melodious or beautiful males, according to their standard of beauty, might produce a marked effect" (Darwin 1859 p. 8, Odom *et al*. 2014).

Um segundo pressuposto nos estudos sobre evolução do canto em aves é de que os mesmos mecanismos de seleção atuantes em machos podem ser extrapolados para explicar a evolução do canto em fêmeas (Clutton-Brock e Huchard, 2013). O papel da seleção sexual na evolução de ornamentos e exibições mútuas nos dois sexos ou apenas em fêmeas, nas espécies em que não há reversão dos papéis sexuais, é discutível e controverso (Lyon e Montgomerie, 2012; Roughgarden, 2012; Rubenstein, 2012;

Clutton-Brock e Huchard, 2013). Enquanto teoria e estudos empíricos indicam que o acesso a acasalamentos é o principal limitante do sucesso reprodutivo de machos (Bateman, 1948), o sucesso reprodutivo de fêmeas parece depender principalmente do acesso a recursos que influenciam a fecundidade dessas fêmeas (Brown *et al*., 2009). Portanto, o sucesso de acasalamento pode ter um papel importante na evolução do canto em machos, enquanto a fecundidade seria uma pressão seletiva importante para a evolução do canto em fêmeas.

Em estudo comparativo, que incluiu 1141 espécies e 44 famílias de pássaros, Odom e colaboradores (2014) mostraram que o canto em fêmeas é presente em 71% das espécies estudadas (em 32 famílias) e que o canto em ambos os sexos muito provavelmente seria o caráter ancestral dos pássaros modernos. Esses resultados sugerem que as diferenças sexuais na ocorrência de canto entre as espécies de pássaros atuais decorrem principalmente da perda do canto em fêmeas e, portanto, de pressões seletivas contrárias ao canto em fêmeas (Odom *et al*., 2014). Esses resultados também destacam a necessidade de mais esforço de pesquisa sobre o canto em fêmeas, não somente para uma compreensão mais abrangente da evolução do canto em aves (Price, 2015), mas para entender a evolução de qualquer característica ornamental em ambos os sexos (Tobias *et al*., 2012b; Soma e Garamszegi, 2015).

Além da seleção sexual, outras formas mais abrangentes de seleção, como seleção social e seleção natural, são igualmente candidatas para explicar a evolução do canto em fêmeas (Lyon e Montgomerie, 2012; Tobias *et al*., 2012b; Webb *et al*., 2016). A teoria de seleção social abrange a teoria de seleção sexual e é definida como sendo a pressão seletiva que resulta em variação no sucesso reprodutivo decorrente da competição social (West-Eberhard, 1983). A teoria de seleção social sugere que interações sociais, não necessariamente relacionadas ao acasalamento (i.e., seleção sexual), podem prever a evolução do canto em fêmeas (Lyon e Montgomerie, 2012; Tobias *et al*., 2012b; Webb *et al*., 2016). A competição por recursos alimentares não direcionados à prole (Chaine *et al*., 2011), territórios não reprodutivos e dominância social são exemplos de pressões de seleção social que podem atuar na evolução de ornamentos em fêmeas (Rubenstein e Lovette, 2009).

Diversidade e estrutura do dueto

Ainda mais intrigante do que a evolução de ornamentos em fêmeas, é a evolução de exibições sociais envolvendo mais de um indivíduo, como duetos e coros (i.e. coordenação de vocalizações entre três ou mais indivíduos) (Farabaugh, 1982; Langmore, 1998, 2002; Hall, 2004). O dueto consiste na coordenação temporal de cantos de dois indivíduos, normalmente o casal reprodutor (Farabaugh, 1982). O dueto evoluiu diversas vezes em aves, está presente em aproximadamente 18% (1830 espécies) das espécies de aves modernas, e apresenta uma grande diversidade estrutural (Farabaugh, 1982; Hall, 2004, 2009; Dahlin e Benedict, 2013; Tobias *et al*., 2016).

O dueto também evoluiu em insetos, anfíbios e primatas. Duetos em insetos são caracterizados por respostas rápidas de fêmeas aos chamados iniciados por machos, e por funcionarem no contexto de encontro com o parceiro para a cópula (Bailey, 2003; Bailey e Hammond, 2003; Hall, 2009). O dueto em anfíbios é raro e também funciona no contexto de localização do parceiro (Tobias *et al*., 1998). Primatas realizam duetos bem coordenados e de alta amplitude, que refletem a sincronização comportamental e a duração do pareamento do casal (Geissmann e Orgeldinger, 2000; Méndez-Cárdenas e Zimmermann, 2009).

Os duetos podem ser classificados em três tipos quanto à organização acústica dos cantos dos dois indivíduos: antifônicos, simultâneos, ou sobrepostos (Dahlin e Benedict, 2013). Em duetos antifônicos, machos e fêmeas alternam suas notas ou frases no dueto com pouca ou nenhuma sobreposição temporal entre elementos (e.g., *Grallina cyanoleuca*, Hall 2000). Em duetos simultâneos, machos e fêmeas cantam notas ou frases distintas simultaneamente (e.g., *Thamnophilus doliatus*, Koloff e Mennill 2012). Por fim, em duetos sobrepostos, indivíduos cantam as mesmas frases ou notas simultaneamente (e.g., dupla de machos em *Chiroxiphia linearis*, Trainer *et al*. 2002).

Estima-se que na maioria das espécies de aves o dueto possa ser iniciado e concluído por machos e fêmeas, e que os cantos de machos e fêmeas difiram no dueto (Dahlin e Benedict, 2013). Estima-se que em aproximadamente metade das espécies de aves a estrutura do dueto difira consistentemente entre indivíduos ou casais (Dahlin e

Benedict, 2013). Machos e fêmeas podem possuir um repertório de mais de 20 tipos de notas que são comumente combinadas não aleatoriamente para a criação de códigos de dueto (Dahlin e Benedict, 2013). Finalmente, os duetos em aves normalmente envolvem uma exibição visual (Dahlin e Benedict, 2013).

Evolução e função do dueto

A evolução de exibições complexas, como duetos e coros, intriga cientistas há décadas (Farabaugh, 1982; Langmore, 1998, 2002; Hall, 2004). O dueto em aves é comum nos trópicos e raro em regiões temperadas (Slater e Mann, 2004), e sua evolução está associada à ocorrência de territorialidade durante todo o ano e vínculos sociais estáveis (Tobias *et al*. 2016, mas consultar Najar and Benedict 2015, Odom *et al*. 2015). A evolução de dueto e coro em aves não parece estar relacionada à latitude, migração, clima ou habitat (Tobias *et al*., 2016). Esses estudos sugerem que a defesa conjunta de territórios por parceiros reprodutores pode ser determinante para a evolução do dueto em aves (Logue e Hall, 2014; Tobias *et al*., 2016).

Por que um indivíduo cria o dueto, ou seja, responde a um canto iniciado por outro indivíduo, ao invés de permanecer em silêncio (Hall 2009)? A função adaptativa primária do dueto é foco de considerável discussão, porque não existe um consenso se o dueto surge através de cooperação sexual (Logue, 2005; Hall, 2009), conflito sexual (Tobias e Seddon, 2009) ou ambos (Benedict, 2010; Dahlin e Benedict, 2013). Atualmente, existem oito hipóteses, não mutualmente excludentes, para explicar a função primária do dueto, e mais quatro hipóteses sobre funções secundárias do dueto (Hall, 2004).

As possíveis funções primárias do dueto baseadas em cooperação sexual são: manter o contato entre parceiros (Thorpe 1963; e.g., Logue 2007), garantir a sincronia reprodutiva (Armstrong, 1947), evitar que o parceiro seja substituído no par social, defesa conjunta de território ou recursos (Robinson, 1949), e sinalização de comprometimento com o parceiro (Smith, 1994; Hall, 2004). As possíveis funções primárias do dueto baseadas em conflito sexual são: guarda de parceiro (Levin, 1996), guarda de paternidade (Sonnenschein e Reyer, 1983) e sinalização de qualidade (Smith, 1994; Hall, 2004). Funções secundárias do dueto incluem reconhecimento sexual, manutenção de isolamento reprodutivo, apaziguamento ritualizado e proteção contra predadores (Hall, 2004). Funções secundárias do dueto não são suficientes para explicar a sua evolução (Hall, 2004). A seguir, descrevo as principais hipóteses para a função primária do dueto, detalhando suas previsões e evidências.

A hipótese de defesa conjunta de recursos sugere que parceiros realizam duetos para defenderam um recurso ou território comum de outros coespecíficos (Robinson, 1949; Hall, 2004). Essa hipótese sugere que a probabilidade de manutenção ou ocupação de um território é maior se os parceiros o defenderem em conjunto (permanecendo próximos um do outro e realizando duetos) do que se eles não coordenarem a defesa (permanecendo distantes um do outro e realizando cantos solo) (Levin, 1996; Hall, 2009). A defesa conjunta de recurso prevê que o dueto por intrusos represente uma maior ou equivalente ameaça à posse do território em comparação com cantos solo (Bradley e Mennill, 2009; Dahlin e Wright, 2012; Koloff e Mennill, 2013).

Alternativamente, o dueto pode representar conflito sexual. Segundo a hipótese de defesa de parceiro, um indivíduo guarda o parceiro de outros indivíduos do mesmo sexo através da criação do dueto (i.e., resposta a um canto iniciado pelo parceiro) (Levin, 1996; Seddon *et al*., 2002; Hall, 2009). Nesse caso, a guarda tem um custo para o parceiro, por exemplo, evitando a atração de indivíduos para cópulas extrapar (Tobias e Seddon, 2009). Evidências indiretas para a hipótese de defesa de parceiro advêm de estudos que mostram que indivíduos respondem mais agressivamente a cantos solo de indivíduos do mesmo sexo, do que a cantos solo de indivíduos do sexo oposto ou duetos (Rogers *et al*., 2006).

A hipótese de defesa de paternidade para explicar a função do dueto é similar à hipótese de defesa de parceiro. No entanto, a primeira restringe-se aos casos em que o macho cria o dueto, e ao período fértil da fêmea (Sonnenschein e Reyer, 1983; Hall, 2004). De acordo com a hipótese de defesa de paternidade, a criação do dueto pelo macho restringe a fêmea de cópulas extrapar, porque sinaliza o estado pareado da fêmea, afastando outros machos (Hall, 2004; Topp e Mennill, 2008; Baldassarre *et al*., 2016; Dowling e Webster, 2017). No entanto, em nenhuma espécie de ave com dueto, este se restringe ao período fértil da fêmea, sugerindo que essa hipótese, mesmo que explicando parcialmente o fenômeno do dueto, não é suficiente para explicar sua evolução (Hall, 2009).

O dueto pode funcionar como um sinal de comprometimento com o parceiro, uma vez que o dueto normalmente exige alto nível de coordenação com o parceiro (Wickler, 1980). Esse comprometimento com o parceiro deve refletir o investimento que o indivíduo faz em funções ligadas à do parceiro, como no cuidado da prole do casal (Wickler, 1980). O investimento no vínculo do par social pode levar a um investimento recíproco pelo parceiro (Wickler, 1980; Hall, 2004). Alguns estudos sugerem que essa hipótese é coerente com a baixa taxa de paternidade extrapar em espécies de aves socialmente monogâmicas que cantam em dueto. Um exemplo ocorre na cambaxirra *Pheugopedius felix* (Troglodytidae), em que a fêmea opta por responder ao *playback* do canto do parceiro ao invés de sobrepor seu canto com o *playback* de outra fêmea, quando os dois estímulos são emitidos simultaneamente (Templeton *et al*., 2013). Por fim, o dueto pode sinalizar a qualidade do indivíduo se a habilidade individual de percepção e produção rápida de som estiver relacionada à qualidade individual (Smith, 1994). Essa qualidade pode ser objeto de seleção de parceiro ou mediar lutas por defesa de territórios (Hall, 2004).

A dificuldade em compreender a evolução do dueto talvez ocorra porque o dueto tenha múltiplas funções adaptativas na maioria das espécies (Marshall-Ball *et al*., 2006; Benedict, 2010; Dahlin e Benedict, 2013). Múltiplas funções podem ocorrer em uma mesma espécie em diferentes circunstâncias sociais e reprodutivas (Dahlin e Benedict, 2013). Estudos extensos, de longo prazo, testando múltiplas hipóteses em uma mesma espécie, poderão contribuir para compreensão da multifuncionalidade do dueto.

ESPÉCIE DE ESTUDO

O João-de-barro (*Furnarius rufus*; Aves: Furnariidae) é uma espécie de ave Neotropical, sexualmente monocromática (aparentemente), forrageadora de artrópodes de solo, e socialmente monogâmica (Fraga, 1980; Sick, 2001; Remsen e Bonan, 2017). Ambos os pais investem no cuidado parental (Braga, 2012; Massoni *et al*., 2012), desde a construção de um ninho maciço de matriz de barro (Shibuya *et al*., 2015), até um longo período de compartilhamento de território com os filhotes (~8 meses) (Bobato, 2012). O casal de João-de-barro tende a permanecer unido por mais de uma estação reprodutiva, e constrói o ninho normalmente durante pelo menos dois a três meses antes da postura dos ovos (Fraga, 1980). O período de nidificação ocorre entre setembro e dezembro, tanto no centro-leste da Argentina (Fraga, 1980) como no sudeste do Brasil (Braga, 2012).

O comportamento vocal do João-de-barro é ainda pouco conhecido. Estudos anteriores sugerem a existência de dois tipos de canto nessa espécie, um do macho e outro da fêmea (Laje e Mindlin, 2003; Amador *et al*., 2005; Roper, 2005). Acreditava-se que os cantos seriam sempre executados juntos, sobrepostos temporalmente, em um dueto (Burger, 1979). Machos contribuem com uma nota grave e curta, repetida em ascensão no dueto, enquanto fêmeas cantam uma ou duas notas agudas e longas (Laje e Mindlin, 2003; Amador *et al*., 2005; Roper, 2005).

Esses estudos também sugerem que apenas machos iniciam o dueto e que os ritmos dos cantos de machos e fêmeas se relacionam não linearmente nos duetos e são produzidos por um substrato neural relativamente simples (Laje e Mindlin, 2003; Amador *et al*., 2005). Entretanto, esses estudos não relatam a possibilidade de existência de cantos solo realizados por machos ou fêmeas (Laje e Mindlin, 2003; Amador *et al*., 2005; Roper, 2005). Adicionalmente, esses estudos não investigaram o dueto em contextos reprodutivos, sociais ou ecológicos.

O João-de-barro é um modelo ideal para estudos do dueto, por fazer parte de um grupo filogenético (subordem Tyranni) com muitas espécies que cantam em dueto, mas que é relativamente pouco conhecido em todos os aspectos do dueto (ontogenia, mecanismos, função e evolução) (Seddon e Tobias, 2006; Koloff e Mennill, 2012). O João-de-barro faz parte da família Furnaiidae, uma das mais ricas em espécies e menos conhecidas do mundo com relação aos sistemas de acasalamento social e genético (Tobias *et al*., 2012a). O João-de-barro é abundante em áreas urbanas na região centro-meridional da América do Sul (Marreis e Sander, 2006), e os casais defendem territórios e cantam durante todo o ano em poleiros conspícuos em habitats abertos (Burger, 1979; Sick, 2001), tornando-os modelos ideias para o estudo do dueto.

OBJETIVOS DA TESE

Neste estudo, investiguei a função do canto em fêmeas e machos de João-de-barro, com atenção especial aos cantos coordenados em duetos. Investiguei diversas hipóteses para explicar a função do canto no João-de-barro, com destaque para as hipóteses de defesa de território e guarda de parceiro. Testei se a função do canto varia entre os cantores, sexos, papéis no dueto (i.e., se o indivíduo inicia o dueto ou responde a um canto iniciado pelo parceiro), nível de organização do dueto (individual ou do casal) e estrutura do grupo social. Além disso, investiguei a relação entre a expressão do canto e o sucesso reprodutivo de fêmeas e machos, e o papel do nível de coordenação do dueto na mediação de interações agressivas entre grupos. Para atingir esses objetivos, utilizei diversas abordagens que incluíram observações focais, gravações de vocalizações e experimentos de *playback* em campo, análises acústicas e análises genéticas de paternidade.

No primeiro capítulo, avaliei a variação sazonal nas interações territoriais e na expressão de cantos nos níveis individual (início de canto e resposta ao parceiro) e do par reprodutor (e.g., quantidade de duetos), abrangendo as estações reprodutiva e não-reprodutiva. Se esses tipos de canto têm funções similares, previ que as expressões deles variariam em paralelo ao longo do tempo. Também avaliei o pico sazonal esperado para cada tipo de canto de acordo com cinco hipóteses funcionais: coordenação do cuidado parental, garantia da sincronia reprodutiva, defesa territorial, guarda de parceiro e guarda de paternidade. Por exemplo, de acordo com a hipótese de guarda de paternidade, esperava que um macho responderia mais aos cantos iniciados pela parceira (criando duetos) no período fértil dessa fêmea.

No segundo capítulo, observei a resposta agressiva de machos e fêmeas adultos de casais reprodutores à invasão simulada (i.e., *playback*) de território por macho (solo), fêmea (solo) e casal (dueto). Adicionalmente, avaliei se essa resposta dos adultos à invasão territorial variava entre casais focais com jovens e casais focais sem jovens, assumindo que a presença de jovens poderia influenciar a função do canto. Nesse capítulo, testei as hipóteses de defesa territorial e guarda de parceiro sobre função do dueto, incluindo variações dessas duas hipóteses. Por exemplo, de acordo com a hipótese de guarda unilateral de parceiro, esperava uma resposta mais agressiva ao playback de canto solo do mesmo sexo do que ao playback de dueto ou canto solo do sexo oposto.

No terceiro capítulo, investiguei o sistema de acasalamento genético do João-de-barro e as consequências do canto na aptidão dos adultos dessa espécie, explorando a relação entre expressão de canto, qualidade do território e sucesso reprodutivo dos cantores. Esperava encontrar uma relação positiva entre a expressão do canto e a aptidão dos cantores, que poderia ocorrer por meio da prevenção de cópulas extra-par do parceiro (i.e., guarda de parceiro), sucesso de acasalamento extra-par (i.e., atração de parceiros), aquisição e/ou defesa de territórios de alta qualidade (i.e., defesa conjunta de territórios).

No quarto capítulo, explorei se o nível de coordenação dos cantos no dueto do João-de-barro teria um papel em mediar interações territoriais. Para cumprir esse objetivo, realizei um experimento de *playback*, expondo os indivíduos focais a estímulos de duetos com níveis manipulados de coordenação e sobreposição dos cantos. A ‘hipótese de qualidade da coalisão’ (Hall e Magrath, 2007) assume que a coordenação do dueto requer uma atenção especial ao comportamento do parceiro e, portanto, pode sinalizar a habilidade conjunta ou motivação dos parceiros em engajarem em interações territoriais. De acordo com a ‘hipótese de qualidade da coalisão’, preví uma resposta mais agressiva a duetos com cantos altamente coordenados e/ou sobrepostos em comparação com duetos com cantos pouco coordenados e/ou sobrepostos e cantos não coordenados e/ou não sobrepostos.

No quinto e último capítulo, investiguei a possibilidade de existência de sinais sexuais crípticos no João-de-barro, explorando outra modalidade sensorial: a visual. Utilizei medidas morfométricas, sexagem molecular, espectrofotometria e modelos visuais para testar se o João-de-barro é uma espécie sexualmente dimórfica em relação ao tamanho e à coloração da plumagem. Adicionalmente, testei se existe pareamento seletivo (*assortative mating*) com relação a essas características, ou seja, se fêmeas e machos de tamanhos ou cores de plumagem similares tenderiam a se parear mais do que o esperado ao acaso.

ÁREA DE ESTUDO

Estudei uma população urbana de João-de-barro no campus Darcy Ribeiro da Universidade de Brasília, Brasil (15°45’ S, 47° 51’ O). O campus Darcy Ribeiro foi fundado em 1962 e possui uma área de 395 ha. A área total construída e a área com vegetação abrangem, respectiva e aproximadamente, 13% e 42% da área do campus (Universidade de Brasília, 2012). A área com vegetação é composta principalmente por árvores ornamentais de médio e grande porte isoladas em uma matriz composta por um baixo estrato de gramíneas, que é periodicamente podado. O campus Darcy Ribeiro também comporta uma área de vegetação nativa de Cerrado típico no Centro Olímpico. Pares de João-de-barro estão distribuídos por todo o Campus, nidificando em árvores ou postes.

EQUIPE DE TRABALHO

O presente estudo foi realizado no Laboratório de Comportamento Animal da Universidade de Brasília, coordenado pela Dra. Regina Macedo, e em colaboração com o laboratório do Dr. Michael Webster da Universidade de Cornell, Estados Unidos da América. Realizei uma visita técnica ao laboratório do Dr. Michael Webster entre junho e agosto de 2016 para a realização de análises genéticas de parentesco (Capítulo 3).

Gianlucca S. Rech, Edvaldo F. da Silva Júnior, MSc. Pedro H. L. Ribeiro e Desirée M. Ramos também colaboraram com o presente estudo. Gianlucca Rech realizou um projeto de iniciação cientifica (PIBIC, CNPq) entre 2013 e 2014 sobre a função do dueto na estação não reprodutiva do João-de-barro (tema do Capítulo 2), e colaborou com a coleta de dados para o Capítulo 5. Pedro Ribeiro, técnico bolsista (CNPq) do laboratório, colaborou com a coleta de dados para os Capítulos 2 e 5. Edvaldo da Silva Júnior realizou um projeto de iniciação científica através do Programa Jovens Talentos para a Ciência (JTCic) – CAPES, em que investigou a variação sazonal no canto do João-de-barro (tema do Capítulo 1). Desirée Ramos colaborou na conceptualização, coleta de dados e revisão do texto do Capítulo 4.

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CAPÍTULO 1

Duetting attributes in a Neotropical bird: seasonal variation and adaptive signaling functions[2]

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[2] Uma versão deste capítulo será submetida para publicação em *Journal of Avian Biology* como "Diniz P, Silva-Júnior EF, Webster MS & Macedo, RH. Duetting attributes in a Neotropical bird: seasonal variation and adaptive signaling functions."

ABSTRACT

An important step toward understanding the adaptive function of bird song is to determine if and how singing behavior varies seasonally. This leads to particularly insightful information when considering duetting species, where song function may vary according to the level of organization (individual vs. pair) and singing role (initiator vs. responder). We tested the idea that seasonal variation in duetting behavior depends on sex, level of duetting organization and singing role. We studied social pairs of a Neotropical bird species (Rufous Hornero *Furnarius rufus*) for seven consecutive months, recording vocal and territorial behaviors. Overall, partners coordinated most of their songs (61%) into duets and many song traits (song initiation rate, song output and duet rate) peaked in territorial contexts. Males engaged in territorial interactions more often, initiated more songs, and answered proportionately more of their partners' songs than did females. At a finer scale, seasonal variation in singing behavior depended on a complex interaction among sex, singing role and levels of duetting organization. Male song initiation rate peaked during the pre- and post-breeding stages, whereas females initiated more songs during the non-breeding season. Both sexes answered partner songs faster and at higher rates during the pre-breeding and female fertile stages. Partners duetted at a higher rate during the pre- and post-breeding stages. Finally, song initiation rates and duet rate, but not song answering rates, correlated with frequency of territorial interactions. Our findings indicate that song function may vary with sex, singing role and level of duet organization in duetting species.

Key words: **bird song, female song, duet, territoriality, joint territory defense, *Furnarius rufus***.

INTRODUCTION

Understanding the adaptive function of bird song relies on knowing the phenology and context in which the bird sings (Catchpole and Slater 2008). This is well documented for males in north-temperate bird species, whose song rate usually peaks during the spring before pairing (Catchpole 1973, Amrhein et al. 2002) and is associated with territoriality (Nowicki et al. 2016), suggesting that songs function to attract females and repel rivals (i.e., the dual function of bird song; Catchpole and Slater 2008). However, the singing and breeding phenology of tropical birds differ largely from north-temperate birds: females sing year-round and join male songs to create duets in many tropical species (Slater and Mann 2004, Odom et al. 2014, Tobias et al. 2016). To better understand variation in the phenology of singing effort, we must study species that exhibit female song (Langmore 1998, Cain and Langmore 2015) and coordinated song, such as duetting (Hall 2006, 2009, Topp and Mennill 2008, Bradley and Mennill 2009a, Dowling and Webster 2013, Odom et al. 2016).

Duetting occurs mainly when mated pairs coordinate vocal behaviors (Farabaugh 1982). The function of duetting is often difficult to discern (Hall 2004), because duetting is a collective behavior composed of two levels of organization: the individual level and the pair level (Logue and Krupp 2016). The individual level includes behaviors such as song initiation (solo songs plus initiated duets) and song answering (proportion of partner’s songs answered), whereas the pair level includes behaviors such as duet rate and duration (Logue and Krupp 2016). Either or both levels may influence individual fitness independently or as a whole. For example, duet rate may vary due to changes in song initiation, song answering, or both behaviors, in one or both sexes, but only duet rate may affect fitness for these individuals (Logue and Krupp 2016). Song initiation can be considered as a solo if not answered by the partner, and thus may be under similar selection as the usual solo songs (Hall 2009, Logue and Krupp 2016). Therefore, it is important to consider the possibility that each “duetting” behavior (song initiation, song answering and pair-level duetting) may have different functions (Logue and Krupp 2016). However, most studies investigate the seasonal variation only at the pair-level (e.g., duet and solo song rates) (Bradley and Mennill 2009a, Benedict

2010, Tobias et al. 2011, Koloff and Mennill 2012, Dowling and Webster 2013, but see Odom et al. 2016).

Duetting can reflect conflict or cooperation between partners and may be used in within- or extra-group communication (Hall 2004, 2009). At the pair level, duetting may function to defend common resources (Bradley and Mennill 2009b, Koloff and Mennill 2013) or as a mutual mate guarding display (van den Heuvel et al. 2014). At individual and inter-group levels, song answering might have evolved as a mate guarding strategy, whether to prevent the partner from attracting extra-pair mates or to repel same-sex rivals (Grafe and Bitz 2004, Rogers et al. 2006) – thus song answering and song initiation could have different functions for each sex in this scenario. Finally, duetting may have a role in communication between partners, such as stimulating and coordinating breeding activities in environments that have little seasonality (Dilger 1953; Hall 2009).

Mate guarding and territorial defense hypotheses predict higher signal expression during aggressive interactions with conspecifics (Hall 2004). A few species exhibit peaks in duet rate in the pre-breeding stage, which decrease as the breeding season progresses (Sonnenschein and Reyer 1983, Hall 2009, Dowling and Webster 2013, Odom et al. 2016), generally resulting from a decreasing female song rate (Hall 2006, Topp and Mennill 2008). However, studies rarely assess if these peaks match a peak in aggressive interaction with strangers (Dowling and Webster 2013). Without recording seasonal variation in aggressive interactions, it is difficult to distinguish if seasonal peak in singing effort is due to communication within (e.g., to ensure reproductive synchrony) or between social pairs (e.g., territory defense).

Here, we investigated seasonal variation in territory interactions and singing behavior in the Rufous Hornero *Furnarius rufus*, a socially monogamous, Neotropical bird species, in which males and females sing solo songs and also can combine their songs into duets. This species breeds seasonally (Fraga 1980, Massoni et al. 2012), but both sexes apparently sing year round, allowing us the opportunity to study the selective pressures shaping the evolution of bird song (Odom et al. 2016). If a signal is used only in the breeding season, it is probably shaped by sexual selection (Price et al. 2008, Illes and Yunes-Jimenez 2009, Odom et al. 2016). However, if a bird also sings during the non-breeding season, this may represent social or natural selection (Tobias et al. 2012, Odom et al. 2016).

We studied social pairs of horneros across seven months and recorded vocal behavior and aggressive interactions with strangers throughout this period. At the individual level, we measured the number and duration of territorial interactions, singing effort (rates of song initiation, song output and phrase duration in coordinated songs) and song attentiveness to partner songs (i.e. song answering rate and latency to answer). At the pair level, we recorded duet rate and duration. We tested the general hypothesis that seasonal variation in singing behavior depends on sex, singing role and level of duet organization. Our study innovates by treating three signals, song initiation, song answering and pair-level duetting, as fairly independent behaviors (Logue and Krupp 2016; but see Odom et al. 2016) and brings together hypotheses from both the solo song and duetting literatures (Table 1-1). If these three signals are evolving under the same selective forces, we expect them to vary in parallel across breeding and non-breeding stages.

METHODS

Study species

The Rufous Hornero is a sexually monochromatic and socially monogamous bird species from southern South America (Sick 2001, Diniz et al. 2016, Remsen and Bonan 2016). Social pairs maintain territories year-round, build a domed nest and normally produce a single brood (3-4 eggs) per year (Fraga 1980, Massoni et al. 2012). Both parents contribute similarly to parental care (Massoni et al. 2012) and nesting success is high (Fraga 1980). Little is known about their vocal behavior: male and female overlap sex-specific phrases in duets, which can be initiated by males (Laje and Mindlin 2003, Roper 2005, Amador et al. 2005) or females (P Diniz, unpublished data). In addition, adults and juveniles can coordinate their songs into chorus (i.e. three or more individuals coordinating songs; P Diniz, unpublished data). Vocal repertoire is determined by variations of one or a few song types for each sex, and duet phrases are similar to solos in structure, but differ in tempo (P Diniz, unpublished data).

Study area and field methods

We studied 12 social groups from an urban and banded population of the Rufous Hornero on the campus of the Universidade de Brasilia, central Brazil (15°45’S, 47°51’W). The study area has a seasonal climate, which includes a dry season (from May to September) and a wet season (from October to April). The Rufous Hornero breeds seasonally across its distribution, mainly between September and December (Fraga 1980, Massoni et al. 2012) (Supplementary material, Figure 1-5s).

Group size varied from two to six individuals (mean ± SD = 2.83 ± 0.82, n = 163 trials), which at the beginning of the study were composed of adults only (social pair, 2 groups) or adults and juveniles (10 groups). All studied juveniles hatched in the previous breeding season and stayed in their natal territories (see Fraga 1980). Although the juveniles may help the parents to build a new nest during the year, they disperse before the nesting stage and never incubate or feed nestlings (Fraga 1980, Massoni et al. 2012). Group size varied across the study period due to juvenile dispersal and recruitment of new offspring (Supplementary material, Figure 1-5sB, Figure 1-5sC).

We monitored 11 social groups for seven months (June-December 2015), which covered non-breeding and breeding seasons (Supplementary material, Figure 1-5sC). One additional group was monitored for 3.5 months (seven focal sessions), from June to September, when this group lost its territory to an unbanded pair. We observed each of these groups during one hour at 15-day ± 0.15 (mean ± s.e., n = 149) intervals, for a total of 14 focal sessions per group (except the one group that lost its territory). Before starting the trials, we determined the order of observation at random. We maintained this order during the study period to preserve equality of sampling intervals within groups. All focal sessions occurred from zero until five hours after sunrise (preliminary observations reveal that there is no dawn chorus in the species).

Observations were generally carried out by two observers (range = 1-4) and always focused on the mated pairs. During each 1h-focal session, we followed the pair and recorded their vocalizations using a Marantz PMD 660 recorder combined with a Sennheiser ME66 or Yoga HT-81 microphone. We also recorded behaviors, including aggressive interactions (chasing, approach followed by vocalization, song overlapping, displacement and/or fights) involving adult focal individual(s) against strangers (normally neighbors), and estimated the duration of these interactions whenever possible. We could not measure sex-specific duration of these interactions when both sexes participated and thus considered the same duration for both sexes in this case.

Finally, we recorded the occurrence of breeding behaviors in the same 1-hour focal sessions, which included nest building, incubation, brooding, feeding nestlings or fledglings (Massoni et al. 2012). Incubation and brooding phases were defined when at least one adult visited its nest, without bringing any nest building material (e.g. mud) or food, and spent five or more minutes inside the nest chamber. When a parent was observed bringing food to the nest, we considered it to be at the nestling phase. We used these behavioral data to estimate the breeding stages (see below).

Breeding phenology

We classified the study period in five stages: non-breeding, pre-breeding, fertile, nesting and post-breeding. We considered the non-breeding stage from 31-120 days before the first sign of incubation; pre-breeding from 16-30 days before the first sign of incubation; and fertile stage from 1-15 days before the first sign of incubation. The nesting stage comprised both incubation and nestling stages. The post-breeding stage comprised 1-90 days after fledging, when juveniles stayed in their natal territories. Seven study groups bred once and two groups bred twice during the study period. One group renested after its nest was depredated, and another group renested after the young from the first brood fledged. Because this latter group had juveniles from the current breeding season while the adults were incubating a new clutch, we classified them as being both in the ‘nesting stage’ and ‘post-breeding stage’. Accordingly, we classified them as both ‘fertile stage’ and ‘nesting stage’ in the prior focal session (~15 days before). We did not consider a category for nest building, since Rufous Horneros build their nests throughout the year and building rhythms seem to vary daily as a function of precipitation regimes (Fraga 1980).

Acoustic analyses

Acoustic analyses were performed in Raven Pro 1.5 (Bioacoustics Research Program 2014). Because Rufous Hornero partners overlap sex-specific phrases in duets (Amador et al. 2005), we could identify male and female contributions (Figure 1-1). We used the waveform and the spectrogram (Hann window, window size = 256, overlap in time = 50%) to demarcate the start and end of each phrase and song of each adult whenever possible. Then, we counted the number of initiated and answered songs in each 1h recording, obtained the duration of phrases and songs, and the latency to answer the partners’ songs in coordinated songs.

We classified initiated songs as solos plus initiated duets or chorus (Hall and Peters 2008a), where solo songs were those not coordinated with the social partner. Song answering was computed when the focal individual answered partner-initiated duets or chorus (reviewed by Logue and Krupp 2016). Importantly, since we focused on the adults, we ignored the phrase contributions and the solos of juveniles (see Dowling and Webster 2013). We computed adult initiated songs even when a juvenile initiated the song and was answered by the adult. Similarly, we computed adult answered songs of its partner even if a juvenile phrase preceded the partner phrase. Therefore, we classified all choruses involving both adults as duets. We adopted this approach because Rufous Horneros have a very low song output (< 2% of the time singing, see Results), juveniles initiated a minority of group songs (< 5%) and rarely answered a parent song before the other parent had sung. We argue that by overlooking the juvenile songs we increased our statistical power to explain seasonal variation of singing behavior without a detrimental effect on the reliability of results.

Rufous Hornero females, and especially males, can emit long solo song bouts (P Diniz, unpublished data) composed of subunits of monosyllabic and accelerated trills (Figure 1-1A, Figure 1-1B). We considered such a solo song bout as a single initiated song, if the intervals between their consecutive subunits were no longer than 30s (an interval value rarely reached by consecutive subunits) – otherwise we classified these subunits, separated by more than 30s intervals, as separately initiated solo songs. We considered as two independent solos those cases where partners overlapped their long monosyllabic song bouts if there was a very low degree of overlap (<5%) and no clear coordinating structure (Hall 2004; P Diniz, unpublished data). When an individual joined a partner solo song bout longer than 1 min, we computed this as both a solo song bout and a duet, using as a marker the beginning of the partner's last accelerated trill before the individual joined the vocalization. Low amplitude solo songs lasting less than 2s and short songs emitted during chases across territories in aggressive territory interactions were not counted. Individual's songs overlapping by only one or two partner notes were classified as solos.

Since Rufous Horneros may emit long solo song bouts, song rate does not fully represent song output in this species. We measured song output as the total time each adult spent singing at each 1h focal session. We could not measure phrase or song duration from 15% of the vocalizations recorded (*N* = 208 of 1395) due to poor quality of some recordings. Thus, we estimated the missing phrase durations using the mean duration of the other phrases during the same 1h recording. We then used the real measured duration plus the estimated duration values to obtain the song output for each recording.

Statistical analyses

Statistical analyses were performed in R (R Core Team 2015). We analysed separately each of the following seven individual-level response variables: (1) number and (2) duration of territorial aggressive interactions, (3) song initiation and (4) answering rates, (5) song output, (6) latency to answer partner's song, and (7) phrase duration in duets. We also analyzed two response variables at the pair-level: (8) duet rate (number of duets/h) and (9) duet duration. Song initiation rate means the number of initiated songs in a 1h focal session. Song answering rate was considered as the proportion of partner's initiated songs that were answered by the focal bird (reviewed by Logue and Krupp 2016).

We analyzed our dataset with linear (LMM) or generalized mixed modelling (GLMM) depending on the scale of our response variable (according to Zuur *et al.* 2009). We modelled the response variables (1), (3) and (8) assuming a Poisson distribution of errors, and the variable (4) according to the Binomial family for proportional data. The remaining response variables were analyzed according to the Gaussian family. We log-transformed the response variable when necessary to achieve a normal distribution of errors.

We included breeding phenology as a fixed effect in all models. We included sex to model individual-level song behaviors. We also included the identity of the social pair (random factor), group composition (adults or adults plus juveniles) and decimal hour at the beginning of each focal trial (covariates) in all models. Additionally, we added aggressive context as a fixed effect to model song variables. Aggressive context during the 1h focal sessions was a factor composed by three levels: aggressive, non-aggressive and unknown role. Aggressive context occurred when the focal bird engaged in one or more agonistic interactions with strangers whereas non-aggressive context consisted of the lack of such agonistic interactions. Unknown role meant the focal bird engaged in aggressive interaction, but we did not know if the bird interacted with strangers or other group member(s). We kept this “unknown role” level to avoid decreasing sample size and statistical power.

We added song type (i.e., duet or chorus) as a fixed effect to model latency to answer partner’s songs and phrase duration in duets. We included the identity of the focal session nested within the identity of the social pair as a random nested effect to model the variables (2), (6), (7) and (9). Finally, we added the interaction sex × breeding phenology in all models, and the interaction sex × aggressive context to model song variables.

We tested the ‘attraction of extra-pair mates hypothesis’ for male song initiation rate and male song output. We created two new global models (one for each response variable), exclusively for males, to perform these analyses. To these models we added all the variables included in the global models for both sexes, except the variable ‘sex’. We also added the proportion of studied females that were fertile in each 15-day study period (e.g., August 1-15) as an estimate of the number of fertile females in the studied population.

We used the function ‘dredge’ from the ‘MuMIn’ (Barton 2015) package to select the best-fit models based on the AICc criteria (ΔAICc<2; Burnham and Anderson 2002).

For continuous predictors, we obtained β estimates for the retained variables from the top model in which the variable occurred. For categorical predictors, we used the commands ‘glht’ (multcomp package, Hothorn et al. 2008) and ‘lsmeans’ (lsmeans package, Lenth 2015) to obtain post-hoc comparisons among factor levels, using the top model in which the variable occurred. We controlled for false discovery rate in post-hoc comparisons according to Benjamini and Hochberg (1995).

RESULTS

Territorial interactions

We recorded 162 territorial aggressive interactions involving focal adult birds and strangers (mean ± s.e. = 0.73 ± 0.05 interactions/h). Most interactions involved song by the focal individuals or strangers (74%, n = 150 focal sessions) and/or chasing (64%, n = 148); a few interactions involved displacement (without song or chasing) (8%, n = 162) or physical fights (3%, n = 151). We identified the aggressive role in 65 (40%) of these territorial interactions. Focal males performed most aggressive behaviors towards strangers (40%), but social pairs also coordinated attacks (25%). Strangers, normally neighbors, also started some aggressive interactions (26%). Unaccompanied females conducted a few aggressive interactions directed towards strangers (9%).

For both sexes, the number and duration of aggressive interactions with strangers did not vary across breeding and non-breeding stages (Table 1-2). On average, males engaged in aggressive interactions 1.5 times more than did females (β = 0.43 ± 0.13; mean ± s.e.; male = 0.89 ± 0.08; female = 0.58 ± 0.07), although the sexes did not differ in the time spent in each territorial interaction (mean ± s.e.; male = 32.89 ± 6.49 s; female = 41.09 ± 9.75 s; Figure 1-2).

Song rate

We recorded, on average, 10 adult songs/h (n = 1611 adult songs, 12 social groups), including solos and duet phrases. For 184 recorded songs (10% of total songs recorded, n = 1795), we could not determine if the singer(s) was (were) from the focal group. We determined the singing role (i.e. initiator or responder) in 88% of the recorded and identified songs (n = 1611). Overall, most songs (61%) were duets.

Song initiation

Song initiation rate was associated with breeding phenology and aggressive context, but sexes differed in how their song behavior varied relative to breeding phenology (Table 1-3; Figure 1-3A). Female song initiation rate peaked during non-breeding (mean ± s.e. = 1.94 ± 0.22 songs/h) and post-breeding stages (mean ± s.e. = 2.51 ± 0.25 songs/h), and gradually decreased from the non-breeding season through the breeding season. In contrast, male song initiation rate peaked in the post-breeding stage (mean ± s.e. = 5.10 ± 0.54 songs/h) and, less noticeably, in the pre-breeding stage (mean ± s.e. = 4.42 ± 0.68 songs/h). Females tended to initiate fewer songs in the nesting stage (mean ± s.e. = 0.75 ± 0.15 songs/h), and males initiated songs at their lowest rate in the non-breeding stage (mean ± s.e. = 2.84 ± 0.36 songs/h).

Males and females initiated more songs in contexts that included at least one territorial aggressive interaction (mean ± s.e: female = 2.02 ± 0.21 songs/h, male = 4.53 ± 0.34 songs/h) compared to those without any territorial interaction involving the focal individual (β ± s.e. = 0.25 ± 0.08; mean ± s.e: female = 1.56 ± 0.16 songs/h, male = 2.02 ± 0.16 songs/h). The third best-ranked model (ΔAICc = 1.77) indicates this result is exclusively for males, which suggests that females initiate fewer songs than males in response to aggressive contexts. Although sexes differed in their singing initiation behavior relative to breeding phenology, males initiated, on average, twice the number of songs that females initiated, irrespective of breeding stage and the occurrence of aggressive interactions (pooled data, β ± s.e. = 0.90 ± 0.10; mean ± s.e.: male = 3.74 ± 0.24 songs/h; female = 1.76 ± 0.13 songs/h).

In the subset model that included only male song data, we found a negative correlation between male song initiation rate and the estimated proportion of females that were fertile in the population (β ± s.e. = –0.16 ± 0.07). The parameter, estimated proportion of fertile females, occurred in the two top-ranked models for male song initiation rate (ΔAICc < 2); the results for the other variables of interest (breeding phenology and aggressive context) remained qualitatively unchanged.

Song answering

Male and female song answering rates differed but were similarly associated with breeding phenology (Table 1-3; Figure 1-3B). On average, males answered 73% (± 2.97 s.e.) of partner songs, whereas females answered 61% (± 2.59 s.e.) of male-initiated songs (β ± s.e. = 0.56 ± 0.16). For both sexes, song answering rate clearly peaked during pre-breeding (mean ± s.e.; male = 0.97 ± 0.03, female = 0.77 ± 0.08) and female fertile stages (mean ± s.e.; male = 0.97 ± 0.03, female = 0.78 ± 0.08). Song answering rate did not differ between non-breeding, nesting and post-breeding stages (sexes pooled, mean ± s.e.: non-breeding = 0.62 ± 0.03; nesting = 0.65 ± 0.05; post-breeding = 0.62 ± 0.03). Finally, song-answering rates did not correlate with territorial interaction for either males or females.

Song output

Males differed from females in time spent singing, irrespective of breeding phenology or aggressive context (Table 1-3; Figure 1-3C). Males spent, on average, twice the amount of time singing compared to females (β = 0.40 ± 0.10; mean ± s.e.; male = 63.62 ± 8.46 s/h; female = 30.64 ± 2.70 s/h). For both sexes, song output varied with aggressive context and across breeding and non-breeding stages. First, individuals spent more time singing when engaged in aggressive interactions with strangers (sexes pooled, β ± s.e. = 0.37 ± 0.11; mean ± s.e.: aggressive context = 59.89 ± 8.04 s/h; not aggressive context = 35.87 ± 4.57 s/h). Second, individuals spent less time singing in the nesting stage compared to pre-breeding and post-breeding stages.

We found no correlation between male song output and the estimated number of fertile females in the study population (ΔAICc > 2), for the subset of models including only male song data.

Duet rate

Duet rate varied with breeding phenology and aggressive context (Table 1-3; Figure 1-3D). Duets peaked in the pre- (mean ± s.e = 4.67 ± 0.79 duets/h) and post-breeding stages (mean ± s.e = 4.20 ± 0.31 duets/h), and were less frequent in the nesting stage (mean ± s.e = 2.69 ± 0.31 duets/h). Duetting peaked during the pre-breeding stage was mainly a result of high song initiation by males and high answering rates by both sexes. Differently, the duetting peak during the post-breeding stage was mainly a result of high song initiation by both sexes despite the moderate song answering rates at this stage.

Partners duetted at highest rate when one pair member engaged in one or more aggressive interactions with strangers (β ± s.e. = 0.21 ± 0.10; mean ± s.e: non-aggressive context = 2.45 ± 0.28 duets/h, aggressive context = 3.74 ± 0.20 duets/h). However, the second best-ranked model (ΔAICc = 0.37) does not indicate this relationship.

Song latency and duration

Latency to answer partner's songs

Song latency to answer partner's initiated songs varied with sex, breeding phenology and song type (i.e., duet or chorus), but did not vary with the occurrence of aggressive encounters (Table 1-3; Figure 1-4A). Males answered partner's initiated songs faster than females, irrespective of breeding phenology or song type (β = 0.56 ± 0.11; mean ± s.e.; male = 0.84 ± 0.08 s; female = 1.46 ± 0.10 s). Both sexes answered partner initiated songs more quickly during the female fertile and nesting stages (and, less clearly, in the pre-breeding stage), compared with the post-breeding stage. Both sexes tended to have lower latency in answering their partner's song in duets compared with chorus (sexes pooled, β ± s.e. = 0.27 ± 0.15; mean ± s.e.: duets = 1.13 ± 0.07 s; chorus = 1.85 ± 0.24 s).

Song duration

The duration of phrases emitted in duets varied with sex and breeding phenology (Table 1-3; Figure 1-4B). Males emitted longer phrases than females, irrespective of breeding phenology (β = 0.95 ± 0.10; mean ± s.e.; male = 6.37 ± 0.08 s; female = 5.41 ± 0.07 s). For both sexes, the duration of phrases peaked in the pre-breeding and female fertile stages (mean ± s.e.; pre-breeding = 6.43 ± 0.16 s; fertile = 6.63 ± 0.15 s), and tended to be higher in the nesting stage compared to the non-breeding stage (sexes pooled, β = 0.54 ± 0.21; mean ± s.e.; nesting = 6.03 ± 0.15 s; non-breeding = 5.50 ± 0.10 s).

Duet duration varied with breeding phenology, but not with the occurrence of aggressive interactions with strangers. The duration of duets was higher in the breeding season relative to the non-breeding season (Table 1-3; Figure 1-4C; see Supplementary material for detailed results of model selection).

DISCUSSION

Seasonal patterns of song vary with sex, singing role and duet organization

Both males and females engaged in territorial aggressive interactions, sang solo songs, and initiated and answered songs in duets during both the non-breeding and breeding stages. Overall, partners coordinated most (61%) of their songs into duets, and many song traits (song initiation rate, song output and duet rate) peaked when there was a territorial interaction involving the focal individual or pair. In general, these results indicate that Rufous Hornero males and females use song to defend year-round territories, one of the suggested functions for duets (Hall 2004, 2009).

We found remarkable sex differences in aggressive and singing behavior, regardless of seasonality. Males engaged more in territorial interactions, and sang at higher rates and for longer periods than did females. In addition, males initiated more songs, answered their partner´s songs more quickly to create duets, and did so at higher rates, compared with females. Male bias in song effort is common among other duetting species (Mennill et al. 2005, Rogers 2005, Valderrama et al. 2008, Tobias et al. 2011, Koloff and Mennill 2012, Odom et al. 2016). These results suggest that sexual selection almost certainly plays a role in the evolution of song in the Rufous Hornero and other duetting species (see Mennill et al. 2005; Odom et al. 2016).

Seasonal variation in the Rufous Hornero’s singing behavior also varies between singing roles (initiation vs. answering) and levels of organization of duetting behavior (individual-level vs. pair-level) (see Topp and Mennill 2008). We found sex-specific seasonal variation in song initiation but not in answering behavior, suggesting that the function of duetting components may differ between the sexes. Indeed, the few studies that focus on this issue have shown component-specific seasonal variation in duetting behavior (Topp and Mennill 2008, Odom et al. 2016). For example, in the Rufous-and-white wrens (*Thryothorus rufalbus*), female song answering and output decrease as the breeding season progresses, whereas males keep singing solo songs at high rates through the breeding season (Topp and Mennill 2008). In the Venezuelan troupial (*Icterus icterus*), male song initiation is higher during the breeding season, whereas duetting and female songs occur more often during the non-breeding season (Odom et al. 2016). Therefore, song function may vary with sex, singing role and level of duetting organization (Logue and Krupp 2016).

Song initiation behavior

Song initiation rate peaked during territorial interactions for both sexes, regardless of phenological stage. However, for females, song initiation rate peaked during the non-breeding stage, whereas for males it peaked during the pre-breeding stage, and for both males and females, in the post-breeding stages. Because females initiated songs more often in the non-breeding season, and both sexes initiated songs more often in the post-breeding stage, our results only partially support the ‘territory defense’ hypothesis for the function of male and female song initiation behaviors. Our data also partially support the ‘mate guarding’ and ‘ensure reproductive synchrony’ hypotheses for the function of male song initiation behavior (Table 1-1).

Singing effort in birds usually peaks in the pre-breeding and egg-laying stages (Amrhein et al. 2002, 2004, Dowling and Webster 2013), sometimes in the nesting stage (Cain and Langmore 2015, Chiver et al. 2015), but rarely if ever in the post-breeding stage. The Rufous Honero starts to build a new nest for the next breeding season just after its current seasonal nesting stage has ended (P Diniz, unpublished data; Fraga 1980). Also, some of the juveniles might disperse during the post-breeding stage (P Diniz, unpublished data). These two events may increase the competition for nest sites, territories (for both sexes) and social mates (for males) during the post-breeding stage, which could explain the unexpected peak in male and female song initiation rates in this stage. Alternatively, high song initiation rate may encourage juvenile singing in the post-breeding stage in order to join parents in territory defense or stimulate vocal learning in juveniles.

Male song initiation did not peak during their partner´s fertile stage and it was not associated with the estimated phenological variation in the number of fertile females in the studied population. These results indicate that Rufous Hornero males do not initiate songs to assure paternity or attract extra-pair mates (Table 1-1) (Forstmeier and Balsby 2002). Finally, the peak in male song initiation during the pre-breeding season provides some evidence for the ‘ensure reproductive synchrony’ hypothesis (Table 1-1), indicating that male song initiation could also function to stimulate or respond to their partner´s reproductive activity (Leboucher et al. 1998, Bentley et al. 2000).

Song attentiveness behavior

Both sexes answered their partner’s initiated songs quicker and at much higher rates (100%, males; ~80%, females) during the pre-breeding and female fertile stages. In contrast, other studies have found that males and females do not increase their duet responsiveness during the female fertile stage (Hall and Magrath 2000, Gill et al. 2005, Hall 2006, Hall and Peters 2008b). One exception is the Rufous-and-white wren, where males answered their partner-initiated songs at the highest rate during the fertile stage, although females showed very low responsiveness during this period (Topp and Mennill 2008).

Song attentiveness behaviors (i.e. song answering rate and latency) may have a similar function for male and female Rufous Horneros, since they varied in parallel across non-breeding and breeding stages. Our results provide support for the hypothesis that song attentiveness functions to ensure reproductive synchrony (Table 1-1), and in mutual partnership guarding, despite the fact that we did not find a link between song answering and territorial interactions, required for full support of this last hypothesis (Rogers et al. 2006). We also did not find a higher rate of territorial interactions during the pre-breeding and female fertile stages, providing no support for the territory defense hypothesis (Table 1-1). Thus, song answering in itself should not be enough to promote territory defense in the Rufous Hornero.

Contrary to predictions of the ‘paternity guarding’ hypothesis (Table 1-1), male song answering rate and latency were not higher in the female fertile stage compared with the pre-breeding stage (Hall 2009). Similarly, the proportion of answered songs did not differ between pre-fertile and fertile stages in the Buff-breasted wren (Gill et al. 2005). Thus, acoustic paternity assurance is probably not driving these song components in the Rufous Hornero.

Duets

Duet rate peaked in the pre- and post-breeding stages and was associated with territorial aggressive context, providing partial support for the ‘territory defense hypothesis’ (Table 1-1). Seasonal patterns in duet rate vary among species, but usually peaks in the pre-breeding stage (Rufous-and-white wrens, Topp and Mennill 2008b, Red-backed Fairy-wrens, Dowling and Webster 2013; Rufous Hornero), and other breeding stages (Barred Antshrike, Koloff and Mennill 2012; California towhee, Benedict 2010). Our study confirms that duetting can also peak in the non-breeding season (Venezuelan troupial, Odom et al. 2016), such as the post-breeding stage (Rufous Hornero).

Considering that song answering rates were not associated with territorial interactions, our results also partially support the mutual mate-guarding hypothesis (Table 1-1), and suggest that the pair-level component of duetting should be more important for defending a territory or the pair bond than an individual-level duetting property (i.e. song answering). This means that duetting is important for territory, mate guarding or both, regardless of which sex initiates a duet, and that unilateral acoustic mate-guarding through song answering (Rogers et al. 2006) does not seem to occur in the Rufous Hornero. Finally, the higher duet duration during the breeding season indicates that duet duration should mediate territorial or mate disputes during the breeding season, or facilitate within-pair communication in this period.

Conclusions

Our data suggest that seasonal variation in duetting behavior is dependent upon a complex interaction between sex, singing role (song initiation vs song answering) and levels of duetting organization (individual vs pair-level). Males seem to use song initiation to defend the territory, their mate or both and to stimulate female reproductive activity. In contrast, females seem to use song initiation to defend territorial resources, especially in the non-breeding season. Our study does not support the idea that song answering is associated with territory defense, but instead that it may function in mutual partnership guarding and stimulation of reproduction (e.g., hormonal profiles). Finally, seasonal variation in duetting at the pair-level partially supported the territory defense and mutual mate guarding hypotheses.

Our study supports the concept that singing roles and levels of duetting organization may vary across time in different ways and thus may have unique adaptive functions. In addition, we suggest that the pair-level component of duetting contains information arising from the combination of individual-level components (song initiation or answering). Consequently, we suggest that a better understanding of bird song evolution may be attained through studies of duetting behavior at both the individual and pair levels.

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Figure legends

Figure 1-1. Examples of female solo (A), male solo (B) and male-female duet (C) in the Rufous Hornero. Panel C: lines above and below notes indicate female and male notes, respectively.

Figure 1-2. (A) Number and (B) duration (mean ± s.e.) of territorial, aggressive interactions between focal adults and stranger conspecifics. Phenological stages: Non-B = non-breeding (31-120 days before incubation started); Pre-B = pre-breeding (16-30 days before incubation started); Fertile (1-15 days before incubation started); Nesting (incubation and nestling stages); Post-B = post-breeding (1-90 days after fledgling). Numbers without parentheses indicate sample sizes: total focal sessions in Fig. 1A and total number of interactions in Fig. 1B. Numbers of social pairs are shown within parentheses.

Figure 1-3. Seasonal variation in song rate and output (means ± s.e.) in the Rufous Hornero. Seasonal stages: Non-B = non-breeding (31-120 days before incubation started); Pre-B = pre-breeding (16-30 days before incubation started); Fertile (1-15 days before incubation started); Nesting (incubation and nestling periods); Post-B = post-breeding (1-90 days after fledgling). Different letters indicate post-hoc differences in the response variable between factor levels. Numbers without parentheses indicate total focal sessions. Numbers of social pairs are shown within parentheses.

Figure 1-4. Seasonal variation in song latency and song duration (means ± s.e.) in the Rufous Hornero. Phenological stages: Non-B = non-breeding (31-120 days before incubation started); Pre-B = pre-breeding (16-30 days before incubation started); Fertile (1-15 days before incubation started); Nesting (incubation and nestling periods); Post-B = post-breeding (1-90 days after fledgling). Different letters indicate differences in the response variable between factor levels. Numbers without parentheses indicate total focal sessions. Numbers of social pairs are shown within parentheses.

Figure 1-1

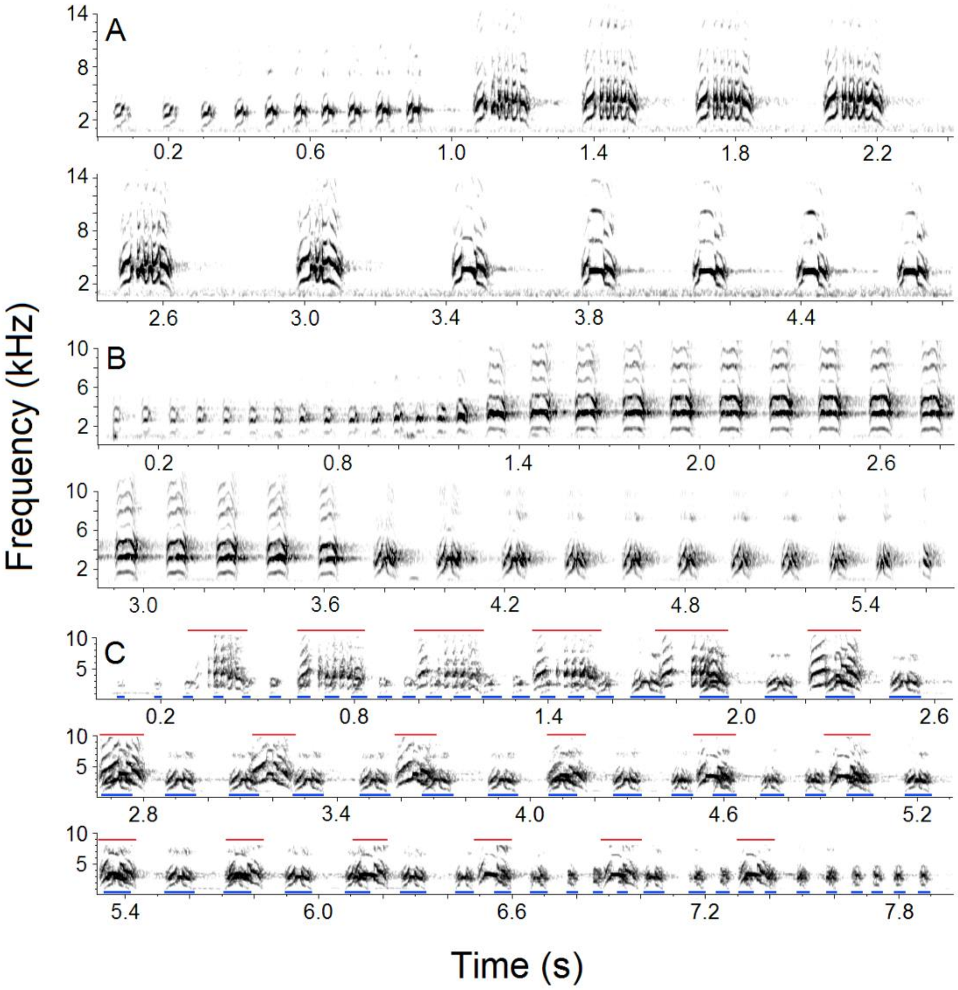

A
B
C
14
8
2
0.2
0.6
1.0
1.4
1.8
2.2
2.6
3.0
3.4
3.8
4.4
10
6
0.8
2.0
4.2
4.8
5.4
5
2.8
4.0
4.6
5.2
6.0
6.6
7.2
7.8
Frequency (kHz)
Time (s)

Figure 1-2

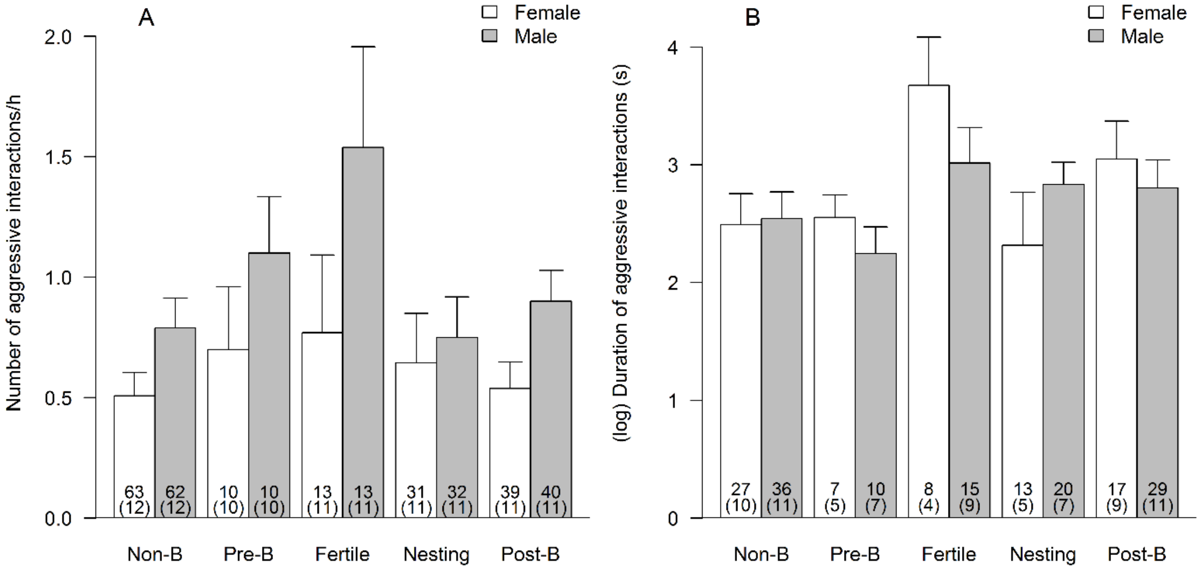
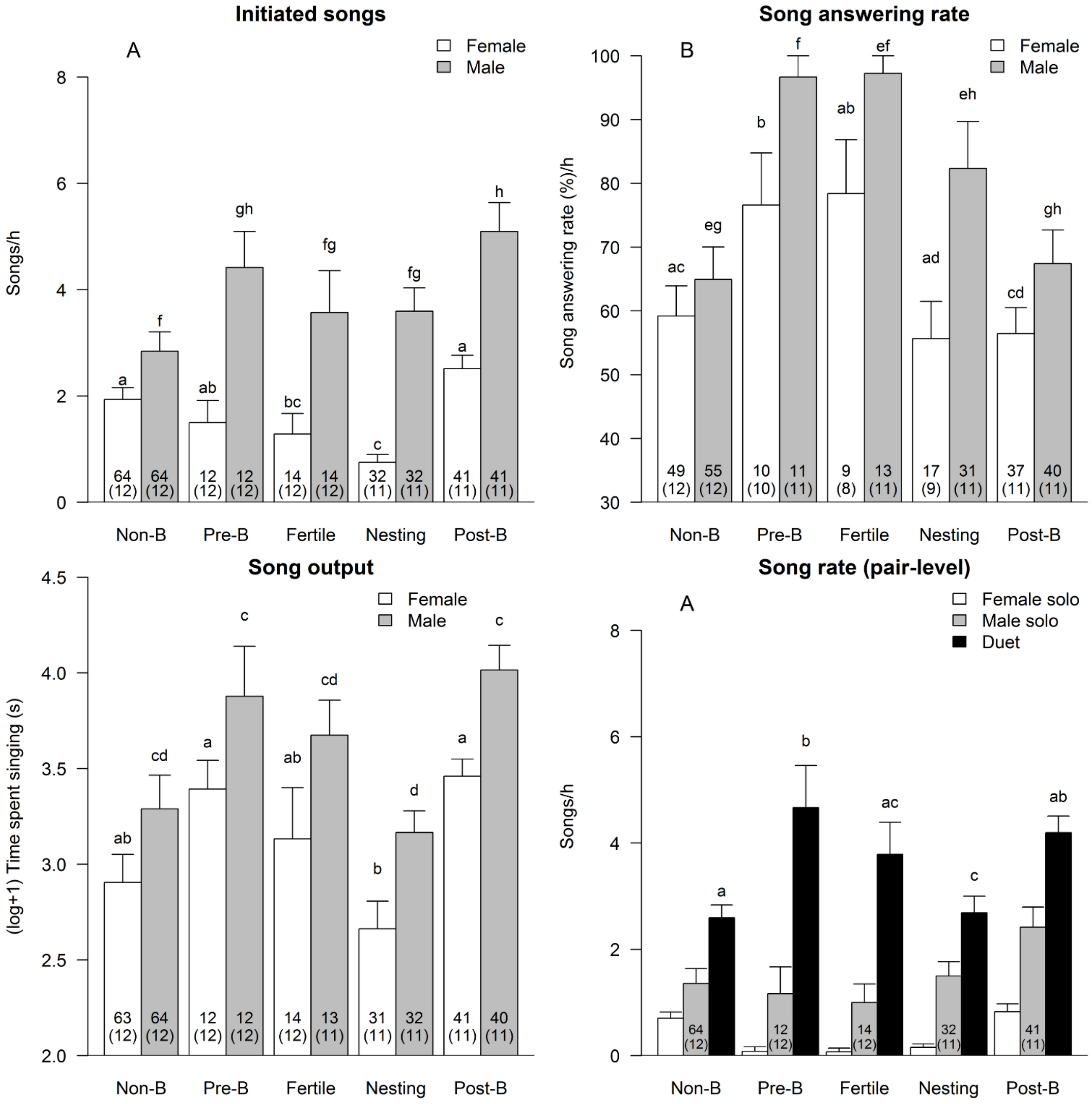
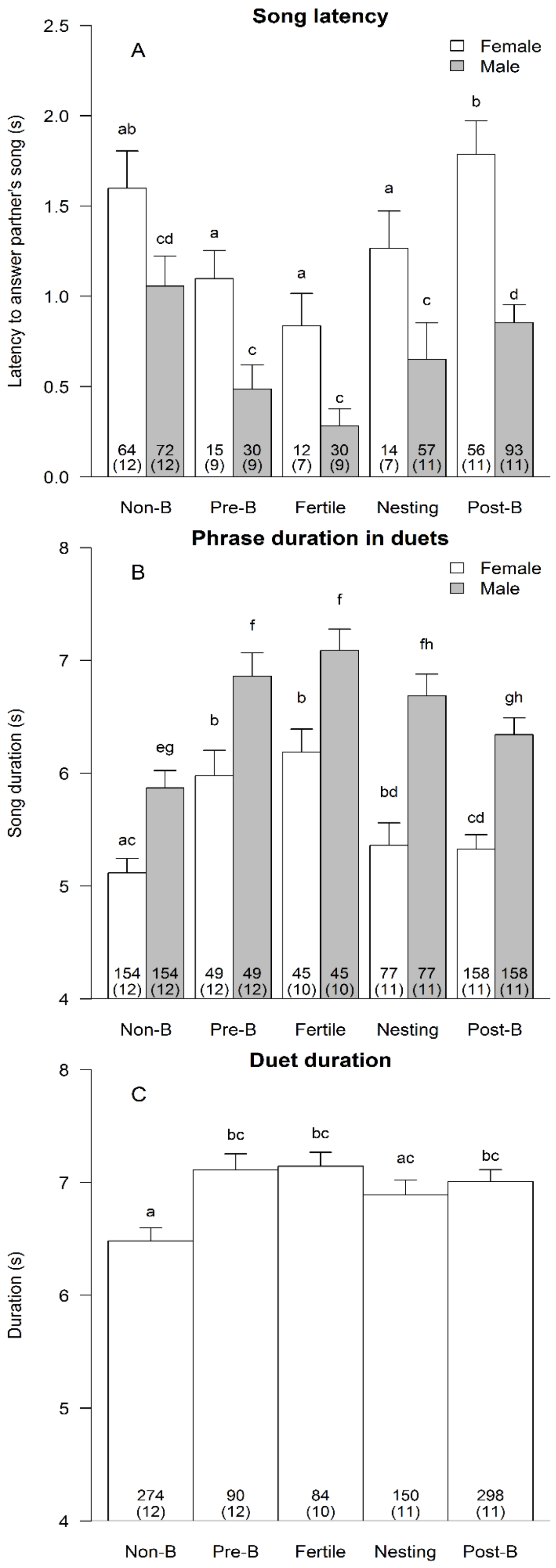

A
Female
Male
Number of aggressive interactions/h
2.0
1.5
1.0
0.5
0.0
63 (12)
62 (12)
10 (10)
10 (10)
13 (11)
13 (11)
31 (11)
32 (11)
39 (11)
40 (11)
Non-B
Pre-B
Fertile
Nesting
Post-B
B
Female
Male
(log) Duration of aggressive interactions (s)
4
3
2
1
0
27 (10)
36 (11)
7 (5)
10 (7)
8 (4)
15 (9)
13 (5)
20 (7)
17 (9)
29 (11)
Non-B
Pre-B
Fertile
Nesting
Post-B

Figure 1-3



Initiated songs
A
Female
Male
Songs/h
0
2
4
6
8
a
f
ab
gh
bc
fg
c
fg
a
h
64 (12)
64 (12)
12 (12)
12 (12)
14 (12)
14 (12)
32 (11)
32 (11)
41 (11)
41 (11)
Non-B
Pre-B
Fertile
Nesting
Post-B
Song answering rate
B
Female
Male
Song answering rate (%)/h
30
40
50
60
70
80
90
100
ac
eg
b
f
ab
ef
ad
eh
cd
gh
49 (12)
55 (12)
10 (10)
11 (11)
9 (8)
13 (11)
17 (9)
31 (11)
37 (11)
40 (11)
Non-B
Pre-B
Fertile
Nesting
Post-B
Song output
Female
Male
(log+1) Time spent singing (s)
2.0
2.5
3.0
3.5
4.0
4.5
ab
cd
a
c
ab
cd
b
d
a
c
63 (12)
64 (12)
12 (12)
12 (12)
14 (12)
13 (11)
31 (11)
32 (11)
41 (11)
40 (11)
Non-B
Pre-B
Fertile
Nesting
Post-B
Song rate (pair-level)
A
Female solo
Male solo
Duet
Songs/h
0
2
4
6
8
a
b
ac
c
ab
64 (12)
12 (12)
14 (12)
32 (11)
41 (11)
Non-B
Pre-B
Fertile
Nesting
Post-B

Figure 1-4


Song latency
A
☐ Female
▨ Male
Latency to answer partner's song (s)
2.5
2.0
1.5
1.0
0.5
0.0
ab
cd
a
c
a
c
a
c
b
d
64
(12)
72
(12)
15
(9)
30
(9)
12
(7)
30
(9)
14
(7)
57
(11)
56
(11)
93
(11)
Non-B
Pre-B
Fertile
Nesting
Post-B
Phrase duration in duets
B
☐ Female
▨ Male
Song duration (s)
8
7
6
5
4
ac
eg
b
f
b
f
bd
fh
cd
gh
154
(12)
154
(12)
49
(12)
49
(12)
45
(10)
45
(10)
77
(11)
77
(11)
158
(11)
158
(11)
Non-B
Pre-B
Fertile
Nesting
Post-B
Duet duration
C
Duration (s)
8
7
6
5
4
a
bc
bc
ac
bc
274
(12)
90
(12)
84
(10)
150
(11)
298
(11)
Non-B
Pre-B
Fertile
Nesting
Post-B

Tables

Table 1-1. Predicted seasonal variation in solos and duets according to six popular functional hypotheses. Song mode: song initiation or solo (I), song answering (A), pair-level duetting (D). Sex: male (M), both (B).

Hypotheses	Description	Song mode	Sex	Expected peak in signal expression	Aggressive context?
Coordination of parental care[1,2]	Parents use song exchange to coordinate nest visits	IA	B	Nesting stage[2]	No
Ensuring reproductive synchrony[3]	Song stimulates and synchronizes reproductive activities	IA	B	Pre-breeding stage[13]	No
Territorial defense[4,5,6]	Song is used to defend resources in a territory	IAD	B	When there are more territorial intrusions[4,6,12]	Yes
Mate guarding (partnership)[6,7,8]	Song is used to guard social partner	IAD	B	Pre-breeding and female fertile stages[6,7,13]	Yes
Mate guarding (paternity) [6,9,10]	Male song prevents female partner engaging in extra-pair copulation	IA	M	Female fertile stage[6,7,13]	Yes
Male attraction of extra-pair mates[7,10]	Initiated and solo songs are intended to attract extra-pair mates	I	M	When most females are fertile in the population[10,11]	No

References: 1 - Langmore (1998), 2 - Halkin (1997), 3 - Dilger (1953), 4 - Catchpole and Slater (2008), 5 - Seibt and Wickler (1977), 6 - Hall (2004), 7 - Moller (1991), 8 - Stokes and Williams (1968), 9 - Sonnenschein and Reyer (1983), 10 - Mace (1987), 11 - Chiver et al. (2015), 12 - Levin (1996), 13 - Hall (2009).

Table 1-2. Modelling comparison of territorial aggressive interactions as a function of breeding phenology and sex.

	df	AICc	ΔAICc	wi
Number of aggressive interactions (GLMM, poisson family)				
sex + decimal hour	4	691.6	0.00	0.59
Duration of aggressive interactions (log) (LMM, gaussian family)				
null model	4	551.7	0.00	0.32
decimal hour	4	552.9	1.28	0.17
group composition	4	553.3	1.67	0.14

We show results from top models (ΔAICc<2) derived from modelling comparisons for all combinations of predicted variables included in the global model (command ‘dredge’ in ‘multcomp’ package). df = degrees of freedom; wi= weight.

Table 1-3. Modelling comparison of song variables as a function of breeding phenology and territorial aggressive interaction.

	df	AICc	ΔAICc	wi
Song initiation rate (GLMM, poisson family)				
aggressive interaction + breeding phenology + sex + decimal hour + breeding phenology × sex	14	1303.4	0.00	0.47
aggressive interaction + breeding phenology + sex + group composition + decimal hour + breeding phenology × sex	15	1304.9	1.50	0.22
aggressive interaction + breeding phenology + sex + decimal hour + aggressive interaction × sex + breeding phenology × sex	16	1305.2	1.77	0.20
Song answering rate (GLMM, binomial family)				
breeding phenology + sex + group composition + decimal hour	9	603.0	0.00	0.52
Song output (log+1) (LMM)				
aggressive interaction + breeding phenology + decimal hour + sex	11	873.7	0.00	0.613
Duet rate (GLMM, poisson family)				
aggressive interaction + breeding phenology + decimal hour + group composition	11	640.7	0.00	0.513
latency (log)				
breeding phenology + decimal hour + sex + song type	11	1328.3	0.00	0.44
breeding phenology + decimal hour + sex	10	1329.2	0.91	0.28
phrase duration				
breeding phenology + sex	9	3862.2	0.00	0.313
breeding phenology + sex + song type	10	3863.5	1.27	0.17
breeding phenology + sex + decimal hour	10	3863.9	1.71	0.13
duet duration				
breeding phenology + decimal hour	9	1746.1	0.00	0.26
breeding phenology + song type + decimal hour	10	1747.0	0.90	0.16

Models about individual-level singing behavior also include sex as a fixed effect. All models contained the identity of the social group as a nested random term. We show results from top models (ΔAICc<2) derived from modelling comparison among all combinations of predicted variables included in the global model (command ‘dredge’ in ‘multcomp’ package). df = degrees of freedom; wi = weight.

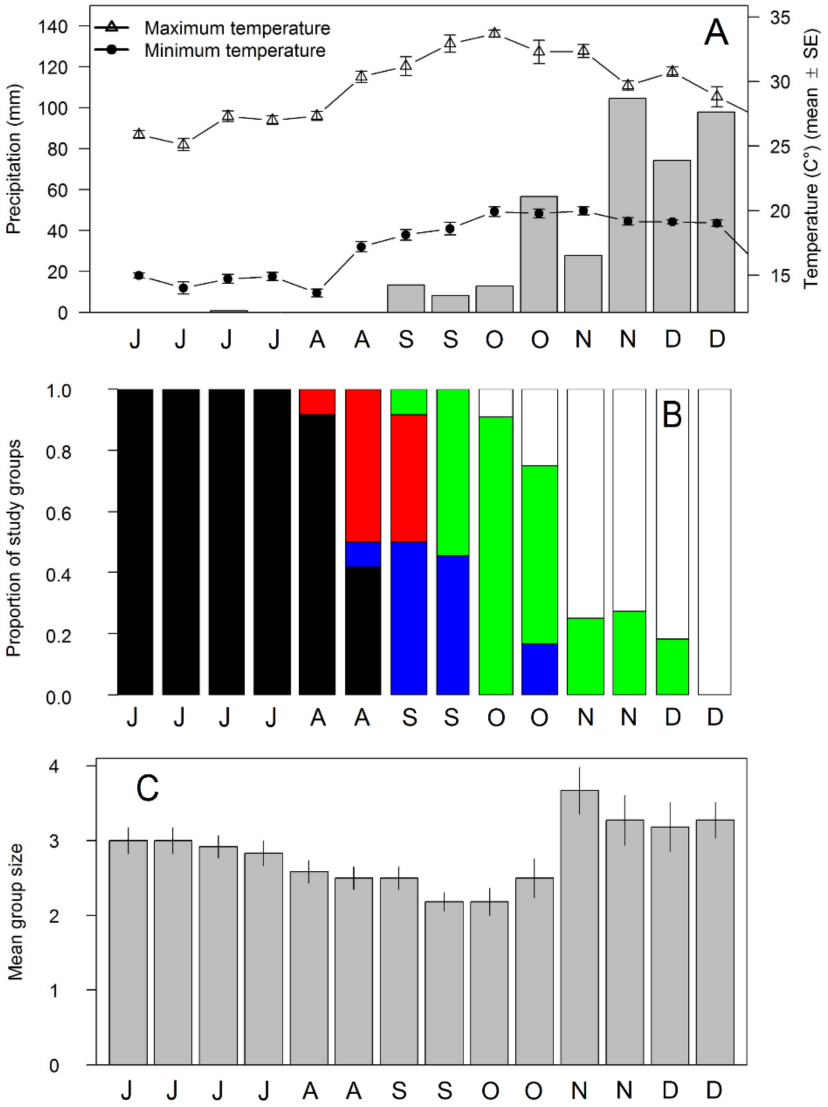


Figure 1-5s. Climate conditions in the study area (A), breeding phenology (B) and size of studied groups (C) during the study period. We show data for half-month periods (equivalent to one focal session), from June to December, on the X-axis. A: cumulative precipitation shown in bars, and temperature in lines. B: Breeding stages: non-breeding (black), pre-breeding (red), female fertile (blue), nesting (green) and post-breeding (white). C: Mean group size (± s.e.). Panels B and C, sample size: 12 groups for the seven first focal sessions and 11 groups for the remaining focal sessions.

Results from model and post-hoc comparisons

We used mixed models to model the variation in territorial aggressive interactions (number and duration) and several song variables relative to variation in breeding phenology and sex. We also modelled song variables as a function of aggressive interactions. We compared the models using AICc criteria. All models contained the identity of the social group as a random term. All models of latency to answer partner's initiated songs, duration of aggressive interactions, duet phrases and songs include the identity of focal session as a random term as well. We show results from top (ΔAICc<2), full and null models, and all the models with weight values higher than 0.01. Results derived from modelling comparison among all combinations of predicted variables are included in the global model (command 'dredge' in 'multcomp' package).

Here, we show the comparison among all models and β estimates of continuous variables obtained from post-hoc comparisons between levels of the predicted variables retained in top models (ΔAICc<2). P values in bold indicate significant results (p < 0.05) after controlling for false discovery rates (Benjamini and Hochberg 1995). P values in bold and italic indicate nearly significant results (p < 0.10).

Table 1-4s. Modelling comparison for variation in the number of aggressive, territorial interactions between focal adults and extra-group individuals (GLMM, poisson family). df = degrees of freedom; wi = weight.

	df	AICc	AICc	wi
sex + decimal hour	4	691.6	0.00	0.585
sex + group composition + decimal hour	5	693.5	1.9	0.227
breeding phenology + sex + decimal hour	8	694.7	3.1	0.124
breeding phenology + sex + group composition + decimal hour	9	696.8	5.21	0.043
breeding phenology + decimal hour + sex + group composition + breeding phenology × sex (full model)	13	703.9	12.37	0.000
null model	2	713.8	22.19	0.000

Table 1-5s. Post-hoc results from the best model to explain variation in the number of aggressive interactions (GLMM, poisson family).

	β ± SE	*z* ratio	p
intercept	–0.65 ± 0.15	–4.32	**<0.001**
sex (male – female)	0.44 ± 0.13	3.29	**0.001**
decimal hour	–0.28 ± 0.07	–3.95	**<0.001**

Table 1-6s. Modelling comparison for variation in the duration of aggressive, territorial interactions between focal adults and extra-group individuals (LMM). df = degrees of freedom. wi = weight.

	df	AICc	AICc	wi
null model	4	551.7	0.00	0.319
decimal hour	5	552.9	1.28	0.168
group composition	5	553.3	1.67	0.138
sex	5	553.8	2.11	0.111
group composition + decimal hour	6	554.4	2.75	0.080
sex + decimal hour	6	555.1	3.41	0.058
sex + group composition	6	555.5	3.81	0.047
sex + group composition + decimal hour	7	556.6	4.92	0.027
breeding phenology + decimal hour	9	558.1	6.42	0.013
breeding phenology	8	558.1	6.42	0.013
breeding phenology + sex + group composition + decimal hour + breeding phenology × sex (full model)	15	565.9	14.21	0.000

Table 1-7s. Modelling comparison for variation in the number of initiated songs (solo songs plus initiated duets) (GLMM, poisson family). df = degrees of freedom. wi = weight.

	df	AICc	AICc	wi
aggressive context + breeding phenology + sex + decimal hour + breeding phenology × sex	14	1303.4	0.00	0.474
aggressive context + breeding phenology + sex + group composition + decimal hour + breeding phenology × sex	15	1304.9	1.50	0.224
aggressive context + breeding phenology + sex + decimal hour + aggressive context × sex + breeding phenology × sex	16	1305.2	1.77	0.196
aggressive context + breeding phenology + sex + group composition + decimal hour + aggressive context × sex + breeding phenology × sex (full model)	17	1306.8	3.34	0.089
breeding phenology + sex + decimal hour + breeding phenology × sex	12	1310.9	7.49	0.011
null model	2	1523.8	220.33	0.000

Table 1-8s. Post-hoc results from the best model to explain variation in the number of initiated songs (GLMM, poisson family).

	β ± SE	*z* ratio	p
intercept	0.56 ± 0.22	2.52	**0.012**
sex (male – female)	0.90 ± 0.10	–9.35	**<0.001**
aggressive context (aggressive – non-aggressive)	0.25 ± 0.08	–3.24	**0.004**
decimal hour	–0.21 ± 0.04	–4.85	**<0.001**
female			
non-breeding – pre-breeding	0.29 ± 0.25	1.16	0.308
non-breeding – fertile	0.60 ± 0.25	2.35	**0.038**
non-breeding – nesting	1.09 ± 0.23	4.84	**<0.001**
non-breeding – post-breeding	–0.08 ± 0.14	–0.59	0.558
pre-breeding – fertile	0.30 ± 0.33	0.91	0.403
pre-breeding – nesting	0.80 ± 0.31	2.54	**0.028**
pre-breeding – post-breeding	–0.38 ± 0.26	–1.45	0.210
fertile – nesting	0.49 ± 0.31	1.57	0.193
fertile – post-breeding	–0.68 ± 0.26	–2.65	**0.027**
nesting – post-breeding	–1.17 ± 0.23	–5.12	**<0.001**
male			
non-breeding – pre-breeding	–0.37 ± 0.16	–2.36	***0.063***
non-breeding – fertile	–0.01 ± 0.16	–0.08	0.997
non-breeding – nesting	–0.09 ± 0.12	–0.76	0.640
non-breeding – post-breeding	–0.37 ± 0.11	–3.32	**0.009**
pre-breeding – fertile	0.36 ± 0.20	1.80	0.144
pre-breeding – nesting	0.28 ± 0.17	1.63	0.172
pre-breeding – post-breeding	0.00 ± 0.16	0.004	0.997
fertile – nesting	–0.08 ± 0.17	–0.45	0.794
fertile – post-breeding	–0.36 ± 0.16	–2.24	***0.063***
nesting – post-breeding	–0.28 ± 0.12	–2.30	***0.063***

Table 1-9s. Modelling comparison for variation in the song answering rates (percentage of partners songs answered by the focal individual) (GLMM, binomial family). df = degrees of freedom. wi = weight.

	df	AICc	AICc	wi
breeding phenology + sex + group composition + decimal hour	9	603	0	0.516
aggressive context + breeding phenology + sex + group composition + decimal hour	11	605.4	2.4	0.155
breeding phenology + sex + decimal hour	8	606.6	3.54	0.088
aggressive context + breeding phenology + sex + group composition + decimal hour + aggressive context × sex	13	607.4	4.38	0.058
breeding phenology + sex + group composition + decimal hour + breeding phenology × sex	13	607.5	4.47	0.055
aggressive context + breeding phenology + sex + decimal hour	10	608.5	5.43	0.034
aggressive context + breeding phenology + sex + group composition + decimal hour + breeding phenology × sex	15	610.1	7.06	0.015
aggressive context + breeding phenology + sex + decimal hour + aggressive context × sex	12	610.3	7.26	0.014
breeding phenology + sex + decimal hour + breeding phenology × sex	12	610.8	7.81	0.010
aggressive context + breeding phenology + sex + group composition + decimal hour + aggressive context × sex + breeding phenology × sex (full model)	17	611.4	8.34	0.008
null model	2	643.3	40.32	0.000

Table 1-10s. Post-hoc results from the best model to explain variation in song answering rates (GLMM, binomial family).

	β ± SE	*z* ratio	p
intercept	0.59 ± 0.25	2.42	**0.016**
sex (male – female)	0.56 ± 0.16	–3.42	**0.0006**
group composition (adults – adults and juveniles)	0.50 ± 0.21	2.36	**0.018**
decimal hour	–0.29 ± 0.09	–3.15	**0.002**
non-breeding – pre-breeding	–0.96 ± 0.33	–2.92	**0.01**
non-breeding – fertile	–0.66 ± 0.35	–1.88	0.10
non-breeding – nesting	0.14 ± 0.28	0.52	0.67
non-breeding – post-breeding	0.20 ± 0.20	1.03	0.43
pre-breeding – fertile	0.30 ± 0.43	0.68	0.62
pre-breeding – nesting	1.10 ± 0.37	2.96	**0.01**
pre-breeding – post-breeding	1.16 ± 0.34	3.40	**0.01**
fertile – nesting	0.81 ± 0.35	2.29	**0.04**
fertile – post-breeding	0.86 ± 0.36	2.41	**0.04**
nesting – post-breeding	0.06 ± 0.29	0.20	0.84

Table 1-11s. Modelling comparison for variation in song output (time spent singing, log + 1) (LMM). df = degrees of freedom. wi = weight.

	df	AICc	AICc	wi
aggressive context + breeding phenology + sex + decimal hour	11	873.7	0.00	0.613
aggressive context + breeding phenology + sex + group composition + decimal hour	12	875.8	2.11	0.214
aggressive context + breeding phenology + sex + decimal hour + aggressive context × sex	13	877.1	3.38	0.113
aggressive context + breeding phenology + sex + group composition + decimal hour + aggressive context × sex	14	879.2	5.51	0.039
aggressive context + breeding phenology + sex + group composition + decimal hour + aggressive context × sex + breeding phenology × sex (full model)	18	887.8	14.08	0.001
null model	3	950.9	77.23	0.000

Table 1-12s. Post-hoc results from the best model to explain variation in song output (time spent singing, log + 1) (LMM).

	β ± SE	*t* value	p
intercept	4.43 ± 0.51	8.75	
sex (male – female)	0.40 ± 0.10	3.87	**0.0001**
aggressive context (aggressive – non-aggressive)	0.37 ± 0.11	3.31	**0.003**
decimal hour	–0.24 ± 0.05	–4.67	
non-breeding – pre-breeding	–0.50 ± 0.20	–2.42	0.11
non-breeding – fertile	–0.07 ± 0.20	–0.35	1.00
non-breeding – nesting	0.32 ± 0.15	2.24	0.16
non-breeding – post-breeding	–0.32 ± 0.14	–2.19	0.18
pre-breeding – fertile	0.43 ± 0.25	1.67	0.44
pre-breeding – nesting	0.82 ± 0.22	3.68	**0.002**
pre-breeding – post-breeding	0.18 ± 0.22	0.82	0.92
fertile – nesting	0.39 ± 0.21	1.88	0.32
fertile – post-breeding	–0.25 ± 0.20	–1.21	0.74
nesting – post-breeding	–0.64 ± 0.16	–4.07	**<0.001**

Table 1-13s. Modelling comparison for variation in duetting rate (number of duets between partners) (GLMM, poisson family). df = degrees of freedom. wi = weight.

	df	AICc	AICc	wi
aggressive context + breeding phenology + group composition + decimal hour (full model)	11	640.7	0.00	0.513
breeding phenology + group composition + decimal hour	9	641	0.37	0.426
aggressive context + breeding phenology + decimal hour	10	646.5	5.81	0.028
breeding phenology + decimal hour	8	646.6	5.92	0.027
null model	3	697.1	56.44	0.000

Table 1-14s. Post-hoc results from the best model to explain variation in duetting rate (GLMM, poisson family).

	β ± SE	*z* ratio	p
intercept	1.15 ± 0.27	4.19	**<0.001**
aggressive context (aggressive – non-aggressive)	0.21 ± 0.10	2.06	**0.04**
group composition (adults – adults and juveniles)	0.34 ± 0.12	2.87	**0.004**
decimal hour	–0.32 ± 0.06	–5.36	**<0.001**
non-breeding – pre-breeding	–0.42 ± 0.16	-2.63	**0.029**
non-breeding – fertile	0.02 ± 0.18	0.12	0.90
non-breeding – nesting	0.35 ± 0.16	2.20	***0.055***
non-breeding – post-breeding	–0.19 ± 0.12	-1.50	0.19
pre-breeding – fertile	0.44 ± 0.20	2.22	***0.055***
pre-breeding – nesting	0.78 ± 0.19	4.15	**0.0003**
pre-breeding – post-breeding	0.23 ± 0.17	1.37	0.21
fertile – nesting	0.33 ± 0.18	1.86	0.10
fertile – post-breeding	–0.21 ± 0.18	-1.17	0.27
nesting – post-breeding	–0.54 ± 0.17	-3.24	**0.006**

Table 1-15s. Modelling comparison for variation in latency to answer partner's initiated songs (LMM). df = degrees of freedom. wi = weight.

	df	AICc	ΔAICc	wi
breeding phenology + sex + song type + decimal hour +	11	1328.3	0	0.239
breeding phenology + sex + decimal hour	10	1329.2	0.91	0.151
breeding phenology + sex + group composition + song type + decimal hour	12	1329.3	1.02	0.143
breeding phenology + sex + group composition + decimal hour	11	1329.5	1.22	0.130
aggressive context + breeding phenology + sex + song type + decimal hour	13	1332.2	3.92	0.034
sex + group composition + song type + decimal hour	8	1332.4	4.11	0.031
breeding phenology + sex + song type	10	1333.1	4.80	0.022
aggressive context + breeding phenology + sex + decimal hour	12	1333.1	4.83	0.021
aggressive context + breeding phenology + sex + group composition + song type + decimal hour	14	1333.3	5.02	0.019
sex + group composition + decimal hour	7	1333.4	5.08	0.019
aggressive context + breeding phenology + sex + song type + decimal hour + aggressive context × sex	15	1333.4	5.15	0.018
aggressive context + breeding phenology + sex + group composition + decimal hour	13	1333.5	5.22	0.018
breeding phenology + sex	9	1333.9	5.61	0.014
breeding phenology + sex + group composition + song type	11	1334	5.74	0.014
breeding phenology + sex + group composition	10	1334.1	5.86	0.013
aggressive context + breeding phenology + sex + group composition + song type + decimal hour + aggressive context × sex	16	1334.4	6.11	0.011
breeding phenology + sex + song type + decimal hour + breeding phenology × sex	15	1334.5	6.19	0.011

Table 1-15s

aggressive context + breeding phenology + sex + decimal hour + aggressive context × sex	14	1334.6	6.34	0.010
sex + group composition + song type	7	1334.7	6.41	0.010
aggressive context + breeding phenology + sex + group composition + song type + decimal hour + aggressive context × sex + breeding phenology × sex (full model)	20	1341.3	13.07	0.000
null model	4	1364.0	35.75	0.000

Table 1-16s. Post-hoc results from the best model to explain variation in latency to answer partner's initiated songs (LMM).

	β ± SE	*t* value	p
sex (male – female)	–0.56 ± 0.11	5.21	**<0.001**
decimal hour	0.16 ± 0.06	2.66	
song type (duet – chorus)	0.27 ± 0.15	1.78	***0.077***
non-breeding – pre-breeding	0.25 ± 0.19	1.31	0.678
non-breeding – fertile	0.48 ± 0.20	2.34	0.134
non-breeding – nesting	0.27 ± 0.17	1.58	0.505
non-breeding – post-breeding	-0.28 ± 0.14	-1.94	0.294
pre-breeding – fertile	0.23 ± 0.24	0.95	0.874
pre-breeding – nesting	0.02 ± 0.21	0.08	1.000
pre-breeding – post-breeding	-0.53 ± 0.20	-2.68	***0.061***
fertile – nesting	-0.21 ± 0.21	-1.00	0.850
fertile – post-breeding	-0.76 ± 0.20	-3.82	**0.002**
nesting – post-breeding	-0.54 ± 0.17	-3.27	**0.012**

Table 1-17s. Modelling comparison for variation in phrase duration in duets (LMM). df = degrees of freedom. wi = weight.

	df	AICc	ΔAICc	wi
breeding phenology + sex	9	3862.2	0.00	0.217
breeding phenology + sex + song type	10	3863.5	1.27	0.115
breeding phenology + sex + group composition	10	3863.8	1.58	0.098
breeding phenology + sex	10	3863.9	1.71	0.092
breeding phenology + sex + song type	11	3865.2	3.00	0.049
breeding phenology + sex + group composition + song type	11	3865.2	3.02	0.048
breeding phenology + sex + group composition	11	3865.5	3.26	0.042
aggressive context + breeding phenology + sex	11	3865.7	3.49	0.038
aggressive context + breeding phenology + sex + aggressive context × sex	13	3865.7	3.54	0.037
breeding phenology + sex + group composition + song type	12	3866.9	4.72	0.020
aggressive context + breeding phenology + sex + song type	12	3867	4.77	0.020
aggressive context + breeding phenology + sex + song type + aggressive context × sex	14	3867.1	4.88	0.019
breeding phenology + sex + breeding phenology × sex	13	3867.1	4.90	0.019
aggressive context + breeding phenology + sex + aggressive context × sex	14	3867.2	5.02	0.018
aggressive context + breeding phenology + sex	12	3867.2	5.03	0.018
aggressive context + breeding phenology + sex + group composition	12	3867.3	5.07	0.017
aggressive context + breeding phenology + sex + group composition + aggressive context × sex	14	3867.4	5.20	0.016
breeding phenology + sex + song type + breeding phenology × sex	14	3868.4	6.19	0.010

Table 1-18s. Post-hoc results from the best model to explain variation in phrase duration in duets (LMM).

	β ± SE	*t* value	p
sex (male – female)	0.95 ± 0.10	-9.34	**<0.001**
non-breeding – pre-breeding	-1.07 ± 0.26	-4.14	**<0.001**
non-breeding – fertile	-1.18 ± 0.26	-4.47	**<0.001**
non-breeding – nesting	-0.54 ± 0.21	-2.54	***0.083***
non-breeding – post-breeding	-0.32 ± 0.18	-1.79	0.371
pre-breeding – fertile	-0.11 ± 0.33	-0.32	0.998
pre-breeding – nesting	0.54 ± 0.29	1.88	0.326
pre-breeding – post-breeding	0.75 ± 0.26	2.88	**0.035**
fertile – nesting	0.64 ± 0.27	2.33	0.135
fertile – post-breeding	0.86 ± 0.26	3.25	**0.012**
nesting – post-breeding	0.22 ± 0.21	1.04	0.831

Table 1-19s. Modelling comparison for variation in duet duration (LMM). df = degrees of freedom. wi = weight.

	df	AICc	AICc	wi
breeding phenology + decimal hour	9	1746.1	0.00	0.256
breeding phenology + song type + decimal hour	10	1747	0.90	0.163
breeding phenology + group composition + decimal hour	10	1748.2	2.07	0.091
breeding phenology	8	1748.5	2.33	0.080
breeding phenology + group composition + song type + decimal hour	11	1749.1	3.00	0.057
breeding phenology + song type	9	1749.3	3.17	0.052
aggressive context + breeding phenology + decimal hour	11	1749.6	3.48	0.045
	4	1750.3	4.14	0.032
breeding phenology + group composition	9	1750.5	4.35	0.029
aggressive context + breeding phenology + song type + decimal hour	12	1750.5	4.41	0.028
decimal hour + decimal hour	5	1751.2	5.06	0.020
breeding phenology + group composition + song type	10	1751.4	5.26	0.018
aggressive context + breeding phenology + group composition + decimal hour	12	1751.7	5.57	0.016
song type	5	1751.7	5.58	0.016
group composition	5	1752.2	6.04	0.012
aggressive context + breeding phenology	10	1752.2	6.11	0.012
song type + decimal hour	6	1752.6	6.49	0.010
aggressive context + breeding phenology + group composition + song type + decimal hour	13	1752.6	6.53	0.010

Table 1-20s. Post-hoc results from the best model to explain variation in duet duration (LMM).

	β ± SE	*t* value	p
decimal hour	0.21 ± 0.10	2.13	
non-breeding – pre-breeding	-0.87 ± 0.31	0.31	**0.04**
non-breeding – fertile	-0.91 ± 0.33	0.33	***0.051***
non-breeding – nesting	-0.50 ± 0.26	0.26	0.33
non-breeding – post-breeding	-0.65 ± 0.23	0.23	0.049
pre-breeding – fertile	-0.03 ± 0.40	0.40	1.00
pre-breeding – nesting	0.38 ± 0.34	0.34	0.80
pre-breeding – post-breeding	0.22 ± 0.32	0.32	0.96
fertile – nesting	0.41 ± 0.34	0.34	0.73
fertile – post-breeding	0.26 ± 0.32	0.32	0.92
nesting – post-breeding	-0.15 ± 0.26	0.26	0.98

CAPÍTULO 2

Aggressive responses to playback of solos and duets vary with the presence of juveniles in a Neotropical ovenbird[3]

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[3] Uma versão deste capítulo será submetida para publicação em *Behavioral Ecology* como "Diniz P, Rech GS, Ribeiro PH, Webster MS & Macedo RH. Aggressive responses to playback of solos and duets vary with the presence of juveniles in a Neotropical ovenbird."

ABSTRACT

Duets in breeding pairs may occur because of conflict, as when an individual answers its partner's song to mate guard acoustically, or because of cooperation, as when the individuals share territory defense. The presence of juveniles, however, may affect the relative cost for territorial adults of either losing a partner or losing a territory, thus affecting how duetting functions. We studied the rufous hornero (*Furnarius rufus*), a socially monogamous, sedentary species with delayed juvenile dispersal. We exposed social pairs with juveniles (groups) and without juveniles (pairs) during the non-breeding season to playbacks of duets, male solos, female solos, and control heterospecific songs. Overall, partners were equivalent and coordinated in their aggressive responses to all conspecific stimuli, especially duets, indicating that the sexes cooperate to defend common territories when duetting. However, birds in pairs responded more strongly to conspecific solos than they did to duets, whereas birds in groups responded more strongly to duets than to solos, particularly male solos. In addition, birds in groups responded more strongly to duets than did birds in pairs. Our results suggest that territory defense is the primary function of duetting for the rufous hornero, but also that duetting seems to work as a mutual mate guarding strategy for birds living in pairs. Our study reveals that aggressive response to solos and duets can indeed vary with group structure and this should be considered in future studies.

Keywords: duetting, female song, delayed juvenile dispersal, joint territory defense, mutual-mate guarding, territoriality, suboscine, *Furnarius rufus*.

INTRODUCTION

Duets are coordinated vocal displays normally performed by breeding partners (Farabaugh 1983). Duetting behaviour has been intensively studied in the last two decades, but there is still no consensus regarding the adaptive function(s) of this singing behaviour (reviews in Hall 2004, 2009). Duetting may be driven by sexual cooperation (Hall, 2009; Logue, 2005), conflict (Tobias & Seddon, 2009) or both (Grafe & Bitz, 2004), and can mediate communication between partners (Logue, 2007) or be targeted at an external audience (neighbors, strangers) (Hall, 2004). Although several non-mutually exclusive hypotheses have been proposed to explain the function of duets (Hall, 2004), two have received the most attention (Dahlin & Benedict, 2013; Hall, 2009): the joint territory defense (Robinson, 1949) and acoustic mate guarding hypotheses (Rogers, Langmore, & Mulder, 2006; Seddon & Tobias, 2006).

The territory defense hypothesis proposes that partners duet cooperatively to establish, maintain or defend common resources or territories (Bradley & Mennill, 2009; Seddon & Tobias, 2003). In this case, duets represent a stronger territorial signal than do solo songs, for example due to a numeric advantage or a quality signal arising from song synchronization (Hall & Magrath, 2007; Kovach, Hall, Vehrencamp, & Mennill, 2014). The mate guarding hypotheses, on the other hand, suggest that duets may arise from conflict between the mated partners, as when an individual answers its partner´s song in an attempt to acoustically mate guard, for example by intimidating rivals or discouraging the partner from pursuing extra-pair mates (Rogers et al. 2006, Seddon and Tobias 2006, Tobias and Seddon 2009). Alternatively, mate guarding can also occur if divorce is costly for both partners (Choudhury, 1995), and they have a common interest in maintaining the pair bond (Griggio & Hoi, 2011; van den Heuvel, Cherry, & Klump, 2014). In this case, duets can be used to safeguard the pairbond itself, as suggested by the mutual mate guarding hypothesis (Sonnenschein and Reyer 1983; Grafe and Bitz 2004; Hall 2009; van den Heuvel et al. 2014).

Researchers have tested these hypotheses through playback experiments, comparing individual aggressive responses towards simulated individual (solos) versus pair intruders (duets) (Douglas & Mennill, 2010). If duet functions in defense of a joint territory, one would expect a stronger and more highly coordinated response to playbacks of duets than to playbacks of solos (Douglas & Mennill, 2010), or at the very least, an equivalently aggressive response to playbacks of duets and solos (Benedict, 2010). Territory defense may be sex-specific (Hall, 2009), when opposite-sex intrusions are less threatening than same-sex and pair simulated intrusions, and duetting facilitates partner division of labor in territory defense (Christopher N. Templeton, Rivera-Cáceres, Mann, & Slater, 2011). In contrast, if duet functions in guarding a mate, one would predict a stronger albeit poorly coordinated response toward same-sex solos and a weaker response to opposite-sex solos (Rogers et al., 2006; Seddon & Tobias, 2006). Finally, if duet functions in mutual mate guarding, a stronger and highly coordinated response to solos versus duets would be expected, assuming that solos would be a greater threat to the partnership than duets (Templeton et al., 2011).

Comparative and empirical studies provide strong support for the joint territory defense hypothesis. However, recent studies indicate that duets may have multiple adaptive functions (Benedict, 2010; Dahlin & Benedict, 2013; Grafe & Bitz, 2004; Mennill & Vehrencamp, 2008), revealing that 65% of the studied species have multifunctional duets, of which more than 20% are both cooperative and conflict-based (Dahlin & Benedict, 2013). For instance, in red-backed fairy-wrens (*Malurus melanocephalus*), seasonal patterns of duetting and stronger responses to playbacks of duets than solos support the joint territory defense hypothesis (Dowling & Webster, 2013; Dowling & Webster, 2016), but unattractive males in this species also answer partner songs to acoustically guard paternity (Dowling & Webster, 2017). The possible multifunctional role of duetting demands investigation in multiple contexts to better understand duet function (Dahlin & Benedict, 2013).

Previous studies suggest that duet function can vary between aggressive and non-aggressive contexts in the same species (Benedict, 2010; Mennill & Vehrencamp, 2008). However, to date no study has assessed variation in duet function in an aggressive context but under varying social circumstances. We suggest that reproductive and social context may influence the relative cost of losing a partner versus losing a territory. For instance, mate guarding may be less necessary during the non-breeding season than during the pre-breeding or breeding seasons, whereas territory defense may be important year round or mainly in the pre-breeding season (Topp & Mennill, 2008). Thus, duet function may vary between breeding and non-breeding seasons (Topp & Mennill, 2008).

Similarly, one of the possible social contexts that may be relevant to duetting behavior is group structure. We hypothesized that the relative cost of losing a territory versus a partner should be higher for pairs with juveniles (hereafter “groups”) than for those without juveniles (hereafter “pairs”), because territory loss may compromise both juvenile and adult fitness. Therefore, we predict a stronger response to playbacks of duets than to solos for birds in groups compared with birds in pairs. We assume that simulated pair intrusions represent a bigger threat to territory tenure than do solo intrusions, whereas simulated solo intrusions are more threatening to the pair bond than pair intrusions. In addition, the presence of juveniles may reflect other mechanisms driving the relative cost of losing a territory versus a partner. For instance, in year-round territorial species, the presence or absence of juveniles may indicate variation in breeding failure among pairs, which in turn can affect sexual conflict, divorce intention (Culina, Radersma, & Sheldon, 2014; Ens, Safriel, & Harris, 1993) and possibly, duet function.

In birds, duetting is evolutionarily related to cooperative breeding (Tobias et al., 2016), and delayed juvenile dispersal is common in socially monogamous, duetting species (Gill & Stutchbury, 2010; Tarwater & Brawn, 2010), allowing us to study the effects of variation in group size and structure on duet function. We performed a single-speaker playback experiment focusing on the rufous hornero (*Furnarius rufus*), a socially monogamous, duetting Neotropical bird. We broadcasted four treatments (i.e., duet, female solo, male solo and a heterospecific control) to each social unit and scored behavioural and vocal responses, as well as the coordination between partners in playback response. We addressed two questions in this study: (1) Do the sexes respond differently to the simulated intrusion of solos (i.e., individual birds) versus duets (i.e., paired birds)? and (2) Does the relative threat of duets and solos to adults vary with group structure (pairs versus groups)? We investigated key predictions for duet function relative to the relevant hypotheses (Table 2-1).

METHODS

Study subjects and area

The rufous hornero (suboscine, Furnariidae) is a ground foraging species inhabiting disturbed open habitats across southern South America (Remsen & Bonan, 2016; Sick, 2001). They live in year-round territories and breed seasonally in domed nests (Fraga, 1980; Shibuya, Braga, & Roper, 2015). Both parents contribute equally to parental care (Massoni, Reboreda, López, & Aldatz, 2012). These birds often sing two sex-specific song types that can be coordinated in duets (Amador, Trevisan, & Mindlin, 2005; Laje & Mindlin, 2003; Roper, 2005) and a few variations of these song types (Figure 2-1; P Diniz, unpublished data). This species is a good model to test variation in playback response with group structure, because juveniles delay dispersal, staying in their parents' territories for four to nine months (Fraga, 1980). Juveniles rarely initiate a song but can join parent-initiated songs.

We studied 16 territorial social units (10 groups, 6 pairs) of the rufous hornero from an urban, partially banded population on the campus of the University of Brasilia, Brasilia, Brazil (15°45' S, 47° 51' O). We carried out a playback experiment in the field from January to April, 2014, which corresponds to the first half of the non-breeding season (Fraga, 1980; Shibuya et al., 2015). Studied adults were molecularly sexed (N = 30) or had their sexes assigned based on their partner´s sex (N = 2). Banding and trapping procedures are described in Diniz et al. (2016).

The 16 studied social units varied from two to five individuals when the experiment started (mean ± SD = 3.06 ± 1.06), and only four juveniles, from two groups, had been banded. We are confident that all unbanded juveniles hatched in the previous breeding season due to their distinctive juvenile morphology (black and short bill, slender body and light plumage coloration; Fraga 1980, Diniz pers. obs.). Social unit size remained stable during the experiment, except for three units, in which juveniles were absent during part of the playback trials, probably due to short-term movements across territories.

Playback stimuli

The rufous hornero emits only 5.38 ± 3.52 songs/h (mean ± SD, *N* = 161 trials; P Diniz, unpublished data). Thus, we used playbacks of only one stimulus per treatment per social group. We recorded non-playback induced songs of sexed adults from the studied population to make the conspecific playback stimuli. These recordings were made with a Marantz PMD660 recorder (settings: WAVE, 48kHz sampling rate, 24-bits accuracy) and a Sennheiser ME66 microphone up to six hours after sunrise, from August 2013 to January 2014. We recorded 59 solos from 23 adults and 15 social units, 137 duets from 18 social units and 34 choruses from 12 social units.

We selected 15 high-quality conspecific recordings (five for each treatment) to make the conspecific stimuli. Each conspecific playback stimulus selected for the experiment came from a social pair other than the focal individuals. We used songs from a syntopic and duetting species, the great kiskadee (*Pitangus sulphuratus*), as a heterospecific control in our playback experiment. Both species are suboscines, abundant (Jebai et al., 2009) and sedentary in our study area. We recorded four high quality great kiskadee songs (solos and duets) from birds of non-contiguous territories (>200m apart) in our study site, and used an additional recording made in a nearby area (27km from the study site, recording: Song Meter SM2, settings: WAVE format, sampling rate = 44.1kHz, 16-bits accuracy). Thus, we prevented a possible confounding effect of stranger sounds on playback response (Searcy, Nowicki, & Hughes, 1997).

We created each playback stimulus in three steps using Raven Pro 1.5 and Audacity: (i) filtering low-frequency (<500Hz), (ii) normalizing the maximum amplitude of each signal (-1.0 dB), and (iii) adding a silent period of 10s before and after each signal. We stored the stimuli in WAVE 16-bits accuracy. Mean signal duration was 5.55 ± 2.01s (SD) across playback stimulus (range = 2.20 – 9.55s). We repeated the same stimulus 2-4 times for each bird unit (mean ± SD = 3.2 ± 0.77 playback trials/stimuli), and analyzed data with mixed models (see Statistical analyses) to deal with pseudoreplication of playback stimuli (McGregor, 2000).

Playback experiment

We played back the four stimuli (heterospecific control, male solo, female solo and duet) to each studied social group in non-consecutive days (mean ± SD = 4.31 ± 3.36 day-intervals; *N* = 52 intervals) to prevent habituation (Harris & Haskell, 2013). All playback trials were carried out 1.89 ± 1.14 hours after sunrise (mean ± SD, *N* = 64 trials). We non-systematically chose the broadcast order of the four stimuli for each group, repeating only two out of 24 possible order combinations. The stimulus for each treatment broadcast at each trial was picked at random. We made sure each stimulus did not come from neighbors (<500m or <5 consecutive territories apart) to avoid neighbor-stranger effects on playback response (Radford, 2005; Wiley, 2013).

To broadcast each stimulus, we placed one speaker (TSI II 1210) on the nest substrate (i.e. tree or light pole), given that intruders may sing on the nest substrate of territorial birds in nature (Diniz, pers. obs.). Moreover, we wanted to make sure birds would hear the broadcast. Our rufous hornero population lives in a noisy environment, and partners defend small territories (~2ha) but seem to spend most of their time near the nest substrate (Diniz, pers. obs.).

We did not use stereo or dual duet playback (Douglas & Mennill, 2010), because males and females overlap phrases in duets in the same frequency range (Figure 2-1), thus we could not extract male and female song contributions (Hall & Peters, 2008). In addition, single and stereo speaker playback may elicit similar playback responses in the rufous horneros, because males and females normally coordinate songs when they are very close to each other (median distance = 0.76m, *N* = 22 social units and 138 duets plus choruses, P. Diniz, unpublished data).

We attached the speaker to a metal rod at an approximate height of 5m, which corresponds to the average height that rufous horneros sing in our population (P Diniz, unpublished data). We positioned the speaker parallel to and facing the ground (birds forage on the ground), and attached the metal rod to the nest substrate. Rufous horneros sing duets at approximately 92 dB maximum amplitude (estimated for 1 m distance from the bird) in the field (91.82 ± 2.63, *N* = 10 pairs; P Diniz, unpublished data) as measured by a sound level meter (model SEW 2310SL) at 20.99 ± 7.96 m from the birds (*N* = 20) (distance effects on amplitude corrected according to van den Heuvel, Cherry, & Klump, 2013). Therefore, we calibrated the speaker volume in silent conditions to broadcast the stimulus at 92 dB maximum sound level at 1 m from the speaker. Finally, we connected the speaker to a cellphone with a 30m cable, and triggered the stimulus with a WAVE player application (Rocket Player) when both focal adults were within 60m of the speaker.

After broadcasting each playback stimulus, two or more observers (mean ± SD = 2.72 ± 0.55, range = 2–4, *N* = 64 trials) recorded adult behaviour and their songs during 15 min (recording apparatus: Marantz PMD660 recorder, Sennheiser ME66 microphone). We were able to track the birds for 92 ± 18% of each focal period (mean ± SD; *N* = 128 trackings x birds). After finishing each trial, we used a measuring tape to estimate the spatial position, movement and specific behaviours of birds, which occurred in response to the playback.

Playback response

Rufous horneros normally respond to conspecific playbacks by approaching and perching high on the speaker substrate (tree or light pole) instead of approaching the speaker itself or branches close to it, which is a typical response for many birds (Hall 2000, Rogers et al. 2006, Dahlin and Wright 2012, Funghi et al. 2015). Rufous horneros then usually sing once and then do not sing again for 5.63 ± 3.81 min (mean ± SD, *N* = 110) after the playback. Therefore, we chose playback response variables based on the unusual playback response of this species. We only estimated playback response from adult birds.

Regarding bird movement responses, we measured: (1) the closest horizontal distance of the bird to the speaker after its first movement toward the playback; (2) the horizontal distance travelled by the bird during the first approach to the speaker; (3) the height of each bird after the first approach to the speaker; and (4) the pre-playback height of each bird. We measured (1), (2), (3), and (4) for, respectively, 98%, 99%, 89% and 95% of occurrences (*N* = 96 birds x trials). We combined these variables and used the Pythagorean theorem to estimate the real distance travelled by each bird during the first approach to the speaker, and the real post-playback distance between each bird and the speaker ("closest approach", hereafter). Distance travelled did not vary among playback treatments (ANOVA, females, $F_{2,35}$ = 1.14, P = 0.33, males, $F_{2,39}$ = 0.70, P = 0.50).

Time budgets were mainly composed of territorial vigilance and foraging. We considered as ´territorial vigilance´ the behaviours where the bird was perched, scanning or singing, relatively immobile or moving among perches in the same substrate (see Tobias and Seddon 2000). Birds often sang at the beginning of a territorial vigilance bout (Diniz, pers. obs.). We considered as ´foraging´ the behaviours where the bird was on the ground searching for or capturing prey, even when alternating these activities with short vigilant bouts. We combined territorial vigilance, foraging and spatial behaviour to estimate behavioural response variables (Table 2-2).

We selected and quantified songs emitted by each bird and assigned the singing role (initiator or responder) for each song. We did not quantify song answering rate (Hall & Peters, 2008; Logue, 2005; Logue & Krupp, 2016; van den Heuvel et al., 2013) since the birds emitted only 1.89 ± 1.10 songs/trial (mean ± SD, N = 122 songs, 61 trials). We classified as song initiator the bird that started a song relative to its partner (Hall & Peters, 2008), regardless of whether it was answered (i.e. duets or chorus) or not (i.e. solos) by its partner. The song responder was the bird that sang after its partner had sung, thus creating a duet or chorus (Logue & Krupp, 2016).

We analyzed vocal behaviour data using Raven Pro 1.5 and acoustic measurements with R (warbleR package, Araya-Salas and Smith-Vidaurre 2016). All acoustic measurements were taken from filtered recordings (<500 kHz deleted; spectrogram window length = 1024, amplitude threshold = 15%). We measured song duration for all songs at the individual level: solos and each contribution to a duet or chorus. We took frequency measurements only for duets between partners, because few playback responses were solos, or chorus and duets involving a juvenile. We measured mean and median frequencies, first and third quantiles, interquantile range, spectral entropy and frequency centroid (see Araya-Salas and Smith-Vidaurre 2016 for details). We combined these frequency measurements with a principal component analysis (PCA, Quinn and Keough 2002) and extracted scores for the first two principal components

(Table 2-3s). Finally, we measured the latency to answer partner-initiated song and degree of song overlap (Table 2-2).

Statistical analyses

We analyzed the effects of playback treatments on approach response of each sex with Fisher's exact test (pooled data from different individuals) using Past 3.14. We analyzed the remaining playback response data with linear mixed models (LMM) or generalized linear mixed models (GLMM) in R (Table 2-4s). We did not use principal component analyses (PCA) to reduce the number of response variables (McGregor, 1992) for two reasons: our response variables differ in sample size, and we would lose power by combining variables; and not all of our response variables are normally distributed, making them inappropriate for PCAs (Quinn and Keough 2002).

We included main effects and interaction playback treatment versus social unit type (pairs vs groups) in all models, and main effects and interaction playback treatment versus sex in all models at the individual level. We included the order of the playback stimulus (e.g. third) and the time that the trial began (measured in hours after sunrise) as covariates in all models. All continuous variables were scaled to obtain comparable β estimates (Zuur, Hilbe, & Ieno, 2013). We added random factors, such as stimulus identity, group and individual identities, and additional predictors to model specific response variables (Table 2-4s).

We also analyzed how the coordination between partners in responses to playback (closest approach, territorial vigilance, song rate and phrase duration) was influenced by playback treatments. We used the same modelling approach described above, but with female playback response as the response variable, and the interaction between playback treatment and the correspondent male playback response as fixed effects. The structure of random terms was changed accordingly.

We applied backward stepwise model selection to choose the top-fitted model. We verified the significance of predictors with likelihood-ratio tests (LRT), keeping the random terms in all models (Zuur, Ieno, Walker, Saveliev, & Smith, 2009). Once we found a significant result in the top-fitted model, we applied post-hoc tests using the packages "lsmeans" and "multcomp" (Hothorn, Bretz, & Westfall, 2008; Lenth, 2015).

The number of response variables in each dataset was used to adjust p-values given by LRT relative to variables of interest retained in the top models. We used false discovery rate as the p-value adjusting method for multiple post-hoc tests and LRTs (Benjamini & Hochberg, 1995).

To model time spent in territorial vigilance, we did not consider the playback trials where the bird was absent for more than 50% of the time (N = 6 out of 128 cases, 4.69%). The proportion of time spent foraging was not included in the analyses due to a high correlation with the proportion of time spent in territorial vigilance (r_P = –0.96, $P < 0.0001$, N = 120 trials). To model latency to sing, we did not correct for the distance between the bird's positions before and after the playback, because there was no relationship between these variables in a pre-modelling scenario ($\chi^2 = 0.86$, $P = 0.35$). Outliers were identified by boxplot inspections (Zuur et al., 2009) and removed before analyzing variation in song duration (N = 2) and in the correlation analysis in song duration between partners (N = 1).

Ethical note

This study was approved by the Brazilian environmental agencies 'Instituto Chico Mendes de Conservação da Biodiversidade – ICMBio' (ICMBio, licence number 40806–1) and 'Centro Nacional de Pesquisa para Conservação das Aves Silvestres – CEMAVE' (licence number 3886). Banding and trapping procedures were conducted as quickly as possible, no bird abandoned its nest or territory after banding procedures, and normally resumed foraging or incubation activities within 10 min. We played back only three conspecific song stimuli to each study social unit, each song broadcasted lasted less than 10s and song stimuli were broadcast in non-consecutive days. Bird often returned to normal activities (foraging, nest building) within 15 min, and no bird abandoned its territory after the experiment. Thus, we believe our playback design generated minimum disturbance to birds.

RESULTS

Behavioral responses

Both males and females approached the speaker in response to nearly all conspecific playbacks (females in 98%, and males in 96% of the 48 trials), and no bird approached the speaker after heterospecific controls (Fisher's exact test, both sexes, $p < 0.0001$). Closest approach did not vary between sexes (LMM: sex: $\chi^2 = 3.05$, df = 1, $P = 0.08$, $N = 48$ trials), but was affected by the interaction between playback treatment and social unit type (LMM: $\chi^2 = 11.02$, degrees of freedom [df] = 2, $P = 0.004$, $N = 48$ trials; Figure 2-2A). Adults in groups approached closer the speaker than did adults in pairs in response to playbacks of duets ($\beta \pm \mathrm{SE} = -0.94 \pm 0.39$, $t = 2.41$, $P = 0.020$), but not to conspecific solos (female solos: $\beta \pm \mathrm{SE} = 0.26 \pm 0.38$, $t = 0.69$, $P = 0.49$; male solos: $\beta \pm \mathrm{SE} = -0.18 \pm 0.37$, $t = 0.47$, $P = 0.64$). Adults in pairs approached the speaker more closely in response to both female ($\beta \pm \mathrm{SE} = -0.84 \pm 0.29$, $t = 2.93$, $P = 0.035$) and male solos ($\beta \pm \mathrm{SE} = -0.69 \pm 0.28$, $t = 2.42$, $P = 0.047$) in comparison with the duet playback (Figure 2-2A). In contrast, adults in groups approached the speaker more closely in response to playbacks of female solos ($\beta \pm \mathrm{SE} = -0.59 \pm 0.21$, $t = 2.76$, $P = 0.048$), and tended (though nonsigificantly) to approach more closely in response to playbacks of duets ($\beta \pm \mathrm{SE} = -0.43 \pm 0.21$, $t = 2.10$, $P = 0.083$; Figure 2-2A), than in response to playbacks of male solos.

Birds often sang after approaching the speaker (females in 90%, and males in 92% of 48 trials). Both sexes sang more often at the nest substrate in response to duet playbacks compared with the heterospecific control (GLMM: treatment: $\chi^2 = 18.34$, df = 3, $P < 0.001$, $N = 59$ trials; duet × heterospecific control: $\beta \pm \mathrm{SE} = 10.45 \pm 3.63$, $z = 2.88$, $P = 0.024$) and, less noticeably (tendencies), when compared with female (duet × female solo: $\beta \pm \mathrm{SE} = 6.84 \pm 2.95$, $z = 2.32$, $P = 0.06$) and male solos (duet × male solo: $\beta \pm \mathrm{SE} = 4.61 \pm 2.43$, $z = 1.90$, $P = 0.087$; Figure 2-2B). Adults also tended to sing more at the nest substrate in response to male solos than to the heterospecific control ($\beta \pm \mathrm{SE} = 5.83 \pm 2.81$, $z = 2.08$, $P = 0.076$; Figure 2-2B). Singing location did not vary with sex

(GLMM: $\chi^2 = 1.95$, $P = 0.16$, df = 1, $N = 59$ trials) or social unit type (GLMM: $\chi^2 = 1.10$, $P = 0.31$, df = 1, $N = 59$ trials).

The time spent in territorial vigilance by adult birds varied with treatment combined with social unit type (LMM: treatment × social unit type: $\chi^2 = 10.83$, df = 3, $P = 0.013$, $N = 63$ trials; Figure 2-2C). Adults in groups spent more time in vigilance than adults in pairs in response to duets ($\beta \pm SE = 0.50 \pm 0.20$, $t = 2.52$, $P = 0.014$), but not in response to other playback treatments (all $\beta < 0.24$, $t < 1.32$, $P > 0.19$). Adults in pairs tended to spend more time in vigilance in response to female solos compared with all the other playback treatments (control: $\beta \pm SE = 0.57 \pm 0.21$, $t = 2.74$, $P = 0.069$; duet: $\beta \pm SE = 0.44 \pm 0.22$, $t = 2.07$, $P = 0.099$; male solo: $\beta \pm SE = 0.50 \pm 0.21$, $t = 2.07$, $P = 0.099$; Figure 2-2C). In contrast, adults spent more time in vigilance in response to duets ($\beta \pm SE = 0.70 \pm 0.17$, $t = 4.01$, $P = 0.003$) and, less noticeably (tendency), to female solos ($\beta \pm SE = 0.41 \pm 0.18$, $t = 2.32$, $P = 0.059$) than in response to heterospecific playbacks. The latency to resume foraging after the playback was not influenced by sex (LMM: $\chi^2 = 0.45$, df = 1, $P = 0.50$, $N = 33$ trials), playback treatment (LMM: $\chi^2 = 0.26$, df = 2, $P = 0.88$, $N = 33$ trials) or social unit type (LMM: $\chi^2 = 1.00$, df = 1, $P = 0.32$, $N = 33$ trials).

Vocal responses

Latency to sing also was affected by playback treatment interacted with social unit type (LMM: playback treatment × social unit type: $\chi^2 = 12.32$, df = 3, $P = 0.006$, $N = 61$ trials; Figure 2-2D). As expected, birds in pairs and groups started to sing more quickly after conspecific than after heterospecific playbacks (all $\beta < -2.66$, $t > 10.84$, $P < 0.0001$), regardless of sex (LMM: playback treatment × sex: $\chi^2 = 0.23$, df = 3, $P = 0.97$, $N = 61$ trials). However, birds in pairs exhibited a longer latency to sing after the playback of duets ($\beta \pm SE = 1.08 \pm 0.40$, $t = 2.66$, $P = 0.011$) and female solo songs ($\beta \pm SE = 0.93 \pm 0.36$, $t = 2.55$, $P = 0.014$) compared with birds in groups. In addition, adults in pairs started to sing more quickly after the playback of male solos than after the playback of duets ($\beta \pm SE = -1.20 \pm 0.36$, $t = 3.31$, $P = 0.005$) and female solos ($\beta \pm SE = -1.08 \pm 0.33$, $t = 3.25$, $P = 0.005$). However, adults in groups started to sing quickly in response to all conspecific playbacks (β ± SE < 0.17, t < 0.64, P < 0.72; Figure 2-2D). The latency to sing did not vary between the sexes (LMM: χ^2 = 0.43, df = 1, P = 0.51, N = 61).

Partners coordinated most of their songs into duets (78%, N = 130 songs). Birds sang at a higher rate after conspecific than after heterospecific playbacks (GLMM: playback treatment: χ^2 = 13.74, df = 3, P = 0.0033, N = 64 trials; all β < –0.60, z < –2.82, P < 0.004; Figure 2-2E), regardless of sex (GLMM: playback treatment × sex: χ^2 = 0.86, df = 3, P = 0.84, N = 64 trials) or social unit type (GLMM: playback treatment × social unit type: χ^2 = 3.82, df = 3, P = 0.28, N = 64 trials). The singing role (song initiator versus song responder) differed between the sexes (GLMM: χ^2 = 7.35, df = 1, P = 0.0067, N = 61 trials), but was not influenced by playback treatment (GLMM: χ^2 = 0.50, df = 3, P = 0.92, N = 61 trials, 130 songs) or social unit type (GLMM: χ^2 = 0.03, df = 1 P = 0.87, N = 61 trials, 130 songs). The probability of initiating a song was higher for males than for females (β ± SE = 1.10 ± 0.39, z = 2.85, P = 0.004), such that most (62.75 %, N = 102 duets) duets were the result of females responding to male songs.

Latency to answer partner-initiated songs tended to be lower in response to conspecific songs than to heterospecific songs, though not significantly (LMM: playback treatment: χ^2 = 6.32, df = 3, P = 0.097, N = 62 trials, 101 songs). Males answered partner-initiated songs more quickly than did females (LMM: χ^2 = 5.35, df = 1, P = 0.02, N = 62 trials, 101 songs; β ± SE = 0.53 ± 0.23, t = 2.34, P = 0.034), regardless of playback treatment (LMM: playback treatment × sex: χ^2 = 3.79, df = 3, P = 0.29, N = 62 trials, 101 songs) and social unit type (LMM: social unit type: χ^2 = 1.91, df = 1, P = 0.28, N = 62 trials, 101 songs). Males also sang longer songs than did females (LMM: χ^2 = 6.29, df = 1, P = 0.01, N = 61 trials, 121 songs; β ± SE = 0.53 ± 0.21, t = 2.50, P = 0.025). Both sexes tended to emit longer songs in response to duets (β ± SE = 1.03 ± 0.41, t = 2.53, P = 0.15) and male solos (β ± SE = 0.92 ± 0.41, t = 2.24, P = 0.15) in comparison to the heterospecific control, though the difference was marginally nonsignificant (LMM: treatment: χ^2 = 7.76, df = 3, adjusted P = 0.051, N = 61 trials, 121 songs) (Figure 2-2F). Frequency measurements of duets produced by pairs, taken from spectrograms, were not affected by playback treatment (LMM, PC1, χ^2 = 0.64, df = 3, P = 0.89; PC2, χ^2 = 0.24, df = 3, P = 0.97, N = 50 trials, 73 duets) or social unit type (LMM, PC1, χ^2 = 0.09, df = 1, P = 0.76; PC2, χ^2 = 0.03, df = 1, P = 0.87, N = 50 trials, 73 duets).

Coordination between partners

The degree of overlap between male and female song contributions in duets or chorus did not vary with playback treatment (LMM: χ^2 = 3.74, df = 3, *P* = 0.29, *N* = 61 trials, 100 duets) or social unit type (LMM: χ^2 = 0.22, df = 1, *P* = 0.64, *N* = 61 trials, 100 duets). Female and male closest approaches to the speaker were positively correlated within pairs (r_{P} = 0.82, *P* < 0.0001, *N* = 41 trials), indicating that partners stay very close to each other after the playback. However, this relation tended to be somewhat stronger in response to duet playbacks (LMM: playback treatment × partner's closest approach: χ^2 = 5.17, df = 2, adjusted *P* = 0.075, *N* = 41 trials; r_{P} = 1.00, *P* < 0.0001 *N* = 12 trials) compared with female (r_{P} = 0.89, *P* < 0.0001, *N* = 14 trials) and male solos (r_{P} = 0.61, *P* = 0.01, *N* = 15 trials, Figure 2-3A). The time spent in territorial vigilance was positively correlated between partners (r_{P} = 0.79, *P* < 0.0001, *N* = 59 trials), but this correlation was not influenced by playback treatment (χ^2 = 5.42, df = 3, *P* = 0.14, *N* = 59 trials). The same pattern of positive correlation between partners was found for song rate (r_{P} = 0.72, *P* < 0.0001, *N* = 64 trials) regardless of playback treatment (GLMM: playback treatment × partner's song rate, χ^2 = 1.67, df = 3, *P* = 0.64, *N* = 64 trials). Finally, the correlation between partners in song duration peaked in response to female solos (r_{P} = 0.76, *P* < 0.0001, *N* = 29 duets) and duets (r_{P} = 0.56, *P* = 0.0009, *N* = 32 duets; χ^2 = 8.37, df = 3, adjusted *P* = 0.052), but did not occur in response to playbacks of male solos (r_{P} = 0.23, *P* = 0.27, *N* = 25 duets). Model selection steps in Table 2-5s, and detailed sample sizes in Table 2-8s.

DISCUSSION

Rufous hornero partners converge remarkably in their playback responses, so that both sexes typically approach the speaker and duet in response to the majority of conspecific playbacks. Playbacks of conspecific songs induced an equivalently aggressive response of territorial females and males in all nine individual-level categories of responses evaluated (Table 2-9s). The probability of initiating a song or answering a partner's song did not differ among playback treatments, but the promptness in song answering was high in response to conspecific songs. We also found a strong correlation between the sexes in several physical and vocal behavioural traits across both aggressive and non-aggressive (i.e. control playback) contexts, indicating higher inter- social unit variation in territorial and vocal behaviours. Taken together, these results suggest that: (i) duetting plays a role in the communication among social units, (ii) both individuals are committed to the pair unit (Templeton, Rios-Chelen, Quiros-Guerrero, Mann, & Slater, 2013; Wickler, 1980), (iii) solos and duets have similar functions in aggressive contexts (Langmore, 1998), and (iv) partners coordinate aggression directed toward intruders (Hall & Peters, 2008).

A high degree of convergence and coordination between the sexes in playback responses has been found for a few other species (Benedict, 2010; Dahlin & Wright, 2007; Hall & Peters, 2008). For example, in yellow-naped amazons (*Amazona auropalliata*), partners did not differ in the approach behaviour or vocal output (Dahlin & Wright, 2012). In purple-crowned fairy-wrens (*Malurus coronatus*), partners coordinate their approach to the speaker and their vocal output in response to playbacks of duets (Hall & Peters, 2008). However, the majority of studied duetting bird species show some sort of sex-specificity in playback responses (e.g. Levin, 1996; Rogers et al., 2006; Seddon & Tobias, 2006; van den Heuvel et al., 2014). Thus, convergence in response to playbacks is not the usual pattern and deserves further investigation.

We evaluated predictions for duetting functional hypotheses of territory defense, mate guarding and their variations (Table 2-1; Table 2-9s). In our study, birds responded most strongly to conspecific songs, especially duets. Specifically, both adults, in both pairs and groups, coordinated their approach to the speaker in response to duets, and sang at higher rates and more often at the nest substrate in response to all the conspecific songs. These results support the widespread hypothesis that duets are a form of cooperation to maintain resources in common territories (Table 2-9s) (Dahlin & Benedict, 2013; Dowling & Webster, 2016; Hall, 2004, 2009; Hall & Peters, 2008; Siefferman & Hill, 2005; Templeton et al., 2011, 2013; Tobias et al., 2016). Intruders singing duets are likely considered greater threats than intruder solos, probably because duets reflect the presence of both pair members, are loud, easy to locate, and may signal quality of each individual or of the pair bond through coordination properties (Dowling & Webster, 2016; Hall, 2009; Kovach et al., 2014).

Further studies may evaluate these possibilities to explain the specific duet elements that appear to represent a bigger threat for the rufous hornero.

Although our results suggest that the joint territory defense strategy is the main function of duetting in aggressive contexts, adults in pairs differed from adults in groups in key playback responses. Playbacks of duets elicited stronger response from adults in groups compared to adults in pairs, in terms of closest approach to the speaker, time spent in territorial vigilance and latency to sing (Figure 2-2). When comparing responses across playback treatments, birds in pairs approached the speaker more closely in response to both conspecific solos, sang more promptly and spent more time in territorial vigilance after the playback of male solos and female solos, respectively. On the other hand, birds in groups approached the speaker more closely and spent more time in territorial vigilance in response to duets and female solos, and sang more promptly after the playback of all conspecific songs. In sum, adults in pairs appear to be more threatened by simulated solo territorial intrusions by either sex than by simulated pair intrusions, whereas adults in groups were more threatened by simulated pair intrusions than by solo intrusions, particularly male intrusions. In addition, these results suggest that: (i) females use duets to repel all conspecific intruders, but mainly other females and especially when in groups, (ii) males in groups sing promptly to help partners in repelling female intruders (mate guarding to avoid mate injury or replacement), and (iii) mutual mate guarding may apply to birds in pairs (Table 2-9s).

Comparing adults in pairs with adults in groups, why would solo intruders be more threatening than pair intruders for adults in pairs, and pair intrusions more threatening than solo intrusions for adults in groups? It is unlikely that lone individuals can invade territories and replace an adult living in a group with independent juveniles, due to simple numeric disadvantage, considering that juveniles can join parents to create choruses (P Diniz, unpublished data) and defend the territory. Thus, lone floaters may invade territories with pairs more often than territories with groups. In addition, if floaters are more interested in replacing one individual than in taking over a territory, pairs may be again more threatened than groups by an individual floater. Our results are consistent with the idea that the relative cost of losing a territory versus losing a mate might be lower for adults in pairs than for adults in groups. However, further investigation on the dynamics of territorial and mate switching in the rufous hornero is needed to evaluate these hypotheses.

Males initiated most songs, answered partner-initiated songs more promptly and sang longer phrases in duets than did females in both aggressive and non-aggressive contexts. This suggests that males have a primary role in territory maintenance and defense, and may be additionally under higher pressure to guard their mates. This is particularly interesting because in addition to the cooperative and strongly united responses of rufous hornero partners to all conspecific stimuli, sexual selection may still play a role in song evolution in this species. Male-biased singing effort and answering rates are common among duetting and non-duetting bird species (Catchpole & Slater, 2008; Hall, 2009) and deserve further investigation.

In conclusion, we found remarkable cohesion and coordination between partners in playback responses to conspecific songs (especially duets), indicating that partners cooperatively duet to defend common territories. However, we found evidence that the relative threat to adults of territorial intrusions by lone individuals versus pairs varies with the social context, i.e., presence or absence of juveniles. Paired rufous horneros responded more strongly to simulated lone intruders, whereas groups responded more strongly to simulated female and pair intruders, suggesting that pairs also use duets to defend the pair bond (i.e. mutual mate-guarding) in addition to the primary duet function of joint territory defense.

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TABLES

Table 2-1. Predicted response to playbacks of solos and duets according to the main hypotheses for duet function (modified from van den Heuvel et al. 2013).

Hypotheses	Territory defense		Mate guarding		
	Joint	Sex-specific	Unilateral	Avoid mate replacement / injury	Mutual
Strongest response to:	duet/conspecific songs	duet or duet & same-sex solo	same-sex solo	opposite-sex solo	solos
Weakest response to:	solos or none	opposite-sex solo	opposite-sex solo	same-sex solo	duet
Response coordination:	high (duet/conspecific songs)	high (duet/conspecific songs)			high (solos)
More likely context:	groups	groups	pairs	pairs	pairs

Table 2-2. Measurements taken at individual level of behavioural and vocal responses to the playback by adult rufous horneros. We indicate if each measurement was made at the individual (ind) or pair level (pair).

Behavioral response	
Approach	Approaching the speaker or not: distance to the speaker reduced by more than 4m during the first 30s after the broadcast stimulus (ind)
Closest approach	Distance (m) between the bird and the speaker after the broadcast stimulus and after the bird approached the speaker (ind)
Singing location	Probability of song at the speaker tree/light post during the 15-min playback trial (ind)
Territorial vigilance	Proportion of time spent perched in vigilance (ind)
Latency to foraging	Latency (s) to resume foraging after the playback stimulus was broadcast and the bird had approached the speaker (ind)
Foraging	Proportion of time spent foraging during each focal trial (ind)
Vocal response	
Latency to sing (s)	Latency to sing after the stimulus was broadcast (ind)
Song rate	Number of songs (solos and duet phrases) by each bird (ind)
Singing role	Song initiator or song responder (ind)
Latency to answer partner-initiated song	Latency to sing (s) after the partner initiated a song (ind)
Song duration	Duration (s) of solos and duet contributions (ind)
Frequency properties	PCA score for five frequency measurements (pair)
Degree of song overlap	Proportion of a duet or chorus in which male and female phrases overlap (pair)

FIGURE LEGENDS

Figure 2-1. Sound spectrograms of female solo (A), male solo (B) and duet (C, female phrase in red, male phrase in blue) in the rufous hornero. Modified from Diniz (2017, Chapter 1).

Figure 2-2. Variation in behavioural and vocal responses to playback (sexes pooled). Bars show means with 95% CI, except panel B that shows proportional data. Sample sizes are shown on the bottom of the bars (panels A, C, D, E: number of trials; panels B and F: number of songs). Comparisons including the social unit type variable were made within and between social levels (pair or group).

Figure 2-3. Variation in the correlation between partners in playback responses (closest approach to speaker and phrase duration in duets). Lines represent model coefficients.

Figure 2-1

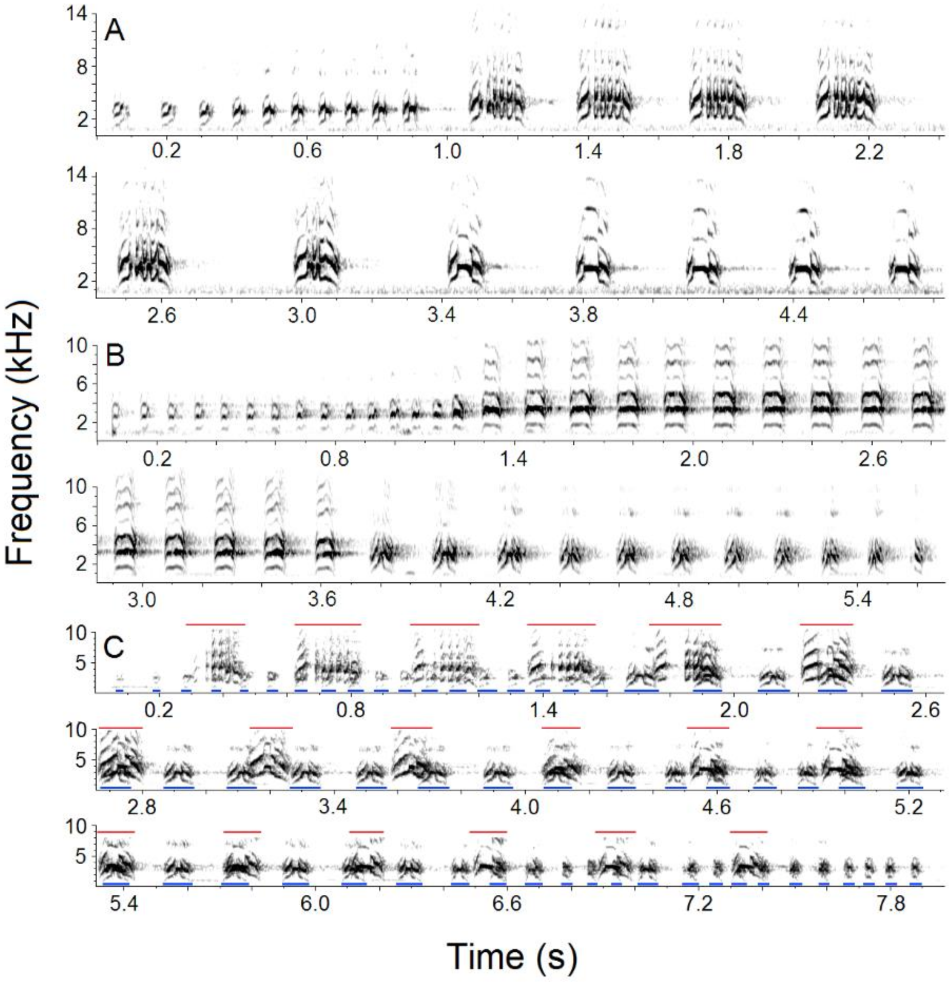

A
14
8
2
0.2
0.6
1.0
1.4
1.8
2.2
14
8
2
2.6
3.0
3.4
3.8
4.4
B
10
6
2
0.2
0.8
1.4
2.0
2.6
10
6
2
3.0
3.6
4.2
4.8
5.4
C
10
5
0.2
0.8
1.4
2.0
2.6
10
5
2.8
3.4
4.0
4.6
5.2
10
5
5.4
6.0
6.6
7.2
7.8
Frequency (kHz)
Time (s)

Figure 2-2

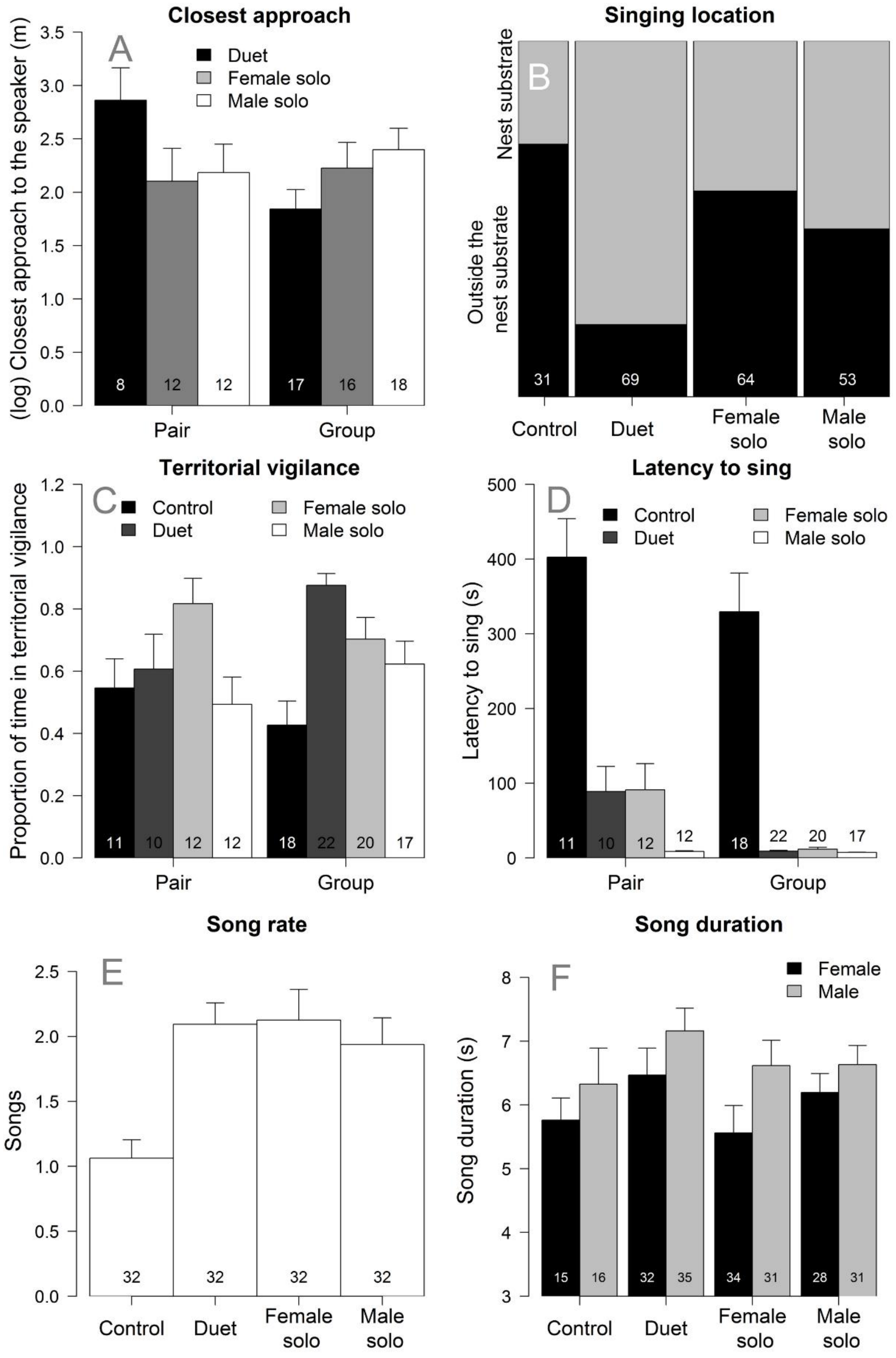
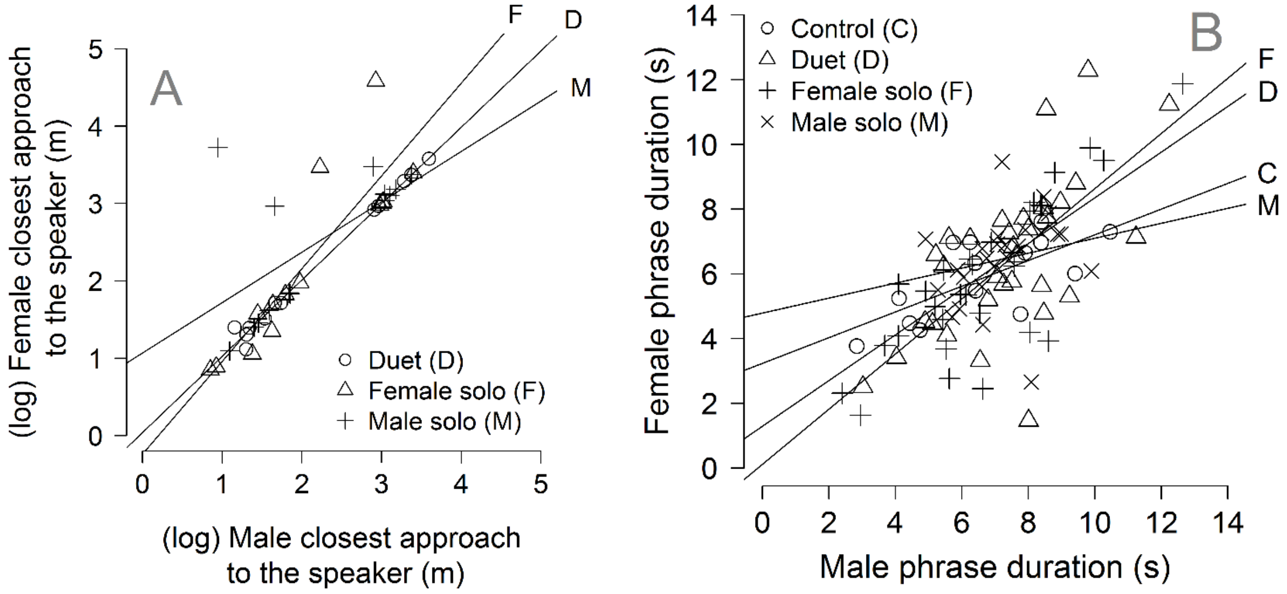

Closest approach
A
Duet
Female solo
Male solo
(log) Closest approach to the speaker (m)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
8
12
12
17
16
18
Pair
Group
Singing location
B
Nest substrate
Outside the nest substrate
31
69
64
53
Control
Duet
Female solo
Male solo
Territorial vigilance
C
Control
Duet
Female solo
Male solo
Proportion of time in territorial vigilance
1.2
1.0
0.8
0.6
0.4
0.2
0.0
11
10
12
12
18
22
20
17
Pair
Group
Latency to sing
D
Control
Duet
Female solo
Male solo
Latency to sing (s)
500
400
300
200
100
0
11
10
12
12
18
22
20
17
Pair
Group
Song rate
E
Songs
2.5
2.0
1.5
1.0
0.5
0.0
32
32
32
32
Control
Duet
Female solo
Male solo
Song duration
F
Female
Male
Song duration (s)
8
7
6
5
4
3
15
16
32
35
34
31
28
31
Control
Duet
Female solo
Male solo

Figure 2-3


A
(log) Female closest approach to the speaker (m)
(log) Male closest approach to the speaker (m)
0
1
2
3
4
5
F
D
M
Duet (D)
Female solo (F)
Male solo (M)
B
Female phrase duration (s)
Male phrase duration (s)
0
2
4
6
8
10
12
14
Control (C)
Duet (D)
Female solo (F)
Male solo (M)
F
D
C
M

SUPPLEMENTARY MATERIAL

Table 2-3s. Results from a principal component analysis for frequency measurements of duets between partners.

	PC1	PC2
Eigenvalue	2.19	1.26
Variance explained (%)	68.37%	22.77%
Loadings		
Mean frequency (kHz)	0.44	
Median frequency (kHz)	0.36	–0.38
First quantile (kHz)	0.25	–0.63
Third quantile (kHz)	0.42	0.22
Interquantile range (kHz)	0.33	0.51
Spectral entropy	0.35	0.35
Frequency centroid	0.45	

Table 2-4s. Structure of full mixed models for behavioral and vocal responses. Playback treatments: [1]all treatments (female solo, male solo, duet, heterospecific song), [2]only conspecific treatments.

Response variable	Family	Predictor variables	Random effects
Closest approach (log)[2]	Gaussian	PT × SE + PT × UT + OR + TI	II nested in GI + SI
Singing location[1]	Binomial	PT × SE + PT × UT + OR + TI + VR	II nested in GI + SI + PI
Territorial vigilance (arc sine)[1]	Gaussian	PT × SE + PT × UT + OR + TI	II nested in GI + SI
Latency to foraging[2]	Gaussian	PT × SE + PT × UT + OR + TI	II nested in GI + SI
Latency to sing (log)[1]	Gaussian	PT × SE + PT × UT + OR + TI	II nested in GI + SI
Song rate[1]	Poisson	PT × SE + PT × UT + OR + TI	II nested in GI + SI
Singing role[1]	Binomial	PT × SE + PT × UT + OR + TI + VR	II nested in GI + SI + PI
Latency to answer partner-initiated song (log + 1)[1]	Gaussian	PT × SE + PT × UT + OR + TI + VR	II nested in GI + SI + PI
Song duration (log)[1]	Gaussian	PT × SE + PT × UT + OR + TI + VR + SR + MD	II nested in GI + SI + PI
Frequency parameters of pairs' duets (PC1)[1]	Gaussian	PT × UT + PT × VR + OR + TI	PI nested in GI + SI
Degree of song overlap (arc sine)[1]	Gaussian	PT × UT + OR + VR + TI	II nested in GI + SI + PI

Predictor variables: PT (playback treatment), SE (sex), UT (social unit type: pair vs group), OR (order that the playback treatment was presented), TI (time after sunrise in hours), VR (order of the vocal response), MD (song mode: solo or duet), SR (singing role: initiator or answered), FS (distance female-speaker), MS (distance male-speaker). Random effects: GI (group identity), II (individual identity), SI (stimulus identity), PI (playback trial identity).

Table 2-5s. Predictor effects in backward stepwise model selection for variation in several playback response variables of adult Rufous Horneros. Predictors are shown in the descending order in which they were removed until the final model, containing only significant predictors. LRT = likelihood ratio test. df = degrees of freedom. Social unit type: pair or group.

Response variable	Predictor variables	*LRT*	df	*P*
Closest approach	Order of the stimulus broadcasted	0.04	1	0.85
	Playback treatment × sex	0.79	2	0.67
	Sex	3.05	1	0.08
	Time after sunrise	8.97	1	0.003
	Playback treatment × social unit type	11.02	2	0.004
Singing location	Playback treatment × social unit type	1.38	3	0.71
	Order of the stimulus broadcasted	0.06	1	0.81
	Time after sunrise	0.49	1	0.48
	Social unit type	1.12	1	0.29
	Playback treatment × sex	4.24	3	0.24
	Sex	1.95	1	0.16
	Order of the vocal response	13.75	1	0.0002
	Playback treatment	18.34	3	0.0004
Territorial vigilance	Order of the stimulus broadcasted	0.22	1	0.64
	Playback treatment × sex	2.64	3	0.45
	Sex	0.11	1	0.74
	Time after sunrise	1.83	1	0.18
	Playback treatment × social unit type	10.83	3	0.013
Latency to foraging	Playback treatment × sex	1.48	2	0.48
	Sex	0.45	1	0.50
	Playback treatment × social unit type	2.11	2	0.35
	Playback treatment	0.26	2	0.88
	Social unit type	1.00	1	0.32
	Order of the stimulus broadcasted	1.73	1	0.19
	Time after sunrise	1.90	1	0.17
Latency to sing	Playback treatment × sex	0.23	3	0.97
	Sex	0.43	1	0.51
	Order of the stimulus broadcasted	3.94	1	0.047
	Time after sunrise	4.12	1	0.04
	Playback treatment × social unit type	12.32	3	0.006
Song rate	Playback treatment × sex	0.86	3	0.84
	Sex	0.21	1	0.65
	Order of the stimulus broadcasted	0.38	1	0.54
	Playback treatment × social unit type	3.82	3	0.28
	Social unit type	1.37	1	0.24
	Time after sunrise	1.38	1	0.24
	Playback treatment	13.74	3	0.0033
Singing role	Time after sunrise	0.005	1	0.95
	Playback treatment × social unit type	0.47	3	0.93
	Social unit type	0.26	1	0.87
	Order of the stimulus broadcasted	0.27	1	0.60

Table 2-5s

	Order of the vocal response	1.64	1	0.20
	Playback treatment × sex	6.25	3	0.10
	Playback treatment	0.50	3	0.92
	Sex	7.35	1	0.007
Latency to answer partner-initiated song	Order of the stimulus broadcasted	0.07	1	0.79
	Order of the vocal response	0.16	1	0.69
	Playback treatment × social unit type	1.83	3	0.61
	Playback treatment × sex	3.79	3	0.29
	Social unit type	1.19	1	0.28
	Time after sunrise	2.96	1	0.086
	Playback treatment	6.32	3	0.097
	Sex	5.35	1	0.02
Song duration	Playback treatment × sex	0.05	3	1.00
	Playback treatment × social unit type	1.11	3	0.77
	Order of the stimulus broadcasted	0.08	1	0.76
	Time after sunrise	3.02	1	0.08
	Social unit type	2.88	1	0.09
	Playback treatment	7.76	3	0.05
	Sex	6.29	1	0.01
	Order of the vocal response	14.79	1	0.0001
	Singing role	17.87	1	<0.0001
	Song mode (solo or coordinated song)	42.05	1	<0.0001
Frequency parameters of pairs' duets (PC1)	Time after sunrise	0.07	1	0.79
	Playback treatment × order of the vocal response	1.18	3	0.76
	Order of the vocal response	0.03	1	0.86
	Order of the stimulus broadcasted	0.49	1	0.48
	Playback treatment × social unit type	4.01	3	0.26
	Playback treatment	0.64	3	0.89
	Social unit type	0.09	1	0.76
Frequency parameters of pairs' duets (PC2)	Time after sunrise	0.08	1	0.78
	Playback treatment × order of the vocal response	2.25	3	0.52
	Order of the vocal response	0.01	1	0.91
	Playback treatment × social unit type	4.28	3	0.23
	Playback treatment	0.24	3	0.97
	Social unit type	0.03	1	0.87
	Order of the stimulus broadcasted	1.27	1	0.26
Degree of song overlap	Order of the stimulus broadcasted	0.17	1	0.68
	Order of the vocal response	0.40	1	0.53
	Playback treatment × social unit type	4.09	3	0.25
	Social unit type	0.20	1	0.65
	Playback treatment	4.65	3	0.20
	Time after sunrise	9.88	1	0.002

Table 2-6s. Beta (β) estimates for predictor covariables retained in the top models for each response variable. The estimates for the main predictors can be found in the main text.

Response variable	Predictor variables	*β* ± SE	*z* ratio or *t* value
Closest approach	Time after sunrise	0.28 ± 0.09	2.99
Singing location	Order of the vocal response	–1.19 ± 0.39	–3.04
Latency to sing	Time after sunrise	0.18 ± 0.09	1.92
	Order of the stimulus broadcast	0.13 ± 0.07	1.73
Song duration	Song mode: coordinated song – solo	3.19 ± 0.47	6.75
	Singing role: initiator – answerer	0.94 ± 0.22	4.29
	Order of the vocal response	–0.47 ± 0.12	2.62
Degree of song overlap	Time after sunrise	–0.10	0.03

Table 2-7s. Standard deviation for random effects in the final models.

Response variable	Playback trial	Individual ID	Group ID	Stimuli ID
Closest approach	0.54	0.00	0.79	0.10
Singing location	4.56	0.00	3.62	0.00
Territorial vigilance		0.00	0.19	0.16
Latency to foraging		0.28	0.58	0.75
Latency to sing		0.00	0.67	0.28
Song rate		0.00	0.22	0.00
Singing role	0.00	0.70	0.00	0.00
Latency to answer partner-initiated song	0.39	0.00	0.00	0.00
Song duration	0.54	0.00	1.07	0.07
Frequency parameters of pair duets (PC1)	1.01		1.38	0.00
Frequency parameters of pair duets (PC2)	0.68		0.74	0.01
Degree of song overlap	0.001	0.00	0.07	0.11

Table 2-8s. General sample sizes for modelling each playback response variable.

Response variable	Playback treatment (duet, female solo, male solo, control)	Social unit type (pairs, groups)	Trials, social units, stimuli
Closest approach	25, 28, 30, no control	32, 51	48, 16, 15
Singing location	69, 65, 53, 31	85, 133	59, 16, 20
Territorial vigilance	32, 32, 29, 29	45, 77	63, 16, 20
Latency to foraging	17, 19, 22, no control	27, 31	33, 16, 15
Latency to sing	32, 32, 29, 24	42, 75	61, 16, 20
Song rate	32, 32, 32, 32	46,82	64, 16, 20
Singing role	68, 68, 62, 34	93, 139	61, 16, 20
Latency to answer partner-initiated song	32, 29, 25, 16	38, 64	62, 16, 20
Song duration	68, 67, 62, 33	93, 137	61, 16, 20
Frequency parameters of pair duets	19, 21, 21, 12	32, 41	50, 16, 20
Degree of song overlap	15, 31, 29, 25	38, 62	61, 16, 20

Table 2-9s. Summary of post-hoc significant ($P < 0.05$) and marginally nonsignificant ($P < 0.1$) results (influenced by playback treatment) and support for each duet functional hypothesis.

	Pairs		Groups	
Behavioral and vocal predicted responses	Female	Male	Female	Male
- Closest approach to both male and female solos (pairs); or to both female solos and duets (groups)	MMG++	MMG++	STD++ UMG+	AMU+
- Sing more in nest substrate in response to duets	JTD++ STD+	JTD++ STD+	JTD++ STD+	JTD++ STD+
- Territorial vigilance higher in response to female solos (pairs); or to both duets and female solos (groups)	UMG+	AMU+	STD++ UMG+	AMU+
- Lower latency to sing in response to male solos (pairs); or to conspecific songs (groups)	AMU+	UMG+	JTD++	JTD++
- Higher song rate in response to conspecific songs	JTD++	JTD++	JTD++	JTD++
- Coordination in approaching the speaker (correlation between sexes in closest approach values) higher in response to duets	JTD++ STD+	JTD++ STD+	JTD++ STD+	JTD++ STD+
- Correlation between female and male phrase durations in duets higher in response to female solos and duets	STD++ UMG+	AMU+	STD++ UMG+	AMU+
Sum of predictions supported	JTD - 6 STD - 4 MMG - 2 UMG - 2 AMU - 1	JTD - 6 STD - 2 MMG - 2 AMU - 2 UMG - 1	JTD - 8 STD - 7 UMG - 3	JTD - 8 AMU - 3 STD - 2

Joint territory defense (JTD), Sex-specific territory defense (STD), Unilateral mate guarding (UMG), Mate guarding to avoid mate usurpation (AMU), Mutual mate guarding (MMG). Symbols: * partial support for one predictions, + support for one prediction, ++ support for the two predictions. Predicted responses in Table 2.1.

CAPÍTULO 3

Female song reflects territory quality in a duetting, Neotropical bird with low extra-pair paternity[4]

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[4] Uma versão deste capítulo será submetida para publicação como "Diniz P, Macedo RH & Webster MS. Female song reflects territory quality in a duetting, Neotropical bird with low extra-pair paternity."

ABSTRACT

Although intensively studied, we still have little consensus about the direct fitness consequences of vocal duetting. Some studies suggest that duetting functions in acoustic mate guarding to prevent cuckoldry, whereas other studies argue that duetting is a cooperative behavior to defend common territories. Thus, duetting parameters presumably could reflect territory quality and a pair's reproductive success. We investigated extra-pair paternity and the relation among song traits, territory quality and productivity in the rufous hornero (*Furnarius rufus*), a Neotropical, socially monogamous bird. We found a lower than average rate of extra-pair paternity (3.33% of 120 offspring and 6.52% of 46 broods), and 100% apparent nest success. Female song (rate, output and latency to answer partner-initiated song) was positively correlated with territory size and quality, as reflected in amount and proportion of territory foraging patches. Duet duration, but not rate, was positively correlated with territory size. Our results suggest that female song and the pair duet are used in the defense of food resources within territories, or enable the acquisition of high quality and large territories. However, neither features of female song, male song, or duets, nor territory features correlated with productivity (number of social fledglings and post-fledging survival) in this species, suggesting that song or territory might affect fitness in other ways, such as in juvenile development or adult survival.

INTRODUCTION

Vocal duets are coordinated songs or calls between partners (Farabaugh 1982). Vocal duetting has fascinated biologists, and studies have been conducted on every aspect of duetting behavior: ontogeny (Hall and Magrath 2007; Rivera-Caceres et al. 2016), proximate mechanisms for coordination (Amador et al. 2005; Logue et al. 2008; Rivera-Cáceres 2015), evolution (Logue and Hall 2014; Tobias et al. 2016) and, especially, adaptive function (reviews in Hall 2004; Hall 2009; Dahlin and Benedict 2013). Adaptive function of duetting has been widely investigated through the interpretation of responses to playbacks of solos and duets by territorial birds (reviews in Hall 2004; Hall 2009; Douglas and Mennill 2010; Dahlin and Benedict 2013), but the fitness consequences of duetting for males and females have seldom been explored (Hall 1999; Hall and Magrath 2007). For example, in magpie-larks *Grallina cyanoleuca*, duet timing is more synchronized in established pairs when compared with new pairs (Hall and Magrath 2007), and established pairs have higher reproductive success than do first time breeders (Hall 1999), suggesting that duets influence or are associated with reproductive success in this species.

Most duetting species are socially monogamous or cooperative breeders (Tobias et al. 2016). To understand the fitness consequences of duetting, we need to know how key fitness characteristics, such as reproductive success, vary among individuals and pairs (Bateman 1948; Jones et al. 2002). Extra-pair paternity (EPP) is prevalent among socially monogamous birds (Griffith et al. 2002; Macedo et al. 2008), but less than 1% of duetting species have been studied in this regard (Table 3-1). Previous studies suggest that extra-pair paternity might be low in duetting species (Gill et al. 2005; Douglas et al. 2012; Koloff and Mennill 2013), probably due to a set of life history and ecological traits presumably associated with both duetting occurrence and low EPP.

Traits common to both duetting species and those with low rates of EPP include: sexual monochromatism, absence of migration, year-round territoriality, strength of social bonds, low divorce rate, and high adult survival (Farabaugh 1982; Stutchbury and Morton 2001; Macedo et al. 2008; Stutchbury and Morton 2008; Benedict 2008a; Logue and Hall 2014; Tobias et al. 2016). However, the very few studies that have examined EPP among socially monogamous, duetting species reveal high interspecific variability (Table 3-1). Intriguingly, two out of the four studied duetting species with high levels of EPP shared most of the above mentioned traits (California towhee *Pyrgisoma crissale*, Benedict 2008b; crimson-breasted shrike *Laniarius atrococcineus*, van den Heuvel et al. 2014) (Table 3-1). The additional two duetting species studied are sexually dimorphic and territorial only in the breeding season (red-backed fairy-wren *Malurus melanocephalus*, Karubian 2002, Baldassarre et al. 2016; pheasant coucal *Centropus phasianinus*, Maurer et al. 2011; Table 3-1). Further information on the genetic mating systems of duetting species would allow broader and more confident inferences about general patterns.

Among species with moderate to high levels of extra-pair paternity, male-created duets (i.e. answered partner songs) might influence male and female fitness through acoustic paternity guarding (Baldassarre et al. 2016). The acoustic paternity guarding hypothesis (Sonnenschein & Reyer 1983, Hall 2009) suggests that duetting should peak in the female fertile period (Hall 2004; Topp and Mennill 2008), and the probability and speed of males in creating duets should signal the pairing status of these males (Hall 2004). This should in turn repel other males from pursuing extra-pair copulations and/or minimize the partner's propensity to pursue extra-pair copulations (Gill et al. 2005; Hall 2009). However, empirical studies found no support for this hypothesis, suggesting that duets do not function to guard partners from extra-pair copulations (Australian magpie-larks, Hall and Magrath 2000; buff-breasted wrens *Cantorchilus leucotis*, Gill et al. 2005; purple-crowned fairy-wren *Malurus coronatus*, Hall and Peters 2008; crimson-breasted shrikes, van den Heuvel et al. 2014). The exception is the red-backed fairy-wren, where a high expression of male song answering rate and speed leads to reduced paternity loss (Baldassarre et al. 2016), a mating tactic adopted especially by unattractive males that results in similar reproductive success in comparison with attractive males (Dowling and Webster 2017).

Most duetting bird species sing throughout the year and the acoustic paternity guarding hypothesis does not apply to females, suggesting this hypothesis is not enough to explain duetting occurrence (Hall 2009). Thus, it remains unclear how male participation in duets could be associated with male fitness in species with low or no EPP and, especially, how duetting could contribute to female fitness. In scenarios of negligible EPP and strong pair bonding, inter-individual variance in mating success may be low, and social selection suggests that individuals should compete for limited resources other than mating opportunities, such as good quality territories (West-Eberhard 1983; Tobias et al. 2012; Lyon and Montgomerie 2012). This interpretation is in line with several playback experimental studies that provided evidence that duets can function in settling territorial disputes (Hall 2009; Dahlin and Wright 2012; Dahlin and Benedict 2013; Koloff and Mennill 2013; Dowling and Webster 2016). Apparently, the coordination aspect of duetting can signal threat level in territorial interactions (Hall and Magrath 2007). If duetting signals competitive ability in acquiring and defending territories in a highly heterogeneous landscape, we could expect that individuals with high expression of duetting attributes would acquire high quality territories and have a lengthy territory tenure, which would in turn influence reproductive success and productivity (Tobias et al. 2012; Cain et al. 2015; Cain and Langmore 2016).

It is well known that male song carries information about a variety of fitness-related traits in the context of sexual selection, such as age (Nemeth et al. 2012), fighting ability and motivation (Ripmeester et al. 2007), aggressiveness (Searcy and Beecher 2009), territory quality (Manica et al. 2014), and parental effort (Buchanan and Catchpole 2000). Thus, variation among males in song expression mediates intrasexual competition and female mate choice in birds (Kroodsma and Byers 1991; Gil and Gahr 2002; Catchpole and Slater 2008) and has proven fitness consequences (Gil and Slater 2000; Bolund et al. 2012; Nelson and Poesel 2013). There are some examples from the female song literature showing that female song mediates territorial interactions (Krieg 2016; reviewed by Cain et al. 2015) and predicts reproductive success, as suggested by social selection theory (Cain et al. 2015; Brunton et al. 2016). In contrast, the link between duetting (or female song), territory quality and reproductive success has never been investigated, to our knowledge, despite widely cited evidence that duet functions in territory defense (Hall 2009; Dahlin and Benedict 2013).

The rufous hornero (*Furnarius rufus*) is a duetting, year-round territorial and socially monogamous Neotropical bird. Previous observational and experimental data suggest that duetting in this species is cooperative-based and functions in the joint defense of territorial resources, strengthening the partnership of social pairs (Diniz 2017) (Chapters 1 and 2). Given that life history and ecological traits linking duetting species with low occurrence of EPP, we predicted a low rate (< 5%) or absence of EPP in this species. Further, we expected that song traits (singing effort and song attentiveness) would exhibit a positive correlation with territory quality and productivity, as reflected in the number of social offspring produced and their subsequent survival. To examine paternity trends in our study population, we conducted genetic analyses of 104 adults and 126 nestlings across three breeding seasons. To test the associations between song traits, territory quality and productivity, we used 12 groups within the same study population.

METHODS

a) Study species and field procedures

The rufous hornero (Furnariidae family) is a monochromatic (Diniz et al. 2016), ground-foraging, socially monogamous and sedentary bird species (Fraga 1980; Sick 2001; Remsen and Bonan 2017), widespread in southern South America (Marreis and Sander 2006). They breed only once, or eventually twice a year, and produce clutches of 2-4 eggs (Fraga 1980; Rodriguez and Roper 2011). Incubation lasts 14-18 days, and the nestling period 23-26 days (Fraga 1980; Remsen and Bonan 2017). Both parents provide parental care, from construction of the heavy globular mud nest (Shibuya et al. 2015) to post-fledging care of the young (Fraga 1980; Massoni et al. 2012). Nest survival is high (Fraga 1980), contrasting with many other Neotropical bird species (Martin 1996), and juveniles may stay in their parents´ territories for seven or more months (Fraga 1980; Bobato 2012).

The rufous hornero sings two song types, one for each sex, as solo songs or overlapping in duets or chorus (i.e., three or more individuals singing) (Roper 2005; Diniz 2017) (Chapters 1and 2) (Figure 3-1). Song rate per sex is low (10 solo songs plus duet phrases/h), most songs are duets (61%) and songs are produced throughout the year (Diniz 2017) (Chapter 1). Males, compared with females, initiate twice as many songs, answer partner-initiated songs at higher rates and more quickly and have longer song duration (Diniz 2017) (Chapter 1).

We studied an urban population of the rufous hornero in 175 ha in the campus of the University of Brasilia, Brazil (15°45’S, 47°51’W) for three consecutive years (2013, 2014 and 2015). In the field, we collected blood samples from adults and nestlings from the study population across the three years for paternity analyses (see below), and conducted focal observations on 12 groups during both non-breeding and breeding seasons in 2015 (seven months, from June to December). From these 12 groups, we recorded vocal behavior, geolocated songs and territorial interactions to estimate territory perimeter, and estimated productivity (number of fledglings produced and post-fledging survival).

b) Banding and blood sampling

We captured and blood sampled 127 adults and 128 nestlings during three breeding seasons (2013, 2014, 2015). All adults and 94 nestlings were banded. Capture methods for adults and nestlings followed Braga et al. (2014) and Shibuya et al. (2015), respectively. Blood samples (~60µl) were obtained from brachial venipuncture for adults and nestlings, and stored in a lysis buffer (100mM Tris HCl, pH = 8.0, 100mM EDTA, 100mM NaCl, 2% SDS) at 4°C. The rufous hornero builds heavy domed nests during the year, which typically are completed before the breeding season (Fraga 1980; Ferreira et al. 1992). Each year, to capture the nestlings with minimal disturbance, we monitored each nest at intervals of up to 15 days from the beginning of the nesting stage or when the parents were captured while brooding (second half of August, Diniz 2017) (Chapter 1). To choose an optimal date to open nests and capture the nestlings, we conducted behavioral observations to determine if the adult birds were just building the nest, or whether they were incubating eggs or feeding nestlings (Shibuya et al. 2015). During the week prior to capturing the nestlings we confirmed the parents' identities.

c) Song, territory quality and productivity

We observed 12 groups for seven consecutive months in 2015 (from June to December) to obtain data on song, territory and productivity. Study groups were composed of adult pairs or pairs plus juveniles (hatched in previous breeding season), but group size also varied across focal trials (mean ± SD = 2.83 ± 0.82, range = 2-6, n = 163 trials). All adults were banded and sexed. In brief, we observed each group at 15-day ± 0.15 (mean ± s.e., n = 149) intervals for one hour, totaling 14 focal sessions/group (excepting one that lost its territory after 7 focal sessions). We focused our observations on adults, recording all the songs they produced using a Marantz PMD 660 recorder coupled with a Sennheiser ME66 or Yoga HT-81 microphone.

We analyzed song data in Raven 1.5 (Bioacoustics Research Program 2014), and detailed acoustic analyses are described elsewhere (Diniz 2017) (Chapter 1). In brief, we extracted the following five variables for each focal session and for each sex: number of initiated songs (solos plus initiated duets or chorus), song output (total time spent singing), song answering rate (proportion of partner songs that were answered), phrase duration in duets or chorus, and latency to answer partner-initiated songs. We averaged these measurements within focal sessions and then within groups. We also counted the number of duets and estimated duet duration for each focal session, averaging these variables within each group.

We geolocated each song produced and each territorial interaction (e.g., chase, fight) involving one focal adult against strangers in each focal session. We used GPS Status 3.0.4. App for Android system (accuracy ~ 3m) to demarcate points. At each focal session, we demarcated the same location only once (e.g., when the bird sang twice in the same tree). We pooled points from multiple focal sessions to obtain territory size (mean ± s.d. = 58.83 ± 12.90 points, n = 12 groups). Coordinated reference system was set to UTM 23S and datum WGS84. We used adehabitatHR package (Calenge 2006) from R 3.2.1 to estimate territory size (at 95% level, in ha) by Kernel utilization distribution function (smoothing parameter computed by 'LSCV'; Worton 1989; Seaman and Powell 1996).

The rufous hornero is an insectivorous and exclusively ground-foraging species, but relies on trees (and less often on light poles) to build their nests (Fraga 1980; Remsen and Bonan 2017). They forage mainly in short grasses or litter, avoiding tall grasses (pers. obs.), and thus may be favored by urban landscapes such as lawns or short-cut grasses. We used QGIS 2.18.3 (QGIS Development Team 2016) to demarcate the contours of trees and short-grass patches at each perimeter-demarcated territory (here, we used 100% minimum convex polygon, Mohr 1947; Odum and Kuenzler 1955) in georeferenced aerial images from the study site (precision = 5m, photos taken in 2015 by Terracap; Figure 3-4s). The non-foraging patches consisted mostly of streets and sidewalks. We calculated the proportion of territory size covered by frequently-managed short grass and litter, and tree canopies, as estimates of available foraging patches and nest sites, respectively. We also computed the absolute area (in ha) covered by these two types of vegetation.

Our focal observations on 11 groups occurred from up to 120 days before to up to 90 days after the nesting stage. This allowed us to estimate productivity based on the number of fledglings produced and post-fledging survival. We have paternity data for only three of these 11 study groups, and we found no extra-pair paternity in these three broods. Considering that we found only a negligible rate of extra-pair paternity in our general study population (see Results), we assume that social productivity reflects genetic productivity in our study groups. Thus, the number of fledglings produced was considered the maximum number of juveniles seen in a territory in 3.73 ± 1.35 focal sessions (mean ± s.d., n = 11 groups) after the first fledgling was recorded. Parents feed juveniles for approximately 22 days and juveniles stay in their natal territory from four to nine months after fledging (Fraga 1980; Bobato 2012). Thus, we classified post-fledging survival in a binary scale: zero, when at least one juvenile disappeared from a territory for at least two consecutive focal sessions post hatching (i.e., ~30 days), and one, when no juvenile disappeared during the observed post-breeding stage (up to 90 days after fledging). The group that lost its territory was assigned a zero relative to number of fledglings produced and post-fledging survival. Individuals from this group were not seen in the study area after losing their territory.

d) Molecular sexing and genetic analysis

Adult birds were sexed with molecular tools (n = 69), by their song phrases (Roper 2005) or using the partner´s known sex (52 birds). We used molecular sexing according to the Griffiths et al. (1998) methodology for 59 adults captured in 2013, and used the Fridolfsson and Ellegren (1999) methodology to sex an additional 10 adults captured across the three study years.

We determined paternity through single nucleotide polymorphisms (SNPs) markers across individuals, since only 11 microsatellite markers tested successfully for members of the Furnariidae family (none from the genus *Furnarius*, Cardoni et al. 2013; Yáñez et al. 2015). In comparison with microsatellite markers, SNPs have been largely used in evolutionary studies but not in parentage analyses (Kaiser et al. 2016). Microsatellites are highly polymorphic, but prone to high genotyping error (Pompanon et al. 2005; Kaiser et al. 2016). Although SNPs are usually not multiallelic and results are low in heterozygosity, they are much more abundant in the genome than microsatellites (reviewed by Kaiser et al. 2016). A few studies that compare both methods reveal that SNP is equivalently successful or outperforms microsatellites in assigning paternity (Anderson and Garza 2006; Cramer et al. 2011; Weinman et al. 2015).

We used the next-generation sequencing-based method named Double Digest Restriction Associated DNA sequencing (ddRAD-seq) to *de novo* SNP development (Peterson et al. 2012). This method provides a reduced-representation and large sample of the genome and does not require previous knowledge on genome sequence or variability (Peterson et al. 2012). The double restriction enzyme digest approach confers advantages in comparison with the previous Restriction Associated DNA sequencing (RAD-seq) method, mainly because the former permits a greater accuracy and repeatability in DNA fragment size-selection for library construction (Peterson et al. 2012).

SNP discovery and genotyping were conducted according to the Peterson et al. (2012) protocol with few changes (see supplementary material for detailed protocol). This protocol involves four steps. First, we isolated, quantified and diluted genomic DNA for 240 samples from 230 individuals. Then we digested genomic DNA samples and ligated short DNA fragments to them, which function as molecular barcodes (i.e. adapters). The third step consisted of pool reactions within each Illumina multiplexing read index (i.e. index group), which assigned a molecular barcode to each group and performed low-cycle DNA amplifications (PCRs). In the last step, a DNA fragment analysis was performed at each index group to calculate molarities and combine diluted index samples. A sample of the final solution of combined DNA from all individuals was submitted to Illumina sequencing read. The reads were checked for quality and filtered (see Bioinformatics in supplementary material), resulting in SNPs 183 loci.

We performed parentage analysis using CERVUS 3.0.7 (Kalinowski et al. 2007) on non-duplicated data (230 individuals). We assumed all social mothers were also genetic mothers. First, we ran an allele frequency analysis to verify loci characteristics. Characterization of SNPs loci revealed mean heterozygosity of 0.45 across all loci (Table 3-6s). Second, we ran the simulation of paternity analysis, which is needed to calculate critical log-likelihood statistics (LOD) to provide confidence for assigned paternity for real data. We used the following settings to run the simulation of paternity analysis: number of simulated offspring (100,000), candidate fathers (178, estimated adult males in our population), proportion of candidate parents that were sampled (0.29), proportion of loci typed (0.979), proportion of loci mistyped (0.1), minimum typed loci (91), confidence calculated using LOD score (relaxed level = 95%, strict level = 99%). Finally, we assigned paternity only at the strict level of confidence (99%) and at positive LOD scores.

e) Statistical analyses

We analyze our data in R 3.2.1 (R Core Team 2015). We reduced the number of song and territory quality variables using principal component analyses (PCA). For song data, we performed PCAs separately for each sex. We retained the two first components (PC, hereafter; eigenvalues > 1) from each of these three PCAs (Table 3-2, Table 3-3). In terms of territory quality, PC1 was positively related to absolute area and proportion of territory size covered by tree canopies, whereas PC2 was positively related to absolute area and proportion of territory size covered by grasses. In terms of female song traits, PC1 was positively related to the number of initiated songs and song output, and negatively related to latency to answer partner songs, whereas PC2 was positively related to phrase duration and song answering rate. Finally, in terms of male song traits, PC1 was positively related to the number of initiated songs and song output, whereas PC2 was positively related to song answering rate and latency to answer partner song, and negatively related to phrase duration.

We used linear models (Gaussian family, 'lm' function) to test whether territory size and quality (PCt1, PCt2) varied as a function of song traits (one model for each response variable). We included the two song PCs for each sex, duet rate and duet duration as predictors in these models. We used generalized linear models ('glm' function) to analyze the variation in productivity (number of fledglings, Poisson family; post-fledging survival, Binomial family) as a function of song traits and territory attributes. To prevent overfitting, we analyzed the effects of territory attributes and song traits in separate models. Moreover, we reduced the model complexity for post-fledging survival, creating two global models for song traits, one with the four PC scores summarizing male and female song traits, and another one with duet rate and duration as predictors. We ranked the models using the corrected Akaike's Information Criterion (ΔAICc<2, Burnham and Anderson 2002) (function 'dredge' from MuMIn package, Barton 2015). All continuous variables were scaled before the analyses to obtain comparable β coefficients from the top model.

RESULTS

Genetic paternity was assigned for 93% of the offspring sampled (n = 126). Extra-pair paternity was infrequent across the study years, and only 4 (3.33%) nestlings from 3 (6.52%) broods were sired by males other than the social father (Table 3-4). We were able to assign paternity for one of the four EP nestlings, which was sired by a male from a contiguous territory. The remaining three EP nestlings had low assignment probability with their social father (LOD score < 0, pair loci mismatches > 8). We were unable to assign paternity for all the young whose social fathers were not DNA sampled (6 young from 2 broods, 7% of offspring sampled, n = 126), probably because the social fathers are the genetic fathers as well.

Mean (± SD) territory size was 0.70 ± 0.23 ha (range = 0.37 – 0.99, n = 12, 95% fixed-kernel). Averaged proportion of territory size covered by estimated tree canopies (i.e. nest sites) and foraging patches was 28.88 ± 11.78 % (SD, range = 12.49 – 54.92) and 55.30 ± 23.72 % (SD, range = 9.29 – 86.35), respectively. Territory quality (i.e. ´PC foraging patches') is correlated with song traits, as reflected in 'PC female singing effort’ (β ± s.e. = 0.90 ± 0.30) (Table 3-5, Figure 3-2). Our results indicate that females that sing at higher rates, for longer bouts (song output), that answer partner songs more quickly (song latency), occupy territories with greater coverage of short grasses, which we presume to be the main foraging patches for this species. Female song traits were not related to territory quality in terms of tree cover (a proxy for nest site availability). The null model was the best-ranked model to explain variation in territory size. However, the second model shows that territory size is positively related to ‘PC female singing effort’ (β ± s.e. = 0.60 ± 0.27) and duet duration (β ± s.e. = 0.61 ± 0.27) when accounting for the covariation between these two predictor variables (Table 3-5, Figure 3-3). Neither territory size nor quality varied with male song traits.

Mean (± SD) number of fledglings produced was 1.75 ± 0.96 (range = 0 – 4, n = 12). The only group with no fledglings lost its territory before the breeding season started. The null model was the best-ranked model to explain variation in the number of fledglings produced (weight = 0.33). Although the ‘PC male song answering’ and duet duration were presented in the second (ΔAICc = 0.52) and third models (ΔAICc = 1.86), respectively, the sizes of these effects had a high degree of uncertainty (β ± s.e., ‘PC male song answering’ = –0.37 ± 0.25, duet duration = 0.23 ± 0.22). At least one juvenile in half of the study groups (n = 12) disappeared in the post-fledging stage, and were assumed to have died. The models containing ‘PC male singing effort’ and duet duration were the top-ranked models to explain variation in post-fledging survival, but the confidence intervals for the coefficients were high (β ± s.e., ‘PC male singing effort’ = 0.90 ± 0.71, duet duration = 1.37 ± 0.95). Neither the number of fledglings nor post-fledging survival were correlated with our proxies of territory quality.

DISCUSSION

According to the acoustic paternity guarding hypothesis (Sonnenschein and Reyer 1983; Hall 2009), males use duets to prevent their mates from engaging in extra-pair copulations (Gill et al. 2005; Hall 2009). However, we found a very low rate of extra-pair paternity for the rufous hornero, which makes it unlikely that male participation in duets functions in acoustic paternity guarding in this species. Instead, our results from paternity analyses in the rufous hornero is consistent with the idea that the occurrence of duetting in birds coincides with low rates of EPP (Table 3-1; Gill et al. 2005; Douglas et al. 2012; Koloff and Mennill 2013). Nevertheless, even if this general pattern holds for a larger number of studied species, there should be other traits associated with duetting that, in an evolutionary context, are better predictors of EPP than the duetting trait itself (Westneat and Stewart 2003). In other words, duetting is probably neither the evolutionarily driver nor the consequence of EPP.

The low rate of EPP found in the rufous hornero supports the concept of cooperative and territorial roles for the duetting behavior (Logue 2005; Hall and Peters 2008b). In this context, we predicted that individual and pair attributes of duet singing would be positively associated with territory size, quality and productivity in this species. We found that females that sang at higher rates and answered their partner´s songs more quickly to create duets had territories that were both larger and richer, in terms of proportion of territory size covered with foraging patches. Considering that, on average, males answer most (73%) of the female songs in this species (Diniz 2017) (Chapter 1), our results suggest that the rate of duets initiated by females predicts territory size and quality. Duet duration also was positively correlated with territory size. However, male song was not associated with territory quality, and neither song traits nor territory quality correlated with our measurements of productivity (number of fledglings and post-fledging survival).

To our knowledge, our results provide the first evidence of a positive association between female song parameters and territory quality. In contrast with our results, Cain and Langmore (2016) found a higher song rate for superb-fairy wrens (*Malurus cyaneus*) living in low-quality habitat compared with high-quality habitat, which suggests a negative association between female song rate and territory quality in that species. Theoretical and empirical studies suggest females are more constrained by dependence upon ecological resources to breed than by mating opportunities (Bateman 1948; Clutton-Brock 2009; Stockley and Bro-Jorgensen 2011; Tobias et al. 2012; Clutton-Brock and Huchard 2013). Thus, female aggressiveness may be important to guarantee access to ecological resources (Robinson and Kruuk 2007; Cain and Ketterson 2013). If female song signals aggressiveness or competitive ability (Tobias et al. 2011; Cain et al. 2015), either of these variables should predict reproductive success or productivity (Cain and Ketterson 2012; Cain et al. 2015; Brunton et al. 2016). Female rufous horneros sing at a higher rate in response to conspecific but not to heterospecific song, suggesting that female song signals aggressiveness in this species (Diniz 2017) (Chapter 2). Taken together, these findings indicate that rufous hornero females with high competitive abilities could acquire high quality territories by means of a higher song investment (i.e. song effort drives territory quality) (Rosvall 2011; Cain et al. 2015). Alternatively, females may need to sing more to defend high quality territories (i.e. territory quality drives song effort) (Cooney and Cockburn 1995; Cain et al. 2015). Future studies could address these two possibilities.

In addition to individual-level song parameters, we also found that duets exhibit two important traits (duration and latency of females to answer partner songs) associated with territory features. To our knowledge, this is also the first evidence of an association between duet song and territory quality. A previous playback study of the rufous hornero revealed that birds tend to answer their partners´ songs more quickly, and partners tend to sing longer duets, in response to conspecific (solos and duets) compared with heterospecific song playbacks (Diniz 2017) (Chapter 2). Although these two tendencies were not statistically significant, when considered in light of the results of the current study, our findings confirm that in rufous horneros, duets function in territory defense and suggest that the performance of duet signals is sensitive to variation in territory quality. Theory and empirical research suggest that duets function in joint territory defense (Seibt and Wickler 1977; Hall 2004; Hall 2009; Dahlin and Benedict 2013; Koloff and Mennill 2013), and we further suggest that duets may more broadly signal territory quality.

It is unclear why male song and territory quality were unrelated. Male fitness is apparently not constrained by extra-pair mating success (given the low rate of EPP), and males sing at higher rates and engage in more territory interactions than do females in this species (Diniz 2017) (Chapter 1). In addition, males duet with females to defend common territories (Diniz 2017) (Chapters 1 and 2). Confounding factors not accounted for here include male size (Ballentine 2009), age (Ferrer and Bisson 2003; Poesel et al. 2006) or experience (Beecher et al. 2000; Hyman et al. 2004), and male quality (Lambrechts and Dhondt 1988; Lampe and Espmark 1994; Christie et al. 2004). These may possibly explain the lack of association between male song and territory features.

We also failed to find a correlation between song or territory quality (and size) and productivity (see also Brunton et al. 2016). One possible explanation for this pattern is that song and/or territory may affect fitness in ways we did not consider in this study, including: offspring quality (Weiss et al. 2009), juvenile development (Komdeur 1992) and dispersal success (Reid et al. 2005), length of territory tenure (Hiebert et al. 1989) or adult survival (Wilson et al. 2000). Another explanation is that song expression (or aggressive-mediated signal expression) trades-off with parental care (Duckworth 2006; McGlothlin et al. 2007; Stiver and Alonzo 2009; Cain and Ketterson 2013), or high singing (or display) effort leads to high nest predation (Kleindorfer et al. 2016). If so, we would expect a negative correlation between song and productivity. In addition, we found no nest predation across our study groups, so it is unlikely that song increases nest predation (Dias et al. 2010; Kleindorfer et al. 2016) in this species. Finally, we did not use a direct measurement of food availability (e.g., ground-arthropod biomass; Maceda-Veiga et al. 2016), which may have masked a relationship between territory quality and productivity (Conner et al. 1986). Thus, the fitness consequences of song and territory quality remain to be understood in the rufous hornero.

In conclusion, in this first description of EPP for the species-rich Furnariidae family (~ 300 species, Derryberry et al. 2011), we found a low rate of EPP for the rufous hornero. Our data also consist of one of the first descriptions of EPP for a duetting species in the Suboscine clade (i.e., birds with small vocal repertoire and low plasticity in song learning and structure, Kroodsma and Konishi 1991; Liu et al. 2013; Touchton et al. 2014). The function and the true benefits of duetting, female song and male song in species where both sexes sing are controversial and still not well understood (Langmore 1998; Hall 2004; Hall 2009; Logue and Krupp 2016; Tobias et al. 2016). Our study demonstrates a relation between song and duet features and territory quality in a duetting species with low rates of EPP. We argue that territory quality is an important pressure shaping the expression of female song and duets in socially monogamous bird species where both sexes sing.

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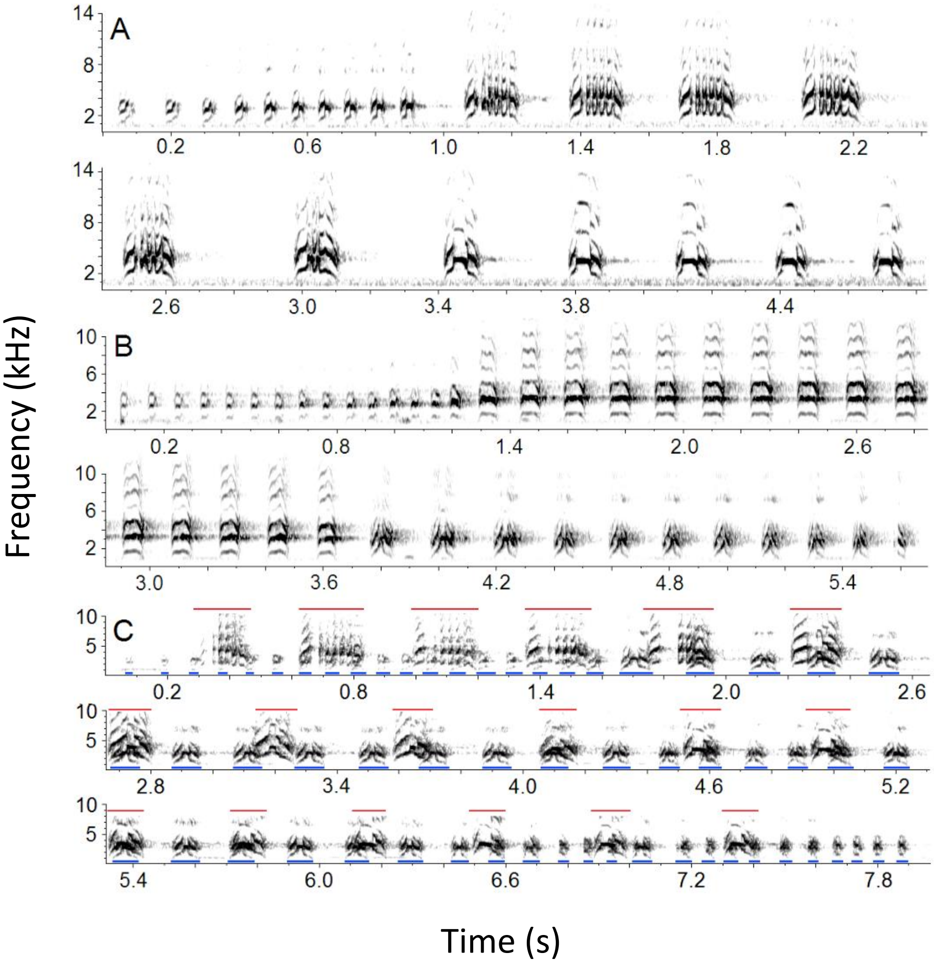

Figure 3-1. Spectrogram of solos (female in A, male in B) and duets (C) of the rufous hornero. Panel C: red and blue lines indicate female and male notes, respectively. Retrieved from Diniz (2017) (Chapter 1).

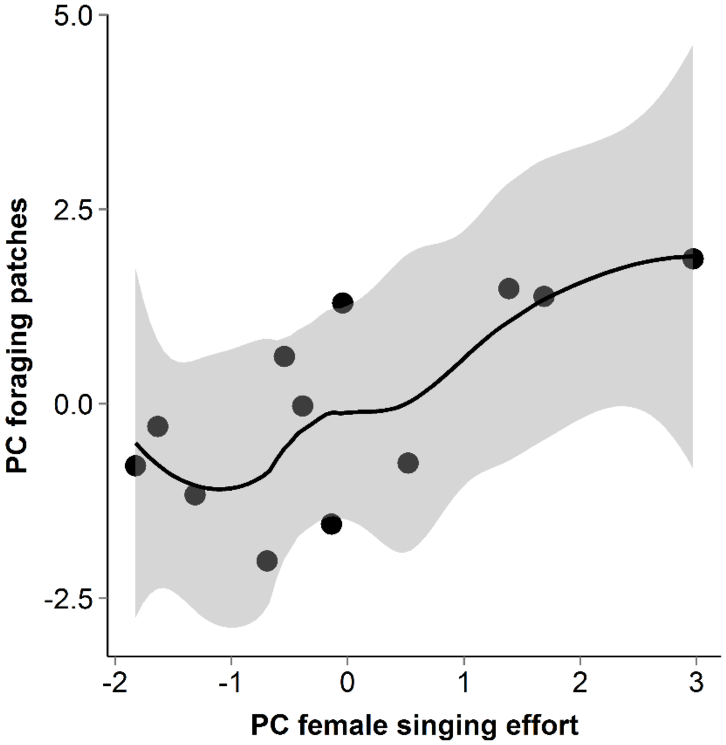

Figure 3-2. Relation between a proxy for foraging patches territory coverage and female song behavior in the rufous hornero. High values of 'PC foraging patches' correspond to absolute area and proportion of territory size covered by short-grasses and leaf litter. High values of 'PC female singing effort' correspond to high song initiation rate and song output, and low latency to answer partner song. Loess smoothing indicates mean trend (black line) and confidence interval (shadow).

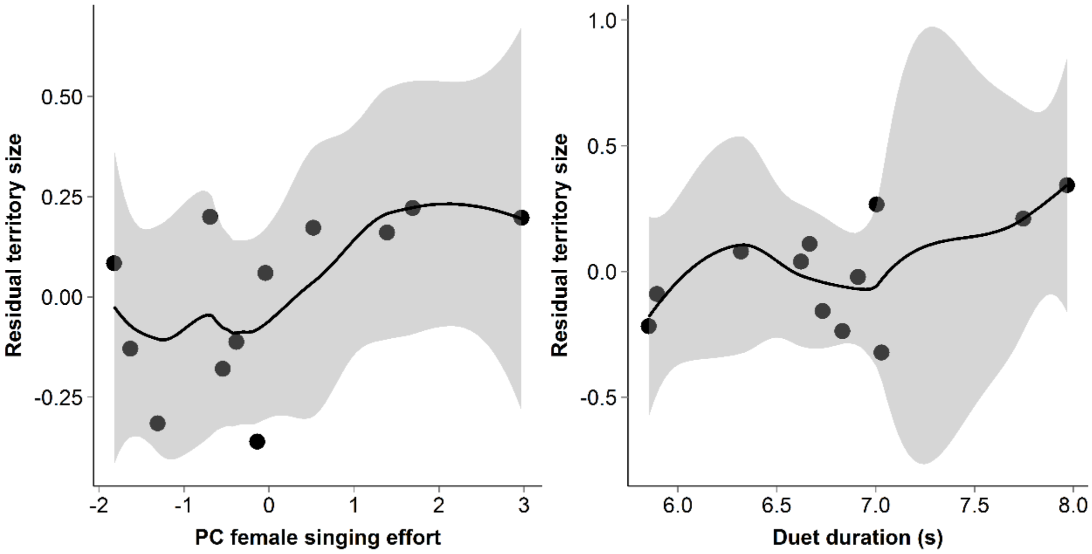

Figure 3-3. Relation between territory size and song traits in the rufous hornero. Territory size is corrected for the effect of duet duration (on left) and "PC female singing effort" (on the right). High values of "PC female singing effort" correspond to high song initiation rate and song output, and low latency to answer partner song. Loess smoothing indicates mean trend (black line) and confidence interval (shadow).

Table 3-1. Extra-pair paternity (EPP) in socially monogamous, duetting, bird species (modified from Douglas et al. 2012, van den Heuvel et al. 2014).

Species	Family	Distribution	EPP (%)	
			Young	Broods
Carolina wren [A]	Troglodytidae	Temperate	0	0
Eastern screech-owl [B]	Strigidae	Temperate	0	0
California towhee [C]	Emberizidae	Temperate	26	42
Crimson-breasted shrike [D]	Malaconotidae	Subtropical	20	30
Purple-crowned fairy-wren [E]	Maluridae	Subtropical	4	6
Red-backed Fairy-wren [F]	Maluridae	Subtropical	47	60
Buff-breasted wren [G]	Troglodytidae	Tropical	4	3
Rufous-and-white wren [H]	Troglodytidae	Tropical	2	6
Pheasant coucal [I]	Cuculidae	Tropical	18.6	47.6
Dusky antbird [J]	Thamnophilidae	Tropical	0	0

[A] *Thryothorus ludovicianus*, Haggerty et al. (2001); [B] *Megascops asio*, Lawless et al. (1997); [C] *Pyrgisoma crissale*, Benedict (2008b); [D] *Laniarius atrococcineus*, van den Heuvel et al. (2014); [E] *Malurus coronatus*, Kingma et al. (2010); [F] *Malurus melanocephalus*, Baldassarre et al. (2016); [G] *Thryothorus leucotis*, Gill et al. (2005); [H] *Thryothorus rufalbus,* Douglas et al. (2012); [I] *Centropus phasianinus*, Maurer et al. (2011); [J] *Cercomacra tyrannina*, Fleischer et al. (1997);.

Table 3-2. Principal component analysis for territory quality variables. Log-transformed variables are indicated.

	'PC nest sites'	'PC foraging patches'
Loadings		
Tree cover – absolute area (ha)	0.66	–0.26
Tree cover – proportion of territory size (%, log)	0.60	–0.31
Grass cover – absolute area (ha)	–0.17	0.71
Grass cover – proportion of territory size (%)	–0.42	0.57
Eigenvalue	1.40	1.25
Variance explained (%)	0.49	0.39

Table 3-3. Principal component analysis for male and female song traits of the rufous hornero. Variables that were log-transformed before the analysis are indicated (log-f = female, log-m = male, log = both sexes).

	Female song		Male song	
	'PC singing effort'	'PC song answering'	'PC singing effort'	'PC song answering'
Loadings				
Number of initiated songs (log)	0.54	–0.27	0.59	0.13
Song output (s, log)	0.60	0.24	0.61	
Song answering rate (%, log-f)	0.38	0.56	–0.27	0.41
Latency to answer partner song (s, log-m)	–0.43	0.21	0.33	0.68
Phrase duration in duets (s)	–0.16	0.72	0.31	–0.59
Eigenvalue	1.37	1.19	1.52	1.04
Variance explained (%)	0.38	0.28	0.46	0.22

Table 3-4. Extra-pair paternity in our study population of the rufous hornero.

Year	% broods with EP nestlings (n broods)	Broods samples	% EP nestlings (n nestlings)	Nestlings sampled
2013	0% (0)	18	0% (0)	52
2014	14.29% (2)	14	6.06% (2)	33
2015	0.06% (1)	14	5.71% (2)	35
Total	6.52% (3)	46	3.33% (4)	120

Table 3-5. Best-ranked models (ΔAICc < 2) resulting from linear models to analyze the interrelation among territory attributes, song traits and breeding success in the rufous hornero. df = degrees of freedom. wi = weight.

Modelling scenario	Predictor variables	df	AICc	ΔAICc	wi
Territory size ~ song traits	null model	2	38.3	0.00	0.37
	'PC female singing effort' + duet duration	4	39.7	1.40	0.18
	duet duration	3	40.2	1.87	0.14
Territory quality ('PC nest sites') ~ song traits	null model	2	38.3	0.00	0.49
	'PC male singing effort'	3	40.3	1.97	0.18
Territory quality ('PC foraging patches') ~ song traits	'PC female singing effort'	3	34.2	0.00	0.56
Number of fledglings produced ~ song traits	null model	1	37.0	0.00	0.33
	'PC male song answering'	2	37.5	0.52	0.26
	duet duration	2	38.8	1.86	0.13
Number of fledglings produced ~ territory attributes	null model	1	37.0	0.00	0.52
Post-fledging survival ~ song traits (individual-level)	'PC male singing effort'	2	19.0	0.00	0.34
	null model	1	19.0	0.08	0.32
Post-fledging survival ~ song traits (pair-level)	duet duration	2	18.7	0.00	0.45
	null model	1	19.0	0.35	0.38
Post-fledging survival ~ territory attributes	null model	1	19.0	0.00	0.51

SUPPLEMENTARY MATERIAL

Figure 3-4s. Perimeters (in yellow) of the 12 study territories in our urban population of rufous horneros, estimated by minimum polygon convex. Aerial image taken on 24 September 2015 at the campus of the University of Brasilia, central Brazil, during the end of the dry season.

Genetic analysis

We used Peterson et al. (2012) protocol to isolate and quantify the SNPs. We extracted the DNA from blood samples using the Qiagen® DNeasy Kit. We added 100µl of blood sample and then 200µl Buffer AL to a solution of 20µl proteinase K and 150µl PBS, and left incubating at 64°C for ~24h. Then, we added 200µl ethanol and retained the digested solution in spin columns. Finally, we watched the solution with buffers AW1 and AW2 and eluted DNA twice (50µl and then 100ul) with warm water (64°C). We measured DNA concentration for all samples with Qubit® dsDNA broad-range assay kit (mixing 2µl of DNA with 198µl Quant-iT^{tm} working solution) in a Qubit® 2.0 fluorometer. DNA samples were diluted or concentrated when necessary to achieve the final, ideal concentrations (mean ± SD = 23.86 ± 5.87ng/ul, range = 15.0 and 35.3ng/ul, n = 240). We polled samples with similar concentration values within the same standard Illumina multiplexing read index (index group, hereafter).

The second step is digest samples and ligate adapters (i.e. short DNA fragments that works as molecular barcordes). We had 20 adapters available to assign to each individual DNA sample, thus we divided our DNA samples into 12 groups of 20 samples (each group corresponded to one index group). A 12.5µl DNA sample from each genomic DNA sample was "double" digest and ligate to a unique adapter (for each index) in a 30µl reaction: 5.75µl water, 210µl CutSmart buffer, 1µl forward adapter (1:20 dilution of 5uM), 2.5µl reverse adapter (undiluted, 25uM), 3µl ATP, 0.75µl *Mspl* enzyme, 0.75µl *Sbfl-HF* enzyme, 0.75 T4 DNA ligase). This solution was incubated in a thermal cycler at 37°C (for 30min) and then at 20° (for 1h).

In the third step, reactions were combined within index groups. We added Serapure beads (a homemade AMPure XP, 1.5x reaction volume) to each reaction and captured beads on magnets (subsequently washed twice with 70% ethanol and eluted with 45µl Qiagen AE buffer, 40µl of supernatant collected). A 2µl of each elution was submitted to the Bioanalyzer at the Biotechnology Resource Center (BRC) at Cornell University for Pippin size selection (450-600bp). DNA fragments of post-pippin samples were amplified (up to five replicates) with Phusion DNA polymerase (reaction: 10µl post-pippin fragments, 12.5µl Phusion master mix, 1.25µl primer P1, 1.25µl index primer). PCRs settings for reactions: 98°C for 30s, 11 cycles at 98° for 5s, 60° for 25s and 72°C for 10s, followed by on cycle of 72°C for 5s. Then we pooled replicates from PCRs products within each index. In this step, each index was assigning to a molecular barcorde (by 12 unique index primers).

In the fourth and last step, we used Serapure beads (0.7x volume of PCR reaction) to discard undesired PCR products following the same procedure described above (except that here beads were re-suspended with 36µl Qiagen AE buffer and 34µl was collected of each of these samples). DNA fragment analysis (for 2µl of each sample) was conducted by the Bioanalyzer at the BRC at Cornell University. Considering concentration and size of DNA at each sample, we calculated and diluted 2nM with water and sent to the BRC for Illumina sequencing read.

Bioinformatics processing of SNPs

All reads from Illumina sequencing were submitted to quality checking and filtering using a series of programs. General quality of reads was accessed with FastQC program. Last 4bp was trimmed and reads with Phred quality score of 10 were discarded. Five percent of reads with Phred quality score of 20 was also discarded. Then, the program process_radtags was used to check for barcodes and demultiplex the data. The program denovo_map.pl was used to execute Stacks pipeline, aligning reads. Corrections to genotype was conducted on individual samples using the program rxstacks (minimum log-likelihood to keep a locus = -50.0). We run “populations” to identify the numbers of loci with potential SNPs. We set r = 0.95 (minimum percentage of individuals to have a locus processed) and m = 10 (minimum stack depth for an individual at a locus). A catalog of 243 identified loci was created (average missing data = 2.22%). We removed the loci that were not in Hardy-Weinberg equilibrium, and 183 out of 243 SNPs loci were kept for paternity analysis.

Table 3-6s. Characterization of 183 SNPs loci isolated from Rufous Hornero genomic DNA (230 individuals) for parentage analysis. N = number of individuals typed, H_{obs} = observed heterozygosity, H_{exp} = expected heterozygosity, NE-1P = average non-exclusion probability for one candidate parent, NE-2P = average non-exclusion probability for one candidate parent (when genotype information from the opposite sex is given).

SNP locus	N	H_{obs}	H_{exo}	NE-1P	NE-2P
119	219	0.370	0.388	0.925	0.844
131	227	0.339	0.388	0.925	0.844
140	225	0.449	0.470	0.890	0.820
164	225	0.440	0.441	0.903	0.828
165	221	0.480	0.501	0.875	0.813
361	228	0.566	0.499	0.876	0.813
465	223	0.381	0.392	0.923	0.843
737	222	0.473	0.500	0.876	0.813
1014	224	0.491	0.484	0.884	0.817
1140	228	0.487	0.496	0.878	0.814
1493	228	0.395	0.393	0.923	0.842
1566	220	0.423	0.443	0.902	0.828
1585	228	0.439	0.426	0.909	0.833
1603	221	0.371	0.375	0.930	0.848
1658	220	0.432	0.419	0.913	0.835
1764	229	0.485	0.443	0.902	0.828
1768	225	0.396	0.424	0.910	0.833
1780	225	0.436	0.396	0.922	0.841
1890	228	0.386	0.409	0.917	0.838
1892	227	0.427	0.392	0.924	0.843
1914	220	0.364	0.393	0.923	0.842
1939	229	0.533	0.499	0.876	0.813
1994	229	0.502	0.469	0.891	0.821
2079	227	0.432	0.499	0.876	0.813
2112	227	0.405	0.398	0.921	0.841
2123	224	0.460	0.481	0.885	0.818
2127	229	0.546	0.501	0.875	0.813
2278	224	0.473	0.437	0.905	0.829
2302	226	0.412	0.423	0.911	0.833
2408	230	0.443	0.395	0.922	0.842
2455	229	0.511	0.464	0.893	0.822
2707	227	0.502	0.497	0.877	0.814
2725	220	0.436	0.441	0.903	0.828
2835	230	0.530	0.501	0.875	0.813
2868	229	0.467	0.462	0.894	0.823
2869	218	0.454	0.414	0.915	0.836
2904	223	0.475	0.497	0.877	0.814
2933	219	0.457	0.448	0.900	0.826
2999	229	0.410	0.404	0.919	0.839

Table 3-6s

3223	227	0.392	0.379	0.928	0.847
3306	228	0.491	0.481	0.885	0.818
3347	223	0.466	0.414	0.915	0.836
3504	230	0.465	0.419	0.913	0.835
3527	223	0.408	0.384	0.927	0.845
3537	220	0.455	0.441	0.903	0.828
3602	228	0.425	0.395	0.922	0.842
3644	221	0.525	0.501	0.875	0.813
3721	229	0.445	0.494	0.878	0.814
3743	228	0.351	0.401	0.920	0.840
3791	224	0.446	0.443	0.902	0.828
3893	226	0.354	0.382	0.927	0.846
3923	225	0.471	0.436	0.905	0.830
3944	229	0.480	0.476	0.887	0.819
3956	220	0.514	0.497	0.877	0.814
3957	230	0.513	0.494	0.879	0.814
3966	218	0.546	0.501	0.875	0.813
4001	229	0.498	0.470	0.890	0.820
4019	224	0.496	0.481	0.885	0.818
4036	230	0.474	0.498	0.876	0.813
4080	229	0.410	0.432	0.907	0.831
4161	227	0.449	0.457	0.896	0.824
4185	227	0.445	0.436	0.905	0.830
4192	223	0.475	0.456	0.897	0.824
4254	225	0.462	0.430	0.908	0.832
4359	223	0.466	0.477	0.887	0.819
4371	224	0.500	0.488	0.881	0.816
4381	218	0.381	0.425	0.910	0.833
4430	229	0.524	0.499	0.876	0.813
4433	230	0.443	0.469	0.890	0.821
4451	226	0.456	0.485	0.883	0.817
4466	225	0.418	0.426	0.910	0.833
4523	228	0.482	0.445	0.901	0.827
4547	222	0.450	0.411	0.916	0.837
4559	221	0.385	0.414	0.915	0.836
4560	229	0.546	0.499	0.876	0.813
4573	229	0.424	0.406	0.918	0.839
4668	223	0.444	0.487	0.882	0.816
4949	221	0.443	0.470	0.890	0.820
4978	220	0.373	0.385	0.926	0.845
5017	220	0.541	0.501	0.875	0.813
5262	228	0.404	0.393	0.923	0.842
5323	222	0.477	0.491	0.880	0.815
5433	219	0.470	0.484	0.883	0.817
5439	221	0.389	0.401	0.920	0.840

Table 3-6s

5453	225	0.400	0.454	0.897	0.825
5479	228	0.465	0.491	0.880	0.815
5488	220	0.468	0.474	0.888	0.819
5503	223	0.395	0.398	0.921	0.841
5508	221	0.480	0.497	0.877	0.814
5531	218	0.463	0.498	0.877	0.813
5558	219	0.447	0.445	0.901	0.827
5575	220	0.418	0.432	0.907	0.831
5580	227	0.419	0.468	0.891	0.821
5583	226	0.527	0.481	0.885	0.818
5596	229	0.528	0.496	0.878	0.814
5666	227	0.427	0.445	0.901	0.827
5686	230	0.522	0.467	0.891	0.821
5695	224	0.411	0.405	0.918	0.839
5719	224	0.406	0.382	0.927	0.846
5731	229	0.511	0.484	0.883	0.817
5733	222	0.523	0.494	0.878	0.814
5812	219	0.438	0.490	0.881	0.815
5941	223	0.439	0.432	0.907	0.831
5970	224	0.357	0.385	0.926	0.845
5984	221	0.398	0.396	0.922	0.841
5986	222	0.441	0.485	0.883	0.817
6099	218	0.417	0.429	0.909	0.832
6101	226	0.372	0.382	0.927	0.846
6111	230	0.430	0.468	0.891	0.821
6254	219	0.502	0.494	0.879	0.814
6353	227	0.432	0.492	0.880	0.815
6360	228	0.496	0.501	0.875	0.813
6433	227	0.449	0.495	0.878	0.814
6435	220	0.495	0.495	0.878	0.814
6470	221	0.443	0.449	0.899	0.826
6507	230	0.483	0.498	0.877	0.813
6593	227	0.498	0.499	0.876	0.813
6650	226	0.447	0.488	0.882	0.816
6654	226	0.482	0.474	0.888	0.820
6702	228	0.447	0.416	0.914	0.836
6745	218	0.436	0.439	0.904	0.829
6748	224	0.464	0.499	0.876	0.813
6760	229	0.459	0.486	0.883	0.816
6763	228	0.412	0.473	0.889	0.820
6769	225	0.422	0.386	0.926	0.845
6782	229	0.467	0.475	0.888	0.819
6800	230	0.391	0.369	0.932	0.850
6851	222	0.374	0.389	0.925	0.844
6948	226	0.442	0.450	0.899	0.826

Table 3-6s					
6976	223	0.475	0.494	0.879	0.814
6995	218	0.422	0.387	0.925	0.844
7037	223	0.516	0.485	0.883	0.817
7041	225	0.516	0.496	0.878	0.814
7059	230	0.452	0.486	0.882	0.816
7089	221	0.475	0.464	0.893	0.822
7101	222	0.509	0.491	0.880	0.815
7146	229	0.415	0.420	0.912	0.834
7166	227	0.441	0.480	0.885	0.818
7230	230	0.448	0.451	0.899	0.826
7387	229	0.467	0.449	0.900	0.826
7400	230	0.526	0.500	0.875	0.813
7452	227	0.489	0.491	0.880	0.815
7490	224	0.415	0.429	0.908	0.832
7527	228	0.364	0.391	0.924	0.843
7572	226	0.504	0.501	0.875	0.813
7810	227	0.410	0.392	0.924	0.843
7828	229	0.454	0.425	0.910	0.833
7998	227	0.515	0.492	0.879	0.815
8185	227	0.392	0.383	0.927	0.845
8347	225	0.431	0.421	0.912	0.834
8448	226	0.451	0.458	0.895	0.824
8499	223	0.408	0.462	0.894	0.823
8565	229	0.498	0.501	0.875	0.813
8708	230	0.478	0.501	0.875	0.813
8723	225	0.484	0.468	0.891	0.821
8784	224	0.531	0.498	0.876	0.813
8813	228	0.522	0.501	0.875	0.813
8901	226	0.465	0.483	0.884	0.817
8946	230	0.452	0.437	0.905	0.829
8985	224	0.455	0.413	0.915	0.836
9081	230	0.422	0.456	0.896	0.824
9089	226	0.456	0.483	0.884	0.817
9162	226	0.527	0.499	0.876	0.813
9223	226	0.504	0.475	0.888	0.819
9250	230	0.448	0.442	0.903	0.828
9270	229	0.432	0.454	0.897	0.825
9283	230	0.396	0.380	0.928	0.846
9294	222	0.414	0.436	0.905	0.83
9296	226	0.491	0.501	0.875	0.813
9306	228	0.461	0.495	0.878	0.814
9314	226	0.491	0.481	0.885	0.818
9348	229	0.463	0.489	0.881	0.815
9401	227	0.414	0.381	0.928	0.846
9498	229	0.546	0.492	0.879	0.815

Table 3-6s					
9501	219	0.365	0.386	0.926	0.845
9632	218	0.468	0.455	0.897	0.824
9722	230	0.448	0.501	0.875	0.813
9752	227	0.330	0.383	0.927	0.845
9838	222	0.441	0.465	0.893	0.822
11505	223	0.363	0.392	0.923	0.843
14446	224	0.424	0.469	0.891	0.821
14516	219	0.470	0.498	0.877	0.813
14533	222	0.491	0.458	0.896	0.824

CAPÍTULO 4

Talking back: responses of a Neotropical bird to duets varying in temporal coordination[5]

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[5] Uma versão deste capítulo será submetida para publicação como "Diniz P, Ramos DM, Webster MS & Macedo RH. Talking back: responses of a Neotropical Bird to duets varying in temporal coordination."

ABSTRACT

Temporal coordination of duets consists of non-random overlap, alternation, or association between rhythms of acoustic elements. Although previous studies suggest that the cooperative nature of duetting behavior is crucial in joint territory defense, it remains unclear whether the temporal coordination of duets plays a role in territorial interactions. Since duet coordination presumably requires high attentiveness between signalers, the coalition quality hypothesis suggests it may indicate the ability or motivation of partners to engage in aggressive interactions. To test this hypothesis, we monitored behavioral responses in a playback experiment conducted with a polyphonal bird duetter, the rufous hornero, *Furnarius rufus*. We used three categories of treatments in the experiment, which totaled six treatments: (1) duet playbacks that varied in phrase overlap and coordination of temporal rhythms; (2) non-overlapped (consecutive) solos; and (3) a control, using heterospecific song. We predicted that birds would respond more strongly to the playback of coordinated duets than to uncoordinated duets, and to uncoordinated duets than to non-overlapping male and female solo songs. Partners coordinated 90% of their song responses into duets across playback treatments. In general, both sexes approached the speaker, sang more quickly and for longer periods in response to conspecific than heterospecific songs. Although birds apparently distinguish songs, varying in rhythmic coordination and overlap (as shown by slight behavioral differences in responses among conspecific playback treatments), they responded with similar aggressiveness to all conspecific songs in terms of closest approach to speaker, time spent in territorial vigilance, latency to sing, song rate, song duration, and acoustic frequency parameters of duets. Our results, therefore, do not support the coalition quality hypothesis for the role of temporal coordination in duets. We suggest that temporal coordination in rufous hornero duets might function in other ways, such as to improve signal propagation or within-pair communication.

Keywords: coalition quality hypothesis, duetting, joint territory defense, polyphonal duets, playback experiment, Rufous Hornero.

INTRODUCTION

Vocal communication is crucial to mediate interactions between organisms that result in differential survival or mating success, thus shaping the evolution of social behavior (Benedict 2010). An interesting case of vocal communication occurs when animals exchange vocalizations with others in a coordinated way (Todt and Naguib 2000; Catchpole and Slater 2008). Vocal coordination of timing involves association of signaling rhythms (Laje and Mindlin 2003; Benichov et al. 2016), alternation (Rivera-Cáceres 2015) and/or overlap of vocal signals (Rehberg-Besler et al. 2016). Examples of vocal coordination are abundant across many different taxa, and include: anuran choruses (Schwartz et al. 2002; Rehberg-Besler et al. 2016), call exchange in bats (Carter et al. 2009), call overlap in whales (Schulz et al. 2008), turn talking in humans (Stivers et al. 2009; Benichov et al. 2016), male-male interactions in birds (Yang et al. 2014; Araya-salas et al. 2017), and vocal duets in many avian taxa (Hall 2004; Hall 2009; Templeton et al. 2013a). Duets are a special case of vocal coordination where partners join their vocalizations for multiple purposes, especially to defend common territories (Hall 2004; Hall 2009; Dahlin and Benedict 2013).

Duets are classified into two groups depending on their degree of timing coordination: antiphonal or polyphonal duets (Hall 2009; Kovach et al. 2014). Antiphonal duets involve precise alternation of male and females notes or phrases with minimum or no overlap (e.g. plain wrens, *Cantorchilus modestus zeledoni*; Mann et al. 2003). In polyphonal duets, male and female overlap phrases temporally and in frequency (e.g. white-eared ground-sparrows, *Melozone leucotis*; Sandoval et al. 2015), or only temporally (e.g. pheasant coulcals, *Centropus phasianinus*; Maurer et al. 2008), without a clear coordination of timing. However, even among polyphonal duetters that exhibit apparently uncoordinated duets, there may be coordination of song rhythms (Todt et al. 1981; Laje and Mindlin 2003; Amador et al. 2005) or overlap itself may be a form of coordination. Highly overlapped duets may demand a fast song response by an individual to its partner´s song when creating or ending a duet. The existence of such a fast response may thus indicate level of attention (Smith 1994) or spatial proximity between members of a social pair (Logue 2007). Hence, duet coordination cannot be evaluated simply as a gradient between polyphonal and antiphonal duets, but should be considered within a social context.

Three behavioral mechanisms have been suggested to explain temporal coordination in duets (reviewed by Rivera-Cáceres 2015). A bird can produce songs in a fixed reaction norm pattern, that is with inflexible tempo, that can be triggered by an initial cue such as song initiated by its partner (e.g. african barbets; Payne and Skinner 1970). In a second mechanism, known as autogenous feedback (Logue et al. 2008), a bird may modify its singing tempo during the development of a duet based on its own preceding note timing. A third mechanism, called heterogeneous feedback (Fortune et al. 2011), is when a bird modifies its singing tempo based on its partner´s preceding rhythm. A few previous studies on this topic have found empirical evidence for both the autogenous and heterogenous hypotheses to explain temporal coordination in antiphonal duetters among species of Neotropical wrens (Logue et al. 2008; Fortune et al. 2011; Templeton et al. 2013; Rivera-Cáceres 2015).

Why should individuals overlap or alternate songs, or coordinate rhythms into duets? Duet timing often involves a high degree of attentiveness to a partner´s behavior (Smith 1994), and this may be informative to territorial rivals (Hall and Magrath 2007; Kovach et al. 2014). In this sense, duet coordination may reflect the coalition quality of cooperative partners or their motivation to fight in territorial contests (Hall and Magrath 2007; Kovach et al. 2014). Alternatively, animals may overlap their songs to enhance the propagation of duet signals across greater distances (Rehberg-Besler et al. 2016), especially in species with polyphonal, loosely coordinated duets. Finally, sexual conflict may drive birds to join a partner’s songs into duets in an attempt to jam or mask their partner’s songs (Seddon and Tobias 2006; Dahlin and Wright 2007; Hall 2009; Tobias and Seddon 2009). In sum, temporal precision could arise as a mechanism to avoid signal interference in contexts involving either sexual conflict or cooperation (Hall 2009; Tobias and Seddon 2009). Therefore, it is crucial to fully understand the causes of temporal precision in duets, as this will allow us to determine whether the nature of duetting is based on conflict or cooperation between partners (Hall 2009).

Some playback studies of territorial birds reveal that from a receiver´s perspective, duets are more threatening than solos, which suggests that duetting may function in the maintenance of territory boundaries (Molles and Waas 2006; Douglas and Mennill 2010; Weng et al. 2012; Dowling and Webster 2016). However, we know very little about what elements of duets make them more threatening or more efficient in defending territories when compared with solo songs (Hall 2009; Kovach et al. 2014). First, duets may reflect the spatial cohesion and readiness of two birds to defend a common territory (Logue 2007; Hall and Peters 2008; Mennill and Vehrencamp 2008), and thus two birds would impose a greater threat to territorial individuals than would a solo intruder (numerical advantage hypothesis) (Molles and Waas 2006; Douglas and Mennill 2010; Kovach et al. 2014). A second explanation, called the coalition quality hypothesis, has been suggested to explain how duets may function in territorial defense ((Hall and Magrath 2007; Kovach et al. 2014). This hypothesis predicts that the degree of threat posed by a rival pair could be estimated by the coordination properties of their duets, which would signal their ability, motivation, cooperation and/or readiness to defend or acquire a territory.

Only two studies have tested the coalition quality hypothesis using a receiver perspective (Hall and Magrath 2007; Kovach et al. 2014). In response to coordinated (precisely alternating notes) versus uncoordinated duets (overlapping notes), magpie-larks (*Grallina cyanoleuca*) sang at higher rates, suggesting that temporal precision in duets increases perceived threat level (Hall and Magrath 2007). In contrast, three studied species of Neotropical wrens did not perceive coordinated duets as more threatening than uncoordinated duets or alternating solos (Kovach et al. 2014).

Duets are common among tropical birds. Rufous horneros (*Furnarius rufus*) are socially monogamous Neotropical suboscines, with year-round territoriality (Fraga 1980; Massoni et al. 2012; Remsen and Bonan 2017). In this species, partners coordinate the majority of their songs into duets, and duetting serves as a cooperative behavior associated with the defense of common territorial resources and mutual mate guarding (Diniz 2017) (Chapters 1 and 2). Their duets are polyphonal with highly overlapped distinct male and female phrases (Roper 2005; Diniz 2017). Rufous hornero duets have non-random degrees of phrase overlap, random note overlap, and variable within-duet, male and female note rates (acceleration, deceleration and fixed tempo) (Laje and Mindlin 2003; Roper 2005; Amador et al. 2005; Diniz unpublished data). Mechanistic studies show that these birds coordinate phrase rhythms in response to their partner´s songs (i.e. heterogeneous feedback; both sexes) and internal tempo (i.e. autogenous feedback; males only) (Laje and Mindlin 2003; Amador et al. 2005; Diniz unpublished data). These patterns make the rufous hornero a useful model to test the effect of rhythm coordination and phrase overlapping in the context of territorial defense.

In this study, we used the rufous hornero to test the coalition quality hypothesis (Hall and Magrath 2007) as an explanation for the temporal precision in duets, which in turn could explain why birds normally respond more aggressively to duets than to solo songs. According to the coalition quality hypothesis, temporal coordination in duets reflects quality or motivation of a coalition to fight for resources, such as territories (Hall and Magrath 2007). Thus, we predicted that birds would respond more strongly to the playback of coordinated duets when compared with playbacks of uncoordinated duets. A second prediction is that they would respond more strongly to uncoordinated duets than to non-overlapping male and female solo songs. Since rufous horneros perform duets that are loosely alternated, highly overlapped, but rhythm coordinated (Laje and Mindlin 2003), we used playbacks of duets varying in the degree of phrase overlap and phrase rhythm coordination as two measures of the umbrella concept of ‘duet coordination’.

METHODS

a) Study area and field methods

We conducted a playback experiment on 13 rufous hornero mated pairs (total of 26 birds) from an urban population on the campus of the University of Brasilia, central Brazil (15°45’S, 47°51’W). Before the experiment, we assigned the sex of each individual based on sex-specific song types (Roper 2005). Both adults were banded in five mated pairs, and only one adult was banded in each of the remaining eight mated pairs (three males banded and five females banded). The experiment occurred during the pre-nesting season in August and September 2015. Descriptions of banding and trapping methods can be found in Diniz et al. (2016). The study area has 395 ha covered by green spaces (42% of the area) and buildings (13% of the area) (Universidade de Brasília 2012). Isolated tall ornamental trees within a matrix of short grasses characterize the green areas. The area has two seasons, a dry (from May to September) and a wet (from October to April).

b) Playback design

The experiment consisted of broadcasting playback duet stimuli varying in the (a) degree of overlap and (b) rhythm coordination between male and female phrases (see Marshall-Ball et al. 2006; Hall and Magrath 2007). Our playback design consisted of six treatments in which subjects were exposed to: (1) highly overlapped natural duet, (2) loosely overlapped natural duet, (3) highly overlapped synthetic duet, (4) loosely overlapped synthetic duet, (5) non-overlapped conspecific solo songs, and (6) a control stimulus of song of great kiskadees (*Pitangus sulphuratus*). The highly and loosely overlapped natural duets were obtained from recordings of spontaneous song of birds in our population. The highly and loosely overlapped synthetic duets, as well as the non-overlapping solo songs, on the other hand, were produced synthetically, as described below (Figure 4-1; Figure 4-2; Table 4-1).

Since rufous hornero partners actively coordinate phrase rhythms in duets (Laje and Mindlin 2003; Diniz unpublished data), we manipulated phrase rhythm coordination by producing synthetic duets containing overlapping solos, and then comparing responses of bird subjects to playbacks of natural duets versus synthetic duets. Natural duets are representing duets with rhythmic coordinated phrases, and synthetic duets are representing duets with rhythmic uncoordinated phrases.

We considered the degree of phrase overlap in duets as the proportion of the duet duration wherein male and female phrases overlapped in the temporal scale. To manipulate phrase overlapping in duets, we first created synthetic duet stimuli of overlapping solos with variable degrees of overlapping. We also retrieved natural duets with extreme degrees of phrase overlapping from our dataset (Diniz 2017) (Chapter 1) obtained from our population to use as playback stimuli.

Natural duet

To create the overlapped natural duet stimuli, we used a dataset of 166 high quality, spontaneous (i.e. non-playback induced) duets recorded from 36 pairs in our study population (recording apparatus: Marantz PMD660 recorder, Sennheiser ME66 microphone, settings: WAVE format, sampling rate = 48kHz, resolution = 24-bits). Synthetic stimuli are often preferred over natural stimuli, because the former control for other acoustic variables correlated with the variable of interest (e.g., Kroodsma et al. 2001; Cator et al. 2010; Reichert and Ronacher 2015). For instance, in rufous hornero natural duets, the degree of song overlapping may be correlated with duet duration (Diniz unpublished data). However, male and female horneros overlap their songs into duets in both temporal and frequency domains (Laje and Mindlin 2003; Roper 2005), which makes it impossible to extract male and female phrases from a duet. Moreover, rufous hornero partners coordinate rhythms in polyphonal duets (Laje and Mindlin 2003; Diniz unpublished data) and the creation of synthetic stimuli might collapse duet coordination.

To create overlapped natural duet stimuli, we first used Raven Pro 1.5 (Bioacoustics Research Program 2014) to apply a high pass filter (500 kHz) to remove background noise from all recordings in our dataset, and then normalized peak amplitude (-0.1 dB) across all the duets using Audacity 2.1.0 (http://audacity.sourceforge.net). Then, we removed duet recordings with extreme values of song overlap and transformed the variable ‘degree of song overlap’ to arc sine in order to achieve a normal distribution, using R (R Core Team 2015). We selected duet recordings in the lower and upper 20% ranges of overlap, and created four subsets of spontaneous duets that varied in degree of song overlap and initiator sex: (i) female-initiated and loosely overlapped, (ii) male-initiated and loosely overlapped, (iii) female-initiated and highly overlapped, and (iv) male-initiated and highly overlapped. Finally, we chose five duets from each of these four subsets to create the stimuli set of natural duets.

Synthetic duet

To create synthetic duet stimuli, which consisted of overlapped solos and non-overlapped solos, we selected nine male and nine female solo songs from our song dataset. All solo recordings were high-pass filtered (500 kHz) and then normalized (-0.1 dB, peak amplitude). We amplified female solo stimuli (0 dB, peak amplitude), because females sing at higher amplitude than do males in duets (Diniz, unpublished data). We created four non-repeated combinations of random male and female solos, totaling 36 synthetic stimuli (18 female-initiated and 18 male-initiated).

We used the above-mentioned dataset of spontaneous duet recordings to set the parameters and create synthetic stimuli. First, we removed outliers and arcsine transformed the variable ‘degree of song overlap in duets’ to achieve normality in data error distribution. Then, we obtained the 20% and 80% quantiles from a normal population distribution. We considered values below the 20% quantile as loosely overlapped duets and those higher than the 80% quantile as highly overlapped duets.

We averaged song overlap values within social pairs separately for each of the two data subsets of natural, highly overlapped and loosely overlapped duets. Then, we calculated the mean and SD for the ratio between degree of song overlap of loosely overlapped natural duets and highly overlapped natural duets. Finally, we used these mean and SD ratio values to create random values of overlap ratio between loosely and highly overlapped synthetic duets from normally distributed data, using R (R Core Team 2015). We also generated random latency values from the 20% range of highly overlapped natural duets in order to increase the similarity between synthetic stimuli and natural duet. We randomly allocated the overlap ratio values across our 36 male-female solos stimuli combinations. In addition, we randomly allocated latency values across our 18 male-female solos combinations for the highly overlapped synthetic duet treatment. To create non-overlapped solos, we added a 1 sec interval between solos at each male-female solos stimuli combinations in Audacity.

To produce the heterospecific stimuli we recorded nine songs from synoptic and different great kiskadee individuals (or pairs), and used an additional song recording from a nearby population of this species (recording apparatus: Song Meter SM2, settings: WAVE format, sampling rate = 44.1kHz, resolution = 16-bits). We randomly allocated each conspecific and heterospecific stimulus to each studied breeding unit. All stimuli for the six treatments were stored at WAVE files for broadcasting (sampling rate = 48kHz, resolution = 16-bits).

c) Playback trials

We subjected four socially paired birds in the field to playbacks of male-initiated duet stimuli, and eight pairs to female-initiated duet stimuli. We subjected one social pair to playback of mixed stimuli in terms of duet initiation. The social pairs were exposed to six playback treatments, except one pair that started to incubate before the last trial and thus was exposed to only five treatments. For each social pair, we played back the six stimuli during the mornings of non-consecutive days (mean ± SD = 4.94 ± 3.69 day-intervals; N = 64 intervals). The broadcast order of playback treatments was randomly taken from a pool of unique set of stimuli for each social pair, and, thus, there was no repetition of playback order across social pairs. The identity of the stimulus set of synthetic duets and non-overlapped solos was randomly taken for each pair from a pool of unique stimulus set. In other words, each pair was exposed to playbacks of synthetic duets and non-overlapped solos made from the unique male-female solos. Finally, we subjected each social pair to randomly taken unique overlapped natural duet stimuli. We made sure all stimuli came from birds of non-contiguous territories relative to the focal pairs (Radford 2005; Wiley 2013; Diniz 2017) (Chapter 2).

We used a single-speaker design to produce playbacks of the stimuli songs. This method has disadvantages compared with the dual-speaker design, which provides a spatially more realistic scenario (Douglas and Mennill 2010). However, it is impossible to extract male and female contributions from natural rufous hornero duets to make stereo stimuli (Roper 2005; Hall and Peters 2008; Diniz 2017) (Chapter 2). In addition, the single-speaker playback design was previously tested in rufous horneros, revealing that these birds are able to successfully distinguish among playbacks of duets, male and female solos (Diniz 2017) (Chapter 2). Finally, rufous hornero partners normally sing close to each other (<1m) in aggressive and non-aggressive contexts, producing results from the single-speaker design similar to those from dual-speakers.

We used a Harman Kardon Onyx Studio, Bluetooth speaker to broadcast the stimuli. For each trial, we positioned the speaker inside the territory, faced upward at 0.5 m above the ground. The maximum amplitudes of sound stimuli were calibrated to 92 dB in silent conditions. This is approximately the amplitude of rufous hornero duets as measured in the field with a sound level meter (model SEW 2310SL; Diniz 2017) (Chapter 2). We broadcast each sound stimulus (one song per trial) from a cellphone using VLC, a WAVE player application for Android. We only triggered the stimulus when both partners were less than 30m from the speaker.

One or two observers recorded the birds' physical and vocal responses to playbacks using a Marantz PMD660 recorder and a Sennheiser ME66 microphone. We tracked the focal birds until they both resumed foraging or nest building and each one had sung at least one song. Trials lasted 12.95 ± 7.15 min (mean ± SD, n = 77 trials). Fifteen seconds before and after broadcasting the sound, we estimated the distance between birds and the horizontal distance between each bird and the speaker. We could not estimate these distances in a few trials: bird to speaker (both sexes) in pre-playback period (one trial); female to speaker in post-playback period (two trials), male to female in pre-playback period (11 trials); and male to female in post-playback period (one trial).

d) Responses to playback

This species normally sings only once in the 5 min following conspecific playbacks and does not sing very often in non-playback contexts (Diniz 2017) (Chapters 1 and 2). To measure playback responses, we adopted a similar approach used in a previous playback study (Diniz 2017). We measured four variables for physical response, and seven variables for vocal response (Table 4-2). Three responses variables were modelled as functions of conspecific, but not heterospecific, treatments: closest approach, territorial vigilance and song rate.

We did not obtain acoustic frequency measures for solos, because only ~10% of songs emitted in response to playbacks were solos (see also Diniz 2017) (Chapter 2). We obtained the following parameters: mean and median frequencies, first and third quantiles, interquantile range, spectral entropy and frequency centroid (Araya-Salas and Smith-Vidaurre 2016; Diniz 2017) (Chapter 2). These parameters (<500 kHz deleted, spectrogram window length = 1024, amplitude threshold = 15%) were reduced with a principal component analysis (PCA, Quinn and Keough 2002), and two scores with eigenvalues greater than 1 were retained (Table 4-3s).

We analyzed field recordings in Raven Pro 1.5 (Bioacoustics Research Program 2014), and obtained acoustic frequency measures of duets in R (warbleR package, Araya-Salas and Smith-Vidaurre 2016).

e) Statistical analyses

Statistical analyses followed a similar approach as described in Diniz (2017) (Chapter 2). We analyzed data in R (R Core Team 2015) with mixed modelling (lme4 function, Bates et al. 2015; Table 4-4s). All playback response variables were analyzed separately and assuming a Gaussian error distribution, except song rate (Poisson) and singing role (Binomial; Table 4-4s). We did not combine our response variables with a PCA analysis (McGregor 1992), except the frequency parameters, because these variables differ in sample size and not all are normally distributed (Quinn and Keough 2002).

We included playback treatment, sex and the interaction between these two variables in all global (i.e. first) models. We also included playback order (e.g. second) and stimulus duration (s) as fixed effects in all global models, the latter to control for varying stimulus duration among playback treatments (Table 4-1). The order of vocal response (e.g. second song in a trial) was added as a covariable to model vocal response variables, except for latency to sing and song rate. Singing role was added as a covariable to model song duration (Table 4-4s). To model song rate, we added the time (h, log) we spent observing the bird as an offset. We included group and stimulus identities as random effects in all global models. Individual identity was added nested within group to model response variables at individual level. Finally, the playback trial was added nested within individual and group identities as random effect to model vocal response variables, except latency to sing and song rate. Response variables were arc sine or log-transformed to achieve normality (see Table 4-4s). Predictors were scaled before model selection to obtain comparable coefficients.

Model selection followed a stepwise backward procedure to find the best-fitted model. We applied likelihood ratio test (LRT) to remove ($p \geq 0.1$) or maintain ($p < 0.1$) predictor variables (Zuur et al. 2009). We made post hoc comparisons of predictor levels retained in the best model using the functions “glht” (Hothorn et al. 2008) and “lsmeans” (Lenth 2015). One outlier detected from boxplot inspection was removed to analyze PC2 for acoustic frequency measures.

RESULTS

Physical responses

Both males and females reduced their distance to the speaker after the playback of conspecific songs (LMM: playback treatment: $\chi^2 = 33.89$, df = 5, $p < 0.0001$, $N = 76$ trials; all $\beta < -9.48$, $t < -4.08$, $p < 0.0002$; mean ± se = 11.18 ± 0.52 m approached, $N = 63$ trials, data pooled), but not in response to heterospecific songs (mean ± se = –0.19 ± 0.45 m approached, $N = 13$ trials). Closest approach to the speaker was negatively correlated with conspecific stimulus duration (LMM: $\chi^2 = 6.16$, df = 1, $p = 0.013$, $N = 64$ trials; $\beta \pm se = -0.28 \pm 0.11$), but did not vary across conspecific playback treatments (LMM: $\chi^2 = 2.12$, df = 4, $p = 0.71$, mean ± se = 4.31 ± 0.42 m, data pooled; Figure 4-3A), when controlling for stimulus duration. Model coefficients reveal that partners stayed closer to each other after the playback of conspecific songs ($\beta < -1.02$, $t < -2.16$, $p < 0.03$), except in highly overlapped synthetic duet ($\beta \pm se = -0.63 \pm 0.48$, $t = 1.33$, $p = 0.19$), in comparison with heterospecific songs (LMM: $\chi^2 = 17.53$, df = 5, $p = 0.0039$, $N = 76$ trials; Figure 4-3B).

For both sexes, the time spent in territorial vigilance was positively correlated to conspecific stimulus duration (LMM: $\chi^2 = 11.44$, df = 1, $p = 0.0007$, $N = 64$ trials; $\beta \pm se = 0.24 \pm 0.07$) and negatively correlated to playback order (LMM: $\chi^2 = 6.17$, df = 1, $p = 0.013$; $\beta \pm se = -0.14 \pm 0.06$). However, the time spent in territorial vigilance for either males or females was not affected by conspecific playback treatments (LMM: $\chi^2 = 2.10$, df = 4, $p = 0.72$; Figure 4-3C), when controlling for stimulus duration and playback order.

Vocal responses

Most of the songs emitted by focal birds across playback treatments were duets (90%, $n = 134$ songs). The latency to sing was lower after the playback of conspecific songs (median < 6.38s, 95% CI = 6.00–6.63s) than after the playback of heterospecific songs (median = 730.56s, 95% CI = 468.32–1016.46; LMM: playback treatment: $\chi^2 = 75.66$, df = 5, $p < 0.0001$, $N = 75$ trials $\beta < 3.98$, $t < 8.13$, $p < 0.0001$; Figure 4-4A), regardless of sex (LMM: playback treatment × sex: $\chi^2 = 4.76$, df = 5, $p = 0.45$). However, the latency to sing did not vary across conspecific playback treatments (post-hoc tests: all $p > 0.05$). Males tended to sing more quickly than females after the playback (LMM: sex: $\chi^2 = 3.27$, df = 1 $p = 0.07$; $\beta \pm se = -0.36 \pm 0.19$; Figure 4-4A).

Neither female nor male song rates varied among conspecific playback treatments (GLMM: playback treatment: $\chi^2 = 0.72$, df = 4, $p = 0.95$, $N = 64$ trials; Figure 4-4B), and song rate did not differ between the sexes (GLMM: $\chi^2 = 0.36$, df = 1, $p = 0.55$). For both sexes, song rate increased with playback order ($\chi^2 = 5.87$, df = 1, $p = 0.015$; $\beta \pm se = 0.16 \pm 0.07$) and tended to be negatively related to stimulus duration ($\chi^2 = 3.68$, df = 1, $p = 0.055$; $\beta \pm se = -0.12 \pm 0.07$).

Males initiated 64% of the duets ($N = 118$ duets) across playback treatments. Singing role (i.e. song initiator or song responder) was affected by the interaction between sex and playback treatment (GLMM: $\chi^2 = 15.92$, df = 5, $p = 0.007$, $N = 75$ trials; Figure 4-4C). Females were more likely to initiate than answer songs in response to the playback of non-overlapped solos compared with synthetic duets (loosely overlapped synthetic duet: $\beta \pm se = 1.78 \pm 0.71$, $z = 2.51$, $p = 0.012$; highly overlapped synthetic duet: $\beta \pm se = 1.71 \pm 0.75$, $z = 2.70$, $p = 0.023$). Accordingly, males were more likely to answer than to initiate songs in response to playbacks of non-overlapped solos compared to loosely overlapped synthetic duet ($\beta \pm se = -1.49 \pm 0.68$, $t = 2.21$, $p = 0.027$). Males were also more likely (though marginally non-significantly) to answer than to initiate songs in response to highly overlapped synthetic duet ($\beta \pm se = -1.40 \pm 0.72$, $t = 1.95$, $p = 0.051$; Figure 4-4C).

Similarly, the latency to answer partner-initiated songs tended to vary with playback treatment (LMM: $\chi^2 = 9.68$, df = 5, $p = 0.085$, $N = 75$ trials; Figure 4-4D), regardless of the bird’s sex (LMM: playback treatment × sex: $\chi^2 = 1.75$, df = 5, p = 0.88). Post-hoc tests indicate that birds answered their partner´s songs more quickly to create duets in response to the playback of loosely overlapped natural duets (β ± se = –0.97 ± 0.35, $t = 2.74$, p = 0.0096) and loosely overlapped synthetic duets (β ± se = –0.72 ± 0.34, $t = 2.11$, p = 0.04) in comparison with heterospecific songs. In addition, birds answered their partner’s songs more quickly after the playback of loosely overlapped natural duets than after the playback of non-overlapped solos (β ± se = –0.64 ± 0.30, $t = 2.13$, p = 0.04; Figure 4-4D).

Song duration (length of duet phrases or solos) varied with playback treatment (LMM: $\chi^2 = 13.92$, df = 5, p = 0.016, $N = 75$ trials; Figure 4-4E), singing role (LMM: $\chi^2 = 17.10$, df = 1, p < 0.0001) and order of the vocal response (LMM: $\chi^2 = 3.91$, df = 1, p = 0.048), and tended to vary with sex (LMM: $\chi^2 = 3.33$, df = 1, p = 0.068). Both sexes sang longer songs in response to the playback of natural duets (highly overlapped natural duets: β ± se = 1.31 ± 0.52, $t = 2.51$, p = 0.016; loosely overlapped natural duets: β ± se = 1.72 ± 0.51, $t = 3.38$, p = 0.001), non-overlapped solos (β ± se = 1.08 ± 0.48, $t = 2.24$, p = 0.030) and loosely overlapped synthetic duets (β ± se = 1.45 ± 0.48, $t = 3.02$, p = 0.004) when compared with heterospecific songs. Males tended to sing longer songs than females (β ± se = 0.41 ± 0.22), regardless of playback treatment (LMM: playback treatment × sex: $\chi^2 = 2.32$, p = 0.80; Figure 4-4E).

The frequency parameters of pairs’ duets were not affected by playback treatment (PC1, LMM: $\chi^2 = 2.01$, df = 5, p = 0.85; PC2, LMM: $\chi^2 = 2.53$, df = 5, p = 0.77, $N = 66$ trials). The degree of phrase overlap by partners in duets tended to be influenced by playback treatment (LMM: $\chi^2 = 9.54$, df = 5, p = 0.089, $N = 75$ trials). Partners tended to show a higher degree of phrase overlap in response to loosely overlapped natural duets compared with non-overlapped solos (β ± se = 0.21 ± 0.07, $t = 2.32$, p = 0.03) and heterospecific songs (β ± se = 0.14 ± 0.06, $t = 2.86$, p = 0.007; Figure 4-4F). Detailed statistic results can be found in the Supplementary Material.

DISCUSSION

The coalition quality hypothesis (Hall and Magrath 2007) suggests that temporal coordination in duets signals ability or motivation of partners to engage in aggressive interactions with outsiders (Hall and Magrath 2007). Thus, we expected a higher response to coordinated than uncoordinated duet playbacks in our experiment with Neotropical rufous hornero pairs. In general, partners responded more strongly to conspecific than to heterospecific songs, approaching the speaker, staying closer to each other and singing promptly and for longer periods. These responses to the playbacks converged between the sexes and across the treatments to which birds were exposed.

Relative to the five conspecific treatments representing uncoordinated and coordinated duets (Table 4-1), we found that neither male nor female responses varied in terms of: closest approach to the speaker, time spent in territorial vigilance, latency to sing, song rate and duration, and acoustic frequency parameters of duets. Therefore, our results do not support the coalition quality hypothesis in attempting to explain two potentially important measures of temporal coordination in polyphonal duetting species: association of phrase rhythms (Laje and Mindlin 2003) and phrase overlap degree (which means rapid reaction time, Smith 1994). The only two other studies of polyphonal duetting species (banded wrens, *Thryothorus pleurostictus*; rufous-and-white wrens, *Cantorchilus modestus*) produced similar results, showing no difference in their responses to coordinated duets, uncoordinated duets and alternated solos (Kovach et al. 2014). In fact, the coalition quality hypothesis has only been supported in magpie-larks, an antiphonal duetter (Hall and Magrath 2007). Our results, taken together with the two studies of polyphonal duetting species, suggest that duet coordination almost certainly does not play a role in threat assessment by territorial polyphonal duetters.

Although rufous horneros apparently responded more strongly to non-overlapped solos, by approaching the speaker more closely and spending more time in territorial vigilance (Figure 4-3), this pattern disappeared when controlling for stimulus duration. Conspecific stimulus duration varied inversely with closest approach to the speaker, and was positively correlated with time spent in territorial vigilance for both sexes. These results suggest that longer duets represent a higher level of threat to rufous horneros, leading them to stay closer to the speaker and spend more time in an alert state. In other birds tested, duration of stimulus affects the response to playbacks and also elicits longer duration of song responses (Lattin and Ritchison 2009; Linhart et al. 2012). Our results suggest that song duration may be relevant in mediating aggressive interactions in rufous horneros and should be considered in future playback studies of duetting species.

Partners responded slightly differently across conspecific playback treatments in terms of singing role, latency to answer partner song and degree of song overlap in duets, suggesting birds were able to distinguish among playback stimuli (see Kovach et al. 2014). For example, females were more likely to initiate songs than males when responding to non-overlapped solos, but not to other conspecific playback treatments. Most non-overlapped solos stimuli used here were initiated by female solo song (see Methods: Playback stimuli). Thus, our results suggest females take the lead in territorial defense against other females in the breeding season. However, females were not more likely to initiate songs in response to female solo songs versus male solo songs in the non-breeding season (Diniz 2017) (Chapter 2), when females show the highest rate of song initiation (Diniz 2017) (Chapter 1). Thus, it remains unclear whether singing role in territorial horneros is sensitive to intruder sex or level of threat.

If temporal coordination does not signal coalition quality, why do rufous horneros (and possibly other polyphonal duetters) actively coordinate phrase timing into duets in such complex ways (Laje and Mindlin 2003; Kovach et al. 2014)? Perhaps the signaling of coalition quality depends upon other measures of coordination not investigated here, such as consistency in reaction times (Thorpe 1963; Farabaugh 1982; Hall 2009). Consistency in reaction times is the variation in timing measures between duets, that is, repeatability in the interval length between consecutive notes in antiphonal duets (Kroodsma et al. 1987; Hall 2009; Rivera-Cáceres 2015). This is particularly plausible if consistent reaction timing across duets is difficult to achieve in comparison with the coordinated phrases within a single duet. However, rufous horneros (Diniz 2017) (Chapter 1) and other Neotropical birds do not sing very often (e.g. plain wrens, Cuthbert and Mennill 2007; rufous-naped wren, *Campylorhynchus rufinucha*, Bradley and Mennill 2009), even when responding to simulated intruders (Diniz 2017) (Chapter 2), which implies that rivals have little opportunity to evaluate precision in reaction times. Future studies could submit polyphonal duetters (with high song rates) to interactive playbacks of duets varying in reaction times, to test the role of consistency in reaction times upon territorial interactions.

Instead of signaling coalition quality, an alternative explanation for phrase overlap is that it could increase signal amplitude, allowing communal signals (such as duets) to propagate to greater distances compared with solo songs (signal enhancement hypothesis: Rehberg-Besler et al. 2016). In this context, perfectly overlapped phrases in duets would be favored. However, although rufous horneros overlap their phrases, their notes are not perfectly overlapped (Laje and Mindlin 2003; Roper 2005), suggesting the maintenance of individual and sex acoustic elements that might be important. Inter-duet variation in phrase overlap may also be a by-product of individual location relative to the partner bird (Hall 2004; Logue 2007; Mennill and Vehrencamp 2008). For example, an individual may take longer to answer a partner song when the partner is farther away, resulting in decreased phrase overlap.

Within-pair communication can be another explanation for temporal coordination in duets. For instance, in zebra finches, partners coordinate private calls during nest relief in the incubation phase (Elie et al. 2010). Call structure and acceleration in duets influence the time parents spend incubating and foraging, suggesting a vocal negotiation over parental care by parents (Boucaud et al. 2016). In rufous horneros, temporal precision in duets, and duet structure in general, may be used by partners to inform and negotiate time budgets in foraging, territorial vigilance and breeding activities, or even to synchronize these activities.

Although several playback studies have compared the response of territorial birds to playbacks of duets versus solos (reviews: Hall 2004; Hall 2009; Douglas and Mennill 2010; Dahlin and Benedict 2013), they have not clarified whether the birds react to the number of simulated intruders (one or two) or degree of coordination of duets.

Our study contributes towards solving this particular issue by isolating these factors, by simulating the intrusion of pairs either overlapping (i.e. duets) or not overlapping their songs (i.e. non-overlapped solos). Since rufous horneros respond more strongly to duets than to solos (Diniz 2017) (Chapter 2), and here we found no effect of temporal coordination of duets upon aggressiveness, our results suggest that the numerical advantage encoded in duets might play a role in territorial interactions. As suggested by Kovach et al. (2014), we advocate future studies about duet function in other species that adopt experimental designs that allow differentiating between coordination of duets and number of intruders.

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TABLES

Table 4-1. Mean ± se and sample sizes for the stimuli created for the six playback treatments. Song overlap is the proportion of overlap between male and female phrases in a temporal scale. Latency is time difference between the start of the first song relative to the second song in a duet.

Playback treatment	Song overlap (%)	Latency (s)	Duration (s)	n stimuli created	n stimuli used
Highly overlapped natural duet[1]	93.22 ± 0.49	0.29 ± 0.03	6.71 ± 0.47	10	10
Loosely overlapped natural duet[1]	57.86 ± 2.86	2.31 ± 0.25	6.98 ± 0.38	10	9
Highly overlapped synthetic duet[2]	74.99 ± 3.62	0.27 ± 0.03	5.01 ± 0.14	20	12
Loosely overlapped synthetic duet[2]	43.02 ± 3.03	1.66 ± 0.18	6.16 ± 0.20	20	13
Non-overlapped solos[2]		5.25 ± 0.22	9.80 ± 0.25	20	12
Heterospecific song			6.71 ± 0.11	10	7

[1] Stimuli created from spontaneous duet; [2] synthetic duet stimuli made from solo songs.

Table 4-2. Playback responses measured for each focal bird or pair. Modified from Diniz (2017) (Chapter 2).

Behavioral response	
Distance travelled to approach the speaker (m)	The distance between the bird's position at the closest approach to the speaker and the bird's position just before (1s) the playback
Closest approach to the speaker (m)[1]	Minimum distance between focal bird and speaker at a 15s-interval after broadcast of stimulus
Closest distance between partners (m)	Minimum distance between partners at a 30s-interval after broadcast of stimulus
Territorial vigilance (s)[1]	Time spent perched in vigilant state after broadcasting the stimulus until resuming foraging or nest building activities
Vocal responses	
Latency to sing (s)	Time taken for each focal bird to sing after the playback
Song rate (songs/h)[1]	Number of songs (solos and duet phrases) divided by the time the bird was observed
Singing role (initiator or responder)	Whether the bird initiated (solo or initiated duets) or answered (duet responder) each song
Latency to answer partner initiated songs (s)	Time taken for the bird answer each partner-initiated duet
Song duration (s)	Duration of each solo and duet phrase
Frequency parameters of pairs' duets (PC1, PC2)	Five acoustic measurements represented in two PCA scores
Degree of song overlap (%)	Proportion of each duet time that male and female phrases were overlapped in temporal scale

[1] Modelled as a function of conspecific, but not heterospecific, treatments.

FIGURE LEGENDS

Figure 4-1. Experimental design showing how we manipulated the (i) proportion of overlap and (ii) rhythm coordination between male and female phrases in rufous hornero duets to produce the playback stimuli. (1) Highly and (2) loosely overlapped natural duets consisted of duet stimuli of which male and female phrases are coordinated rhythmically. (3) Highly and (4) loosely overlapped synthetic duet are synthetic stimuli made overlapped solos and are representing duets of which male and female phrases are not coordinated rhythmically. (5) Non-overlapped solos are synthetic stimuli made from spontaneous solo songs.

Figure 4-2. Examples of playback stimuli of duets varying in the (i) proportion of overlap and (ii) rhythm coordination between male and female phrases. Highly and loosely overlapped natural duets consisted of duet stimuli of which male and female phrases are coordinated rhythmically. Highly and loosely overlapped synthetic duet are synthetic stimuli made overlapped solos and are representing duets of which male and female phrases are not coordinated rhythmically. Non-overlapped solos are synthetic stimuli made from spontaneous solo songs. Female and male phrases indicated by grey and black bars, respectively. Great Kiskadee song was used as a control.

Figure 4-3. Physical responses of mated rufous horneros to the playback of duets varying in (i) phrase overlap and (ii) rhythm coordination. Playback treatments: HN – highly overlapped natural duet (coordinated phrase rhythms), LN – loosely overlapped natural duet (coordinated phrase rhythms), HS – highly overlapped synthetic duet (uncoordinated phrase rhythms), LS – loosely overlapped synthetic duet (uncoordinated phrase rhythms), NS – non-overlapped solos, Control – great kiskadee song. Bars indicate means and 95% CI.

Figure 4-4. Vocal responses of mated rufous horneros to the playback of duets varying in phrase overlap and coordination. Playback treatments: HN – highly overlapped natural duet (coordinated phrase rhythms), LN – loosely overlapped natural duet (coordinated phrase rhythms), HS – highly overlapped synthetic duet (uncoordinated phrase rhythms), LS – loosely overlapped synthetic duet (uncoordinated phrase rhythms), NS – non-overlapped solos, Control – great kiskadee song. Bars indicate means and 95% CI.

FIGURES

Figure 4-1

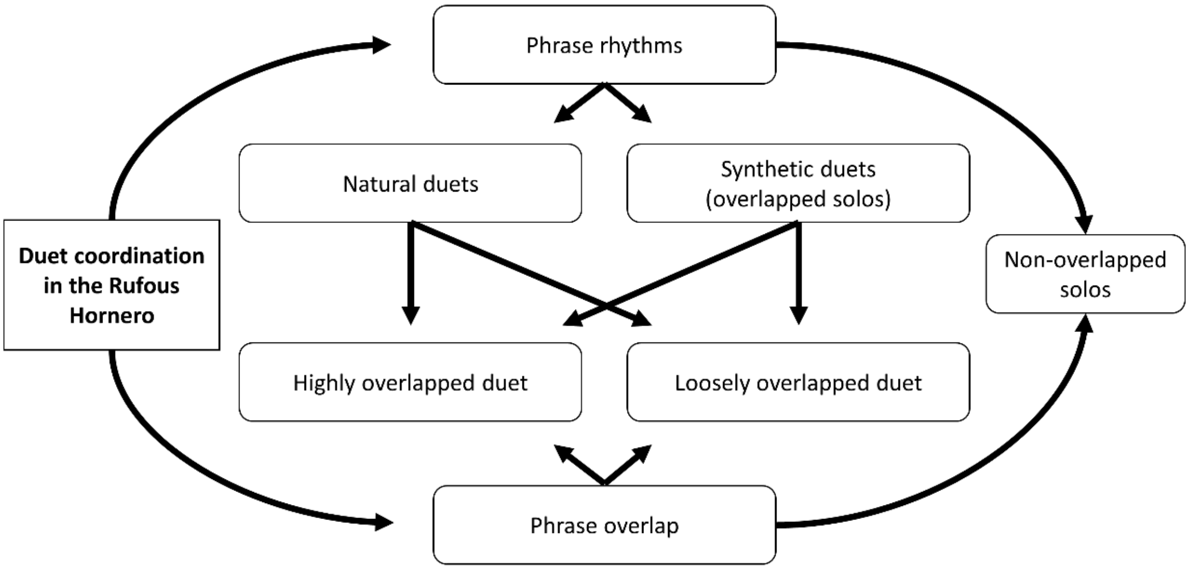


Figure 4-2

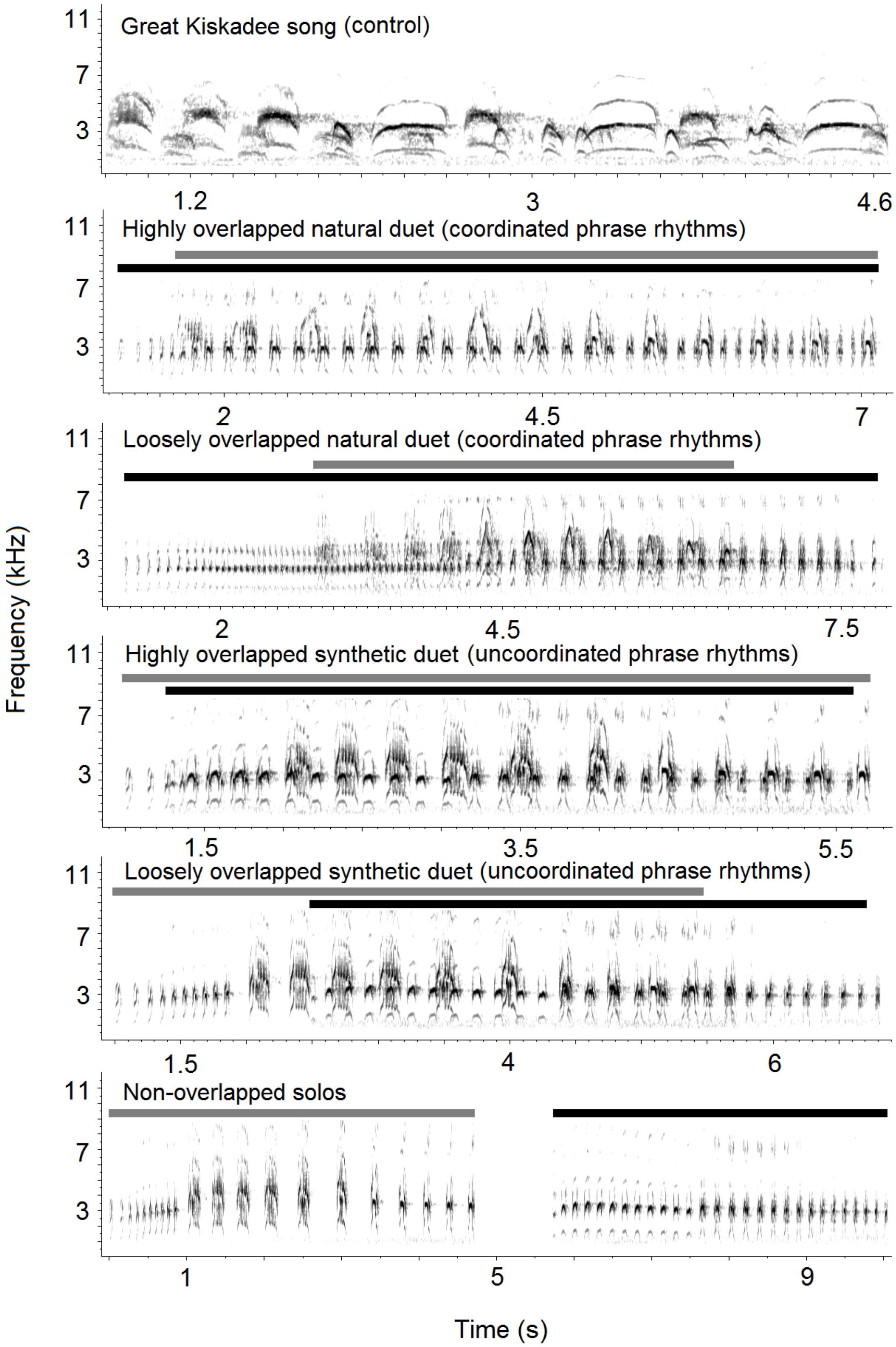

Frequency (kHz)
11
7
3
Great Kiskadee song (control)
1.2
3
4.6
11
Highly overlapped natural duet (coordinated phrase rhythms)
7
3
2
4.5
7
11
Loosely overlapped natural duet (coordinated phrase rhythms)
7
3
2
4.5
7.5
11
Highly overlapped synthetic duet (uncoordinated phrase rhythms)
7
3
1.5
3.5
5.5
11
Loosely overlapped synthetic duet (uncoordinated phrase rhythms)
7
3
1.5
4
6
11
Non-overlapped solos
7
3
1
5
9
Time (s)

Figure 4-3

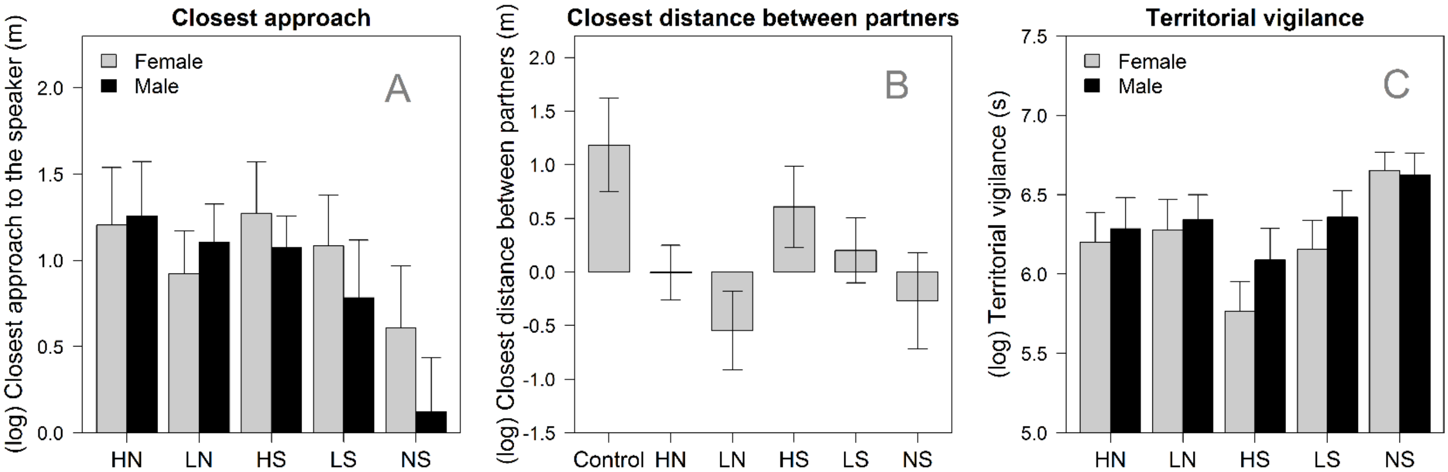
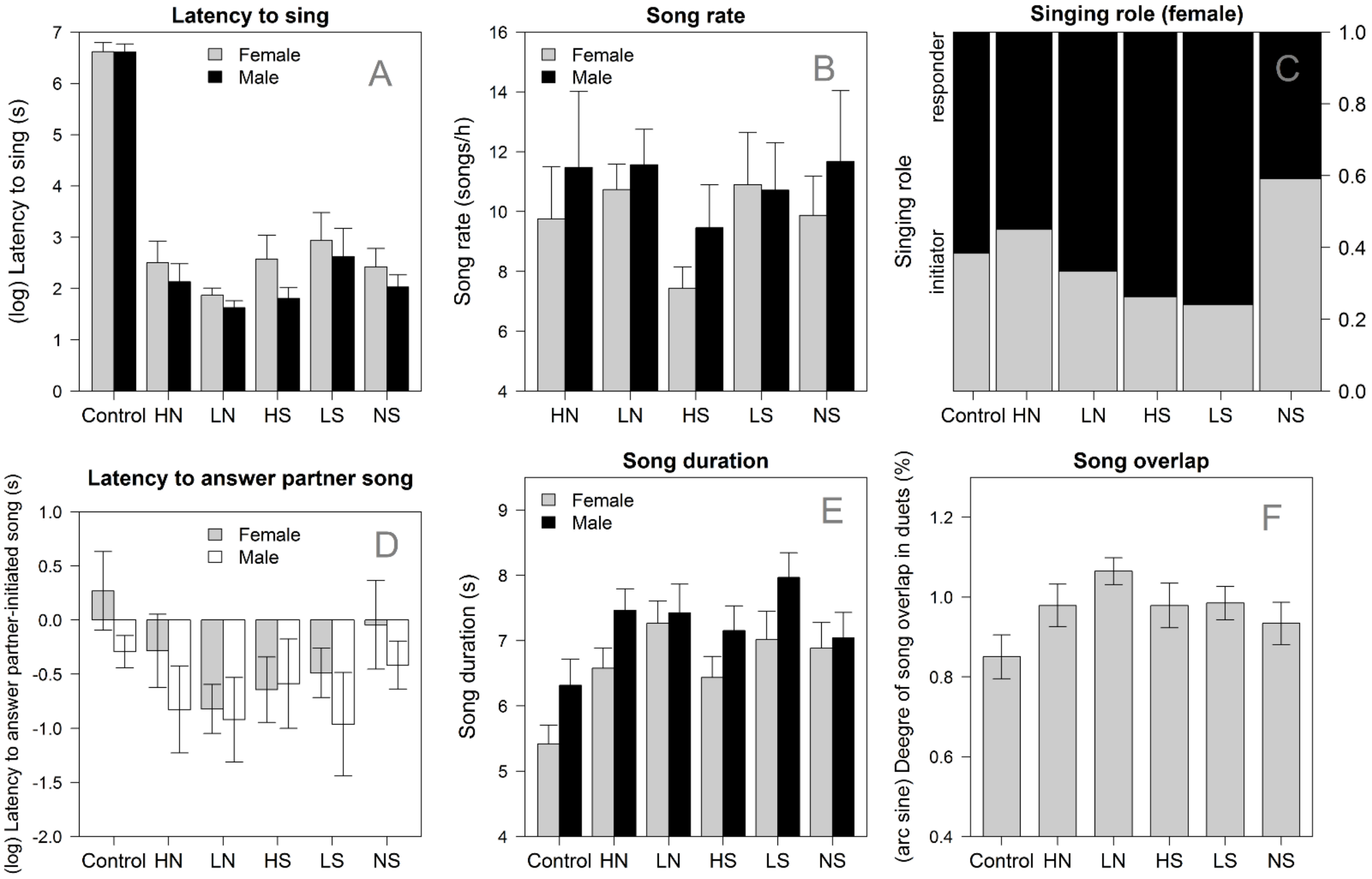

Closest approach
(log) Closest approach to the speaker (m)
2.0
1.5
1.0
0.5
0.0
Female
Male
A
HN
LN
HS
LS
NS
Closest distance between partners
(log) Closest distance between partners (m)
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
B
Control
HN
LN
HS
LS
NS
Territorial vigilance
(log) Territorial vigilance (s)
7.5
7.0
6.5
6.0
5.5
5.0
Female
Male
C
HN
LN
HS
LS
NS

Figure 4-4


Latency to sing
(log) Latency to sing (s)
7
6
5
4
3
2
1
0
Female
Male
A
Control
HN
LN
HS
LS
NS
Song rate
Song rate (songs/h)
16
14
12
10
8
6
4
Female
Male
B
HN
LN
HS
LS
NS
Singing role (female)
Singing role
responder
initiator
C
1.0
0.8
0.6
0.4
0.2
0.0
Control
HN
LN
HS
LS
NS
Latency to answer partner song
(log) Latency to answer partner-initiated song (s)
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
Female
Male
D
Control
HN
LN
HS
LS
NS
Song duration
Song duration (s)
9
8
7
6
5
4
Female
Male
E
Control
HN
LN
HS
LS
NS
Song overlap
(arc sine) Deegre of song overlap in duets (%)
1.2
1.0
0.8
0.6
0.4
F
Control
HN
LN
HS
LS
NS

SUPPLEMENTARY MATERIAL

Table 4-3s. Principal component analysis for frequency parameters of pairs' duets.

	PC1	PC2
Eigenvalue	2.09	1.38
Variance explained (%)	62.39%	27.17%
Loadings		
Mean frequency (kHz)	–0.46	–0.12
Median frequency (kHz)	–0.35	–0.36
First quantile (kHz)	–0.23	–0.60
Third quantile (kHz)	–0.43	0.24
Interquantile range (kHz)	–0.28	0.57
Spectral entropy	–0.38	0.32
Frequency centroid	–0.46	–0.12

Table 4-4s. Global mixed models for physical and vocal playback responses.

Response variable	Family	Predictor variables	Random effects
Physical responses			
Distance travelled to approach the speaker (m)	Gaussian	treatment × sex + playback order + stimulus duration	Individual ID nested in group ID + stimulus ID
Closest approach to the speaker (m, log)	Gaussian	treatment × sex + playback order + stimulus duration	Individual ID nested in group ID + stimulus ID
Closest distance between partners (m, log)	Gaussian	treatment + playback order + stimulus duration	group ID + stimulus ID
Territorial vigilance (s, log)	Gaussian	treatment × sex + playback order + stimulus duration	Individual ID nested in group ID + stimulus ID
Vocal responses			
Latency to sing (s, log)	Gaussian	treatment × sex + playback order + stimulus duration	Individual ID nested in group ID + stimulus ID
Song rate (songs/h)[1]	Poisson	treatment × sex + playback order + stimulus duration	Individual ID nested in group ID + stimulus ID
Singing role (initiator or responder)	Binomial	treatment × sex + order of the vocal response + playback order + stimulus duration	Playback trial ID nested in individual ID nested in group ID + stimulus ID
Latency to answer partner initiated songs (s, log)	Gaussian	treatment × sex + order of the vocal response + playback order + stimulus duration	Playback trial ID nested in individual ID nested in group ID + stimulus ID
Song duration (s)	Gaussian	treatment × sex + order of the vocal response + playback order + stimulus duration + singing role	Playback trial ID nested in individual ID nested in group ID + stimulus ID
Frequency parameters of pairs' duets (PC1, PC2)	Gaussian	treatment × sex + order of the vocal response + playback order + stimulus duration	Playback trial ID nested in group ID + stimulus ID
Degree of song overlap (%, arc sine)	Gaussian	treatment + order of the vocal response + playback order + stimulus duration	Playback trial ID nested in group ID + stimulus ID

ID = identity; [1]the time spent (in h) observing the focal bird was added as an offset in the model.

Table 4-5s. Backward stepwise model selection on global models of playback responses (Table 4-1s). We show the significance of each predictor in descending order as they were removed during the model selection. LRT = likelihood ratio test.

Response variable	Predictor variables	*LRT*	*P*
Physical responses			
Distance travelled to approach the speaker (m)	Order of the stimulus broadcasted	0.46	0.50
	Stimulus duration	0.52	0.47
	Treatment × sex	5.56	0.35
	Sex	1.14	0.29
	Treatment	33.89	<0.0001
Closest approach to the speaker (m, log)	Order of the stimulus broadcasted	0.57	0.45
	Treatment × sex	3.94	0.41
	Treatment	2.12	0.71
	Sex	1.00	0.32
	Stimulus duration	6.16	0.13
Closest distance between partners (m, log)	Order of the stimulus broadcasted	0.04	0.84
	Stimulus duration	1.00	0.32
	Treatment	17.53	0.0036
Territorial vigilance (s, log)	Treatment × sex	3.30	0.51
	Treatment	2.10	0.72
	Sex	2.10	0.15
	Order of the stimulus broadcasted	6.17	0.013
	Stimulus duration	11.44	0.0007
Vocal responses			
Latency to sing (s, log)	Stimulus duration	0.02	0.89
	Treatment × sex	4.76	0.45
	Sex	3.27	0.07
	Order of the stimulus broadcasted	4.28	0.039
	Treatment	75.66	<0.0001
Song rate (songs/h)	Treatment × sex	0.19	1.00
	Treatment	0.72	0.95
	Sex	0.36	0.55
	Stimulus duration	3.68	0.055
	Order of the stimulus broadcasted	5.87	0.015
Singing role (initiator or responder)	Order of the vocal response	0.04	0.84
	Order of the stimulus broadcasted	0.06	0.80
	Stimulus duration	0.06	0.80
	Treatment × sex	15.92	0.007
Latency to answer partner-initiated songs (s, log)	Treatment × sex	1.75	0.88
	Stimulus duration	0.14	0.71
	Order of the stimulus broadcasted	0.20	0.65
	Order of the vocal response	0.17	0.68
	Sex	2.11	0.15
	Treatment	9.68	0.08

Table 4-5s

Song duration (s)	Treatment × sex	2.32	0.80
	Stimulus duration	0.27	0.60
	Order of the stimulus broadcasted	0.43	0.51
	Sex	3.33	0.068
	Order of the vocal response	3.91	0.048
	Treatment	13.92	0.016
	Singing role	17.10	<0.0001
Frequency parameters of pairs' duets (PC1)	Stimulus duration	0.02	0.89
	Order of the vocal response	0.03	0.87
	Treatment	2.01	0.85
	Order of the stimulus broadcasted	0.29	0.59
Frequency parameters of pairs' duets (PC2)[1]	Order of the stimulus broadcasted	0.004	0.95
	Treatment	2.53	0.77
	Order of the vocal response	1.82	0.18
	Stimulus duration	2.23	0.14
Degree of song overlap (%, arc sine)	Order of the stimulus broadcasted	0.08	0.78
	Order of the vocal response	0.54	0.46
	Stimulus duration	1.24	0.26
	Treatment	9.54	0.089

[1] One outlier removed.

Table 4-6s. Beta (β) coefficients for playback treatments when retained in the best model resulting from model selection. P values were adjusted by false discovery rates. Playback treatments: HN – highly overlapped natural duet (coordinated phrase rhythms), LN – loosely overlapped natural duet (coordinated phrase rhythms), HS – highly overlapped synthetic duet (uncoordinated phrase rhythms), LS – loosely overlapped synthetic duet (uncoordinated phrase rhythms), NS – non-overlapped solos, Control – great kiskadee song. Bars indicate means and 95% CI.

Response variable	*β*	SE	*z* ratio or *t* value	*p*
Physical responses				
Distance travelled to approach the speaker (m) (both sexes)				
Control – HN	-9.48	2.32	-4.09	**0.00015**
Control – LN	-11.62	2.36	-4.92	**<0.0001**
Control – HS	-10.92	2.24	-4.88	**<0.0001**
Control – LS	-11.78	2.20	-5.36	**<0.0001**
Control – NS	-13.07	2.23	-5.85	**<0.0001**
HN – LN	-2.15	2.25	-0.95	0.34
HN – HS	-1.44	2.12	-0.68	0.50
HN – LS	-2.31	2.08	-1.11	0.27
HN – NS	-3.60	2.12	-1.70	**0.096**
LN – HN	0.70	2.17	0.32	0.75
LN – LS	-0.16	2.13	-0.08	0.94
LN – NS	-1.45	2.17	-0.67	0.51
HS – LS	-0.87	1.99	-0.44	0.67
HS – NS	-2.16	2.03	-1.06	0.29
LS – NS	-1.29	1.99	-0.65	0.52
Closest distance between partners (m, log) (both sexes)				
Control – HN	1.24	0.49	2.52	**0.058**
Control – LN	1.84	0.49	3.78	**0.007**
Control – HS	0.63	0.48	1.33	0.33
Control – LS	1.02	0.47	2.17	0.11
Control – NS	1.53	0.48	3.18	**0.020**
HN – LN	0.61	0.49	1.25	0.33
HN – HS	-0.60	0.47	-1.27	0.33
HN – LS	-0.21	0.47	-0.45	0.65
HN – NS	0.30	0.48	0.62	0.58
LN – HN	-1.21	0.47	-2.56	**0.058**
LN – LS	-0.82	0.47	-1.75	0.19
LN – NS	-0.31	0.48	-0.65	0.58
HS – LS	0.39	0.46	0.85	0.50
HS – NS	0.90	0.47	1.93	0.15
LS – NS	0.51	0.46	1.10	0.38
Vocal responses				
Latency to sing (s, log) (both sexes)				
Control – HN	4.38	0.46	9.50	**<0.0001**
Control – LN	4.87	0.45	10.75	**<0.0001**
Control – HS	4.54	0.46	9.81	**<0.0001**

Table 4-6s

Control – LS	3.99	0.49	8.13	**<0.0001**
Control – NS	4.43	0.49	9.12	**<0.0001**
HN – LN	0.48	0.41	1.17	0.46
HN – HS	0.16	0.42	0.39	0.81
HN – LS	-0.40	0.45	-0.89	0.52
HN – NS	0.05	0.44	0.10	0.92
LN – HN	-0.32	0.41	-0.78	0.55
LN – LS	-0.88	0.44	-1.98	0.13
LN – NS	-0.44	0.44	-1.00	0.52
HS – LS	-0.56	0.45	-1.24	0.46
HS – NS	-0.11	0.45	-0.26	0.86
LS – NS	0.44	0.47	0.93	0.52
Singing role (probability of sang as responder) (female)				
Control – HN	0.29	0.81	0.36	0.77
Control – LN	-0.36	0.81	-0.45	0.76
Control – HS	-0.72	0.84	-0.85	0.59
Control – LS	-0.79	0.81	-0.98	0.59
Control – NS	0.99	0.80	1.24	0.54
HN – LN	-0.65	0.72	-0.90	0.59
HN – HS	-1.01	0.77	-1.31	0.54
HN – LS	-1.08	0.72	-1.50	0.51
HN – NS	0.70	0.70	1.00	0.59
LN – HN	-0.36	0.76	-0.47	0.76
LN – LS	-0.43	0.72	-0.60	0.75
LN – NS	1.35	0.71	1.90	0.29
HS – LS	-0.08	0.76	-0.10	0.92
HS – NS	1.71	0.75	2.27	0.18
LS – NS	1.78	0.71	2.51	0.18
Singing role (probability of sang as responder) (male)				
Control – HN	-0.56	0.81	-0.69	0.81
Control – LN	-0.02	0.81	-0.03	0.98
Control – HS	0.32	0.84	0.38	0.81
Control – LS	0.42	0.81	0.51	0.81
Control – NS	-1.07	0.77	-1.40	0.54
HN – LN	0.54	0.71	0.75	0.81
HN – HS	0.88	0.77	1.15	0.63
HN – LS	0.97	0.72	1.34	0.54
HN – NS	-0.52	0.67	-0.77	0.81
LN – HN	0.34	0.75	0.46	0.81
LN – LS	0.44	0.71	0.62	0.81
LN – NS	-1.05	0.67	-1.57	0.54
HS – LS	0.09	0.75	0.12	0.97
HS – NS	-1.40	0.72	-1.95	0.38
LS – NS	-1.49	0.68	-2.21	0.38
Latency to answer partner-initiated songs (s, log) (both sexes)				
Control – HN	0.58	0.37	1.59	0.36

Table 4-6s

Control – LN	0.97	0.35	2.74	0.14
Control – HS	0.71	0.36	1.97	0.21
Control – LS	0.72	0.34	2.11	0.21
Control – NS	0.33	0.35	0.93	0.51
HN – LN	0.38	0.31	1.21	0.44
HN – HS	0.12	0.32	0.38	0.76
HN – LS	0.14	0.31	0.45	0.76
HN – NS	-0.26	0.31	-0.84	0.51
LN – HN	-0.26	0.31	-0.83	0.51
LN – LS	-0.24	0.29	-0.84	0.51
LN – NS	-0.64	0.30	-2.13	0.21
HS – LS	0.01	0.30	0.05	0.96
HS – NS	-0.38	0.31	-1.24	0.44
LS – NS	-0.40	0.29	-1.37	0.44
Song duration (s) (both sexes)				
Control – HN	-1.31	0.52	-2.51	**0.078**
Control – LN	-1.72	0.51	-3.38	**0.022**
Control – HS	-0.86	0.50	-1.73	0.23
Control – LS	-1.45	0.48	-3.02	**0.031**
Control – NS	-1.08	0.48	-2.24	0.11
HN – LN	-0.41	0.49	-0.84	0.55
HN – HS	0.45	0.48	0.95	0.55
HN – LS	-0.15	0.46	-0.32	0.75
HN – NS	0.22	0.46	0.48	0.68
LN – HN	0.86	0.46	1.87	0.21
LN – LS	0.26	0.44	0.59	0.68
LN – NS	0.63	0.45	1.41	0.32
HS – LS	-0.60	0.43	-1.38	0.32
HS – NS	-0.23	0.44	-0.52	0.68
LS – NS	0.37	0.42	0.89	0.55
Degree of song overlap (%, arc sine) (both sexes)				
Control – HN	-0.12	0.08	-1.59	0.33
Control – LN	-0.21	0.07	-2.86	0.11
Control – HS	-0.12	0.08	-1.62	0.33
Control – LS	-0.13	0.07	-1.77	0.33
Control – NS	-0.07	0.07	-0.98	0.55
HN – LN	-0.09	0.07	-1.38	0.33
HN – HS	0.00	0.07	-0.01	0.99
HN – LS	-0.01	0.06	-0.10	0.99
HN – NS	0.05	0.07	0.76	0.57
LN – HN	0.09	0.07	1.38	0.33
LN – LS	0.09	0.06	1.38	0.33
LN – NS	0.14	0.06	2.23	0.24
HS – LS	-0.01	0.06	-0.09	0.99
HS – NS	0.05	0.06	0.78	0.57
LS – NS	0.06	0.06	0.92	0.55

Table 4-7s. Beta (β) coefficients of covariables retained in the best model resulting from model selection.

Response variable	Predictor variables	β ± SE	z ratio or t value
Physical responses			
Closest approach to the speaker (m, log)	Stimulus duration	–0.28 ± 0.11	–2.54
Territorial vigilance (s, log)	Stimulus duration	0.24 ± 0.07	3.50
	Order of the stimulus broadcasted	–0.14 ± 0.06	–2.51
Vocal responses			
Latency to sing (s, log)	Order of the stimulus broadcasted	–0.23 ± 0.10	–2.29
Song rate (songs/h)	Stimulus duration	–0.12 ± 0.07	–1.89
	Order of the stimulus broadcasted	0.16 ± 0.07	2.43
Song duration (s)	Order of the vocal response	–0.20 ± 0.10	–1.99
	Singing role (responder relative to initiator)	–0.82 ± 0.19	–4.27

Table 4-8s. Standard deviation for random effects in the top models. ID = identity.

Response variable	Playback trial	Individual ID	Group ID	Stimulus ID
Physical responses				
Distance travelled to approach the speaker (m)		1.87	0.00	4.57
Closest approach to the speaker (m, log)		0.29	0.32	0.66
Closest distance between partners (m, log)			0.65	0.56
Territorial vigilance (s, log)		0.17	0.04	0.39
Vocal responses				
Latency to sing (s, log)		0.41	0.47	0.91
Song rate (songs/h)		0.00	0.00	0.00
Singing role (initiator or responder)	0.00	1.02	0.00	0.00
Latency to answer partner-initiated song (s, log)	0.08	0.29	0.00	0.00
Song duration (s)	0.00	0.30	0.73	0.74
Frequency parameters of pairs’ duets (PC1)	0.97		0.58	0.00
Frequency parameters of pairs’ duets (PC2)	0.16		0.52	0.00
Degree of song overlap (%, arc sine)	0.03		0.08	0.00

Table 4-9s. Pseudo R-squares for fixed and random effects at each top model for each variable.

Response variable	Fixed effects	Random effects
Physical responses		
Distance travelled to approach the speaker (m)	0.38	0.47
Closest approach to the speaker (m, log)	0.07	0.52
Closest distance between partners (m, log)	0.17	0.35
Territorial vigilance (s, log)	0.18	0.49
Vocal responses		
Latency to sing (s, log)	0.63	0.27
Song rate (songs/h)	0.30	0.00
Singing role (initiator or responder)	0.18	0.20
Latency to answer partner-initiated song (s, log)	0.07	0.08
Song duration (s)	0.14	0.33
Frequency parameters of pairs' duets (PC1)	0.00	0.29
Frequency parameters of pairs' duets (PC2)	0.00	0.32
Degree of song overlap (%, arc sine)	0.07	0.14

Table 4-10s. Sample sizes.

Response variable	Playback treatment (HC, HN, LN, HS, LS, NS)	Trials, groups, stimuli
Physical responses		
Distance travelled to approach the speaker (m)	13, 12, 13, 12, 13, 12	76, 13, 64
Closest approach to the speaker (m, log)	no control, 12, 13, 12, 13, 13	64, 13, 56
Closest distance between partners (m, log)	13, 12, 13, 13, 13, 12	76, 13, 64
Territorial vigilance (s, log)	no control, 12, 13, 13, 13, 13	64, 13, 56
Vocal responses		
Latency to sing (s, log)	12, 13, 13, 13, 11, 13	75, 13, 63
Song rate (songs/h)	no control, 13,13,13,12,13	64, 13, 56
Singing role (initiator or responder)	28, 40, 44, 39, 51, 48 (sexes combined)	75, 13, 63
Latency to answer partner-initiated song (s, log)	12, 19, 21, 19, 25, 22 (sexes combined)	75, 13, 63
Song duration (s)	28, 40, 44, 39, 51, 48 (sexes combined)	75, 13, 63
Frequency parameters of pairs' duets (PC1)	14, 17, 13, 12, 14, 16	66, 13, 56
Frequency parameters of pairs' duets (PC2)	14, 17, 13, 11, 14, 16	66, 13, 56
Degree of song overlap (%, arc sine)	12, 19, 21, 19, 25, 22	75, 13, 63

HC – heterospecific control song, HN – highly overlapped natural duet (coordinated phrase rhythms), LN – loosely overlapped natural duet (coordinated phrase rhythms), HS – highly overlapped synthetic duet (uncoordinated phrase rhythms), LS – loosely overlapped synthetic duet (uncoordinated phrase rhythms), NS – non-overlapped solos.

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CAPÍTULO 5

Monochromatism, cryptic sexual dimorphism and lack of assortative mating in the Rufous Hornero (*Furnarius rufus albogularis*)[6]

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ABSTRACT

Neotropical ovenbirds (family Furnariidae) are largely sexually monomorphic and monochromatic, which leads to the assumption that sexual selection has had little effect on the evolution of the morphological and plumage traits of the species in the family. We studied a wild population of the Rufous Hornero (*Furnarius rufus albogularis*) and used morphological measurements, molecular sexing, spectrometer analyses and visual modelling to investigate the assumption of sexual monomorphism and monochromatism in this species. We also tested for assortative mating with respect to these traits. On average, males had slightly longer wings and tails than females but there were no sexual differences in other morphological traits (mass, tarsus and bill) or in the spectral properties of plumage coloration for six body parts. Visual modelling indicated that Rufous Horneros can perceive variation in colour between individuals but colour does not vary with sex. We did not find any evidence of assortative mating for size or colour traits. In conclusion, males from the studied population differ slightly from females in external morphological measurements but not in plumage coloration. This study is among the first to demonstrate complete sexual monochromatism in birds assessed against the avian visual system.

Additional keywords: Furnariidae, neotropical birds, plumage colour, visual modelling.

INTRODUCTION

Sexual selection is the main driver of the evolution of sexual dimorphism and dichromatism in birds (reviews by Owens and Hartley 1998; Dunn *et al*. 2001; Székely *et al*. 2007). Many species, however, show little or no differences in external morphology, including coloration, between the sexes, suggesting low levels of variation in mating success and limited opportunity for sexual selection. This may be the case in the Rufous Hornero (*Furnarius rufus*), a common Neotropical ground-foraging species of ovenbird (family Furnariidae: ovenbirds and wood-creepers) inhabiting rural and urban areas in central and southern South America (Marreis and Sander 2006). Both male and female Rufous Horneros have cryptic reddish-brown plumage coloration (Sick 2001). Rufous Horneros are socially monogamous, territorial (Burger 1979; Sick 2001) with high adult survival rates (Fraga 1980) and parental care of offspring is shared equally (Braga 2012; Massoni *et al*. 2012). It would thus appear that the conditions for sexual selection to generate sexual dimorphism in the Rufous Hornero are lacking.

The species comprising the Furnariidae are widely described as predominantly sexually monomorphic and monochromatic (Skutch 1996; Sick 2001; Remsen 2003). However, this assumption is based mostly on field observations and human perception of colour rather than detailed objective analyses. Sexual monomorphism has been investigated in only a small number of Furnariidae species and those studies have found subtle sexual dimorphism, with males slightly larger than females (Winker *et al*. 1994; Faria *et al*. 2007; Moreno *et al*. 2007; Cardoni *et al*. 2009; Puebla-Olivares and Figueroa-Esquivel 2009).

It has been argued that cryptic sexual dichromatism in the ultraviolet (UV) range – a type of dichromatism perceived as monochromatism by human vision – is somewhat unlikely in ovenbirds (Seddon *et al*. 2010). This is because antbirds (family Thamnophilidae), and probably other tracheophone suboscines (family Furnariidae: woodcreepers, ovenbirds and allies), have a visual system sensitive only to violet within the visible spectrum (and not UV) and low levels of UV reflectance in their plumages (Seddon *et al*. 2010; Tobias *et al*. 2012). However, even among violet-sensitive bird species that are apparently monochromatic there are considerable sexual differences in colour evident to the avian eye (Eaton 2005). To date, sexual dichromatism has been objectively studied in only two furnariids, which showed contrasting patterns: sexual monochromatism in the Thorn-tailed Rayadito (*Aphrastura spinicauda*; Moreno *et al*. 2007) and dichromatism in the Puna Miner (*Geositta punensis*; Eaton 2005).

Assortative mating is the correlation of any phenotypic trait across members of mated pairs, and can evolve through selection on mating preferences or as a consequence of ecological or physiological constraints (reviewed by Jiang *et al*. 2013). Assortative mating in birds has been investigated in mutually ornamented species (e.g. Regosin and Pruett-Jones 2001; Boland *et al*. 2004) and rarely been assessed in birds without obvious ornamental traits (Delestrade 2001), such as the Rufous Hornero. Investigation of assortative mating for colour and size in non-ornamented bird species may contribute to our understanding on the evolution of cryptic sexual dimorphism and dichromatism in birds.

Generating descriptive information about sexual dimorphism in poorly studied avian taxa, such as the Furnariidae, is crucial to substantiating comparative and behavioural research about the evolutionary causes and consequences of sexual dimorphism in birds (e.g. Owens and Hartley 1998; Dunn *et al*. 2001; Székely *et al*. 2007). We used morphometric measurements, molecular sexing, spectrophotometer analyses, and visual modelling to test for sexual dimorphism and dichromatism in a wild population of Rufous Hornero from central Brazil. In addition, we tested for assortative mating by size and plumage colour.

METHODS

Field work and molecular sexing

We studied an urban and wild population of Rufous Hornero on the campus of the Universidade de Brasília, Brazil (15°45’S, 47°51’W). We captured 61 incubating birds (31 males and 30 females) in September and October 2013, using a funnel fish-trap placed over the entrance to the Rufous Hornero's domed nest as described by Braga *et al*. (2014). We captured both members of 23 breeding pairs and one parent of 15 pairs.

We banded individuals with unique combinations of unnumbered coloured plastic (Avinet, Dryden, New York, NY) or, occasionally, metal (Anilhas Capri, São Paulo, Brazil) leg-bands. On capture, we collected ~60mL of blood using brachial venipuncture, and blood samples were transferred to filter paper for later determination of sex. We also recorded mass (using a dynamometer; Pesola AG, model Light Line 10050, Baar, Switzerland; accuracy 0.5 g) and maximum unflattened wing-chord, tarsal-length (from inter-tarsal joint to the base of the toes), tail-length (with one exception; from the uropygial gland to the tip of the longest feather), bill-length (from anterior edge of nostril to tip), and depth and width of bill at junction with skull (all with 150-mm digital callipers, Stainless Hardened, China; 0.01mm graduations). All measurements were taken by one person (P. Diniz). We also collected 3–4 feathers from each of the following body regions: breast, throat, crown, back, rump and undertail-coverts. We wrapped feathers in aluminum foil and stored them at room temperature and dry conditions. Sex was determined for 55 individuals (27 females, 28 males) using molecular methods (Griffiths *et al*. 1998) by a commercial laboratory (Grupo São Camilo – Medicina Diagnóstica, Maringá, Paraná, Brazil); the sex of the remaining six individuals (3 females, 3 males) was based on the sex of their partners as determined by molecular methods.

Measurements of plumage coloration

We used an Ocean Optics USB4000 spectrometer and PX-2 pulsed xenon light-source (Ocean Optics, Dunedin, FL, USA) to assess the spectral characteristics of Rufous Hornero feathers. The PX-2 light-source provides illumination in the visible spectrum for birds (300–700 nm). We positioned the feathers in an overlapping pattern on a non-reflective, black velvet substrate. We placed the optical probe perpendicularly above the feathers (at an angle of 90°) and measured reflectance spectra three times – removing the probe and replacing it upon the feathers between recordings – with the spectrometer and SpectraSuite software (Ocean Optics, Dunedin, FL). We followed the SpectraSuite manual instructions to choose the configuration parameters (integration time = 40µsec, scans to average = 50, boxcar width= 30). These measurements of reflectance spectra (percentages) were obtained relative to a white standard (WS-1-SS) and a dark reference (i.e. the black velvet substrate). We used the combined average spectra for each body region of each individual to prevent pseudoreplication in the analyses described below.

We analysed sexual differences in the colour of Rufous Hornero plumage with visual modelling, which incorporates avian visual sensitivities (cone absorbance; Vorobyev and Osorio 1998; Vorobyev *et al*. 1998). All analyses were performed in the pavo package within R version 3.2.3 (R Development CoreTeam 2015), following the systematic procedure suggested by Maia *et al*. (2013). Furnariids are likely to have a violet-sensitive visual system as other suboscines (Seddon *et al*. 2010) but this has not been studied in any species in the family. We therefore applied visual modelling to consider both the average avian ultraviolet (UVS) and the average violet-sensitive (VS) visual systems. We set the models assuming homogeneous illuminance across wavelengths and absolute quantum catches, which is ideal to contrast colours through ΔS (Vorobyev and Osorio 1998; Maia *et al*. 2013).

We used the visual models to measure the intrasexual and intersexual Euclidean chromatic distances (ΔS) (Vorobyev and Osorio 1998), assuming a noise level of 0.1 (Weber fraction) for the long-wavelength sensitive photoreceptor (Vorobyev and Osorio 1998; Olsson *et al*. 2015) and relative cone proportions for the Blue Tit (*Cyanistes caeruleus*; wavelengths: UV or V = 1, short = 2, medium = 2, long = 4). ΔS is expressed in just noticeable differences (JNDs) and indicates how two spectra are perceived as different, considering the visual space of the receiver; values > 1 are considered discernible by birds (Vorobyev *et al*. 1998; Endler and Mielke 2005). We made 1830 comparisons of chromatic distances for each body region within each visual system (UVS or VS): 435 intra-female comparisons, 465 intra-male comparisons and 930 intersexual comparisons (31 males and 30 females).

We also extracted three colour variables from each spectrum to investigate sexual differences in colorimetric reflectance: mean brightness (mean relative reflectance over all wavelengths), contrast (difference between maximum and minimum reflectances), and red chroma (reflectance of the red spectral range, 605–700 nm, relative to the total brightness) (Montgomerie 2006; Maia *et al*. 2013). We did not use UV chroma (reflectance of the UV spectral range, 300–400 nm, relative to the total brightness) in subsequent analyses, because preliminary analyses showed low UV chroma in feathers of all body regions (<5% for all except throat (12%)) and no sexual differences in this variable (results not shown).

Statistical analysis

All analyses were carried out using R version 3.2.3 (R Development Core Team 2015). We tested for sexual dichromatism with linear mixed modelling and univariate statistics. First, we modelled variation in ΔS (log-transformed) between individuals as a function of the type of comparison (intra-female, intra-male or intersexual) interacting with body region. We included the identities of the two individuals being compared, the identity of the comparison (i.e. the combination of the two individuals being compared) and the paired status of the individuals being compared (i.e. whether or not they belonged to the same breeding pair) as random effects in the model. We tested the existence of the interaction and main effects with analysis of deviance (Wald χ^2 test). We carried out post hoc comparisons of least-squared means among factor levels. If the Rufous Hornero is sexually dimorphic, we would expect ΔS to be greater between sexes than within sexes and, on average, ΔS to be > 1 for intersexual comparisons. Since the comparison of intrasexual and intersexual ΔS has never been conducted for this species, we also modelled the four receptor quantum catches (UV or V, short, medium and long wavelength) as a function of sex interacting with body region (similar to Eaton 2005) in a mixed model (with individual identity as a random factor). We found the same qualitative results as in the previous analyses (i.e. monochromatism; results not presented here). We also used linear mixed modelling to analyse sexual differences in each colorimetric variable (e.g. red chroma). We included sex, body region and their interaction as predictor factors, and individual identity as a random effect. Model inference followed the same protocol previously described.

We tested for sexual dimorphism with multivariate and univariate statistics. We excluded body mass of one female before all analyses because she was thought to be gravid (>60 g; Roper 2005). We used multivariate Hotelling's T-squared test to investigate sexual dimorphism, comparing matrices of size measurements. We identified and removed multivariate outliers (two female size data points) before the analyses using Mahalanobis distances.

We used t-tests with Welch approximation for degrees of freedom to compare colour and size variables between sexes. We identified and sequentially removed univariate outliers before the analyses using Grubbs' test (Grubbs 1950). To express the magnitude of morphological and plumage colour differences between the sexes, we computed the effect size (i.e. magnitude) of mean differences between sexes for each size variable using Cohen's d values and respective confidence intervals (see Nakagawa and Cuthill 2007). For example, Cohen's d values of 0.2 and 0.8 are considered small and large difference, respectively (reviewed by Nakagawa and Cuthill 2007).

We used a discriminant function analysis based on the maximum likelihood estimation method of classification to investigate the accuracy of size measurements to predict sex in the Rufous Hornero. In the discriminant analysis we did not include plumage colour variables because sexes did not differ in colour (see below) or wing-chord, because it was highly correlated with tail-length ($r > 0.5$). Outliers (two female body size data points) were identified and removed before analyses. Finally, to test for assortative mating in relation to size or coloration, we conducted correlation Mantel and Pearson tests of these traits between paired individuals. In the Mantel test, we used correlation of dissimilarity matrices (Euclidean distance) of multiple sexual traits, separately, for colour and size measurements (999 permutations for each one). We controlled for false discovery rates in multiple comparisons (see Benjamini and Hochberg 1995).

RESULTS

We found no differences in plumage coloration between sexes of Rufous Hornero. Although we found high inter-individual perceived chromatic distance (mean $\Delta S \pm$ s.e.: UVS, 11.69 ± 0.14; VS, 5.44 ± 0.07), intersexual ΔS was not greater than intrasexual ΔS

(Wald χ^2 test: UVS $\chi^2 = 0.32$, $P = 0.85$; VS $\chi^2 = 0.32$, $P = 0.85$), and this result was consistent for all body regions from which feathers were collected and analysed. We found an effect of the interaction between body region and type of comparison (i.e. intrasexual or intersexual) on ΔS (Wald χ^2 test: UVS $\chi^2 = 37.06$, $P < 0.0001$; VS $\chi^2 = 47.85$, $P < 0.0001$). This interaction was a result of a tendency of smaller intra-female ΔS compared with intersexual ΔS for undertail-coverts, but there was no difference between intersexual ΔS and intra-male ΔS for these same feathers (Figure 5-1; Table 5-2s in supplementary material), as would be expected in sexual dichromatism. Moreover, we found no difference between sexes in the measurements of colourimetric reflectance (Wald χ^2 test: $\chi^2 < 0.67$, $P > 0.41$), regardless of the body region from which feathers came (Wald χ^2 test: $\chi^2 < 6.57$, $P > 0.26$), though females tended to have brighter breast feathers than males (Figure 5-2; Table 5-3s).

In contrast, male Rufous Horneros differed from females in external measurements (Hotelling's *T*-squared test = 9.24, $P < 0.0001$). Male Rufous Horneros had, on average, slightly longer (~4%) wings and tails than females, although sexes overlapped in measurements (Figure 5-3), and males tended to have longer (1%) tarsi than females, and to be lighter (2%) than females. There were no differences between sexes in bill-depth, bill-length and bill-width (Table 5-1).

The discriminant function analysis had an 86% probability of correctly classifying sex, correctly allocating 22 of 26 females and 27 of 31 males (Figure 5-4s). The analysis generated the following discriminant function of unstandardised measurements:

$$D = (-\,0.28\ \times \text{mass}) + (0.41\ \times \text{tail length}) + (0.20\ \times \text{tarsal length}) + (2.42\ \times \text{bill depth}) - (1.01\ \times \text{bill width}) - (0.91\ \times \text{bill length}) + 13.31.$$

A positive *D* indicates an individual is male, and a negative *D*, female. The largest absolute loadings (i.e. contribution to the predicted sex) of standardised measurements were given by tail-length (1.14) and mass (–0.67), followed by bill-length (–0.70), bill-depth (0.54), bill-width (–0.30), and tarsal-length (0.19). Thus, for example, the longer the tail, the higher the chance of an individual being predicted as male by discriminant analysis.

We found no correlation between paired individuals in colour (Mantel test, $P >$ 0.13) or size (Mantel test, $r = 0.01$, $P = 0.40$, $n = 23$ breeding pairs; see Table 5-4s and Table 5-5s).

DISCUSSION

Our results show that our population of Rufous Hornero males from central Brazil have slightly longer tails and wings (~4%) and tend to have longer tarsi than females, and that females tend to be marginally heavier than males. Despite the slight differences in size between sexes and the overlap in size between sexes, the discriminant analysis correctly classified most of the studied birds (86%). We found no differences in sex for other measurements of size, a pattern of sexual dimorphism also described for the Henna-capped Foliage-gleaner (*Hylocryptus rectirostris*; Faria *et al*. 2007) and similar to the pattern found in other ovenbirds, where males are slightly larger than females (Moreno *et al*. 2007; Cardoni *et al*. 2009; Puebla-Olivares and Figueroa-Esquivel 2009). Montalti *et al*. (2004) found no differences between sexes in length of wing or tail in the Rufous Hornero, but the mean values for both traits for males that they presented were outside the range of their measurements, indicating some error in their analysis. Because of the subtle nature of sexual dimorphism in ovenbirds, we suggest that future studies of horneros (*Furnarius* spp.) use highly precise measurements (e.g. reducing measuring bias) and include measurements of additional morphological traits to increase the accuracy of sex-determination by morphology.

Sexual size-dimorphism in birds may have arisen from differences between sexes in mating competition, display agility and resource division, or female fecundity (Székely *et al*. 2007). Sexual dimorphism in the flight feathers of our studied Rufous Hornero population may have resulted from differences between sexes in territorial competition (Owens and Hartley 1998), in which the reproductive value of a territory is typically higher for males than for females. Alternatively, Rufous Horneros may not be able to recognise sexes based on this small difference in size between sexes, which in turn may have evolved as a by-product of fertility selection for smaller females (Székely *et al*. 2007). Other hypotheses could include sex-specific feather abrasion

(Merilä and Hemborg 2000), for example as a result of the long incubation bouts of female Rufous Horneros at night (Fraga 1980), or age-specific differences in length of feathers (Francis and Wood 1989) coupled with sex-specific adult mortality. We found no assortative mating for size, suggesting that mutual mate-choice is unlikely to drive the evolution of these traits. Future studies could address these functional explanations for the evolution of sexual dimorphism in this Rufous Hornero population and test if these birds can distinguish sexes by size.

Our results suggest the Rufous Hornero is sexually monochromatic. We found high chromatic distances (ΔS) between individuals. However, ΔS was not greater between sexes than within sexes. Since ΔS measures how birds can discriminate colours, in relation to the avian visual colour space (Endler and Mielke 2005), these results suggest that Rufous Horneros can use colour to discriminate between individuals but not between sexes. In addition, we found no differences in plumage reflectance between the sexes of the Rufous Hornero, except a tendency of females to have brighter breast feathers. Finally, we did not find any evidence of assortative mating based on plumage colour. These results suggest that, for this species, sexual selection is unlikely to have been important in the evolution of plumage colour, and that natural selection may have influenced the evolution of this trait in a similar way for both sexes.

Previous studies have suggested that selection for female crypsis may drive the evolution of sexual dichromatism (Burns 1998), with nest predation being among the mechanisms favouring female crypsis (Martin and Badyaev 1996; Götmark *et al*. 1997). However, rates of nest predation appear to be low in the Rufous Hornero (25%, Massoni *et al*. 2012), and another study suggests weaker selection on plumage crypsis in species with concealed nests (i.e. hanging baskets or domed nests, such as the Rufous Hornero) compared with open-nesting birds (Drury and Burroughs 2015). The Rufous Hornero forages on the ground and both sexes have very similar foraging and parental care behaviours (Fraga 1980), suggesting males and females are under similar predation risk. Thus, we suggest that adult predation rather than nest predation may be favouring the evolution of crypsis in both sexes of the Rufous Hornero.

Our study is among the first to demonstrate complete sexual monochromatism in birds in relation to the avian visual system (see also Eaton 2005; Burns and Shultz 2012; Doutrelant *et al*. 2013). Sexual monochromatism is likely to evolve in birds that exhibit negligible UV-reflection (Seddon *et al*. 2010), and such seems to be the case for furnariids. On the other hand, cryptic sexual dichromatism could be more likely in UV-reflecting taxa, like tanagers and cardinals (Burns and Shultz 2012).

It has been suggested that individual recognition of conspecifics may be rare among species of ovenbirds because of their apparent monomorphism and monochromatism (Skutch 1996). However, our study suggests that individual identity may be assessed by plumage colour, with such recognition possibly selected in socially monogamous species with high pair-fidelity and permanent territoriality (Fraga 1980), like the Rufous Hornero. Rufous Horneros also appear to be able to recognise conspecific individuals acoustically, and their song duets are characterised by sex-specific elements (Roper 2005). In summary, male Rufous Horneros in the studied population are slightly larger than females but the sexes do not differ in plumage coloration. Cryptic sexual dimorphism and sexual monochromatism are probably widespread in ovenbirds (furnariids), and more studies on sexual differences in colour and size in other species of Furnariidae are desirable to shed light on this hypothesis.

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TABLES

Table 5-1. Sexual differences in external morphology of adult Rufous Horneros. Cohen's d expresses the effect size (± 95% confidence interval (CI)) of mean differences in morphological attributes between the sexes (Nakagawa and Cuthill 2007). The t-test: all birds is testing means for all males vs all females; the paired t-test is testing means for males vs females of a mated pair; the degrees of freedom (d.f.) for all birds is computed assuming the Welch approximation. Values of P in bold indicate significant results ($P < 0.05$) after controlling for false discovery rates (Benjamini and Hochberg 1995)

Measurement	Mean ± SE			t-test: all birds		Paired t-test ($n = 23$ breeding pairs, d.f. = 22)	
	Male ($n = 31$)	Female ($n = 30$)	Cohen's d (CI)	t (df)	P	t	P
Bill depth (mm)	5.69 ± 0.04	5.64 ± 0.04	0.23 (–0.29, 0.75)	0.90 (57.73)	0.37	1.45	0.16
Bill length (mm)	15.64 ± 0.11	15.70 ± 0.17[A]	–0.08 (–0.60, 0.45)	–0.30 (48.62)	0.77	–1.26	0.22
Bill width (mm)	5.78 ± 0.04	5.84 ± 0.07	–0.24 (–0.76, 0.28)	–0.92 (46.64)	0.36	–0.73	0.47
Mass (g)	52.34 ± 0.40	53.57 ± 0.44[A]	–0.53 (–1.07, 0.0008)	–2.07 (57.10)	0.043	–1.16	0.26
Tail-length (mm)	68.92 ± 0.48	66.12 ± 0.37[B]	1.18 (0.60, 1.75)	4.60 (55.16)	**< 0.0001**	3.78	**0.001**
Tarsal-length (mm)	32.92 ± 0.18	32.45 ± 0.15	0.51 (–0,02, 1.04)	2.01 (58.13)	0.049	1.50	0.15
Wing-length (mm)	92.75 ± 0.58	89.36 ± 0.34	1.28 (0.70, 1.85)	5.02 (48.27)	**< 0.0001**	6.23	**< 0.0001**

[A] $N = 29$, with single outlier removed.

[B] $N = 29$, with data missing for one female.

FIGURE LEGENDS

Figure 5-1. Mean chromatic distances (± s.e.) within and between sexes of the Rufous Hornero. JNDs, just noticeable differences; UVS, ultraviolet-sensitive; VS, violet-sensitive.

Figure 5-2. Mean reflectance (± s.e.) of the reddish-brown feathers from six body regions of male (*n* = 31, dark shade) and female (*n* = 30, light shade) Rufous Horneros.

Figure 5-3. Covariation between lengths of wing and tail of male (*n* = 31) and female (*n* = 29) Rufous Horneros.

FIGURES

Figure 5-1

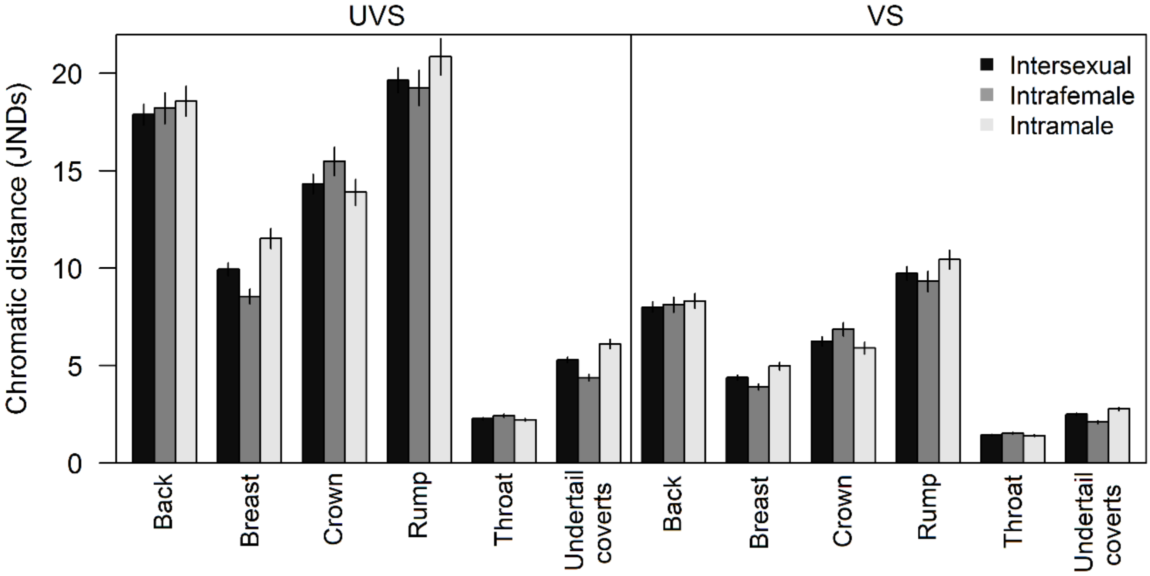


Figure 5-2

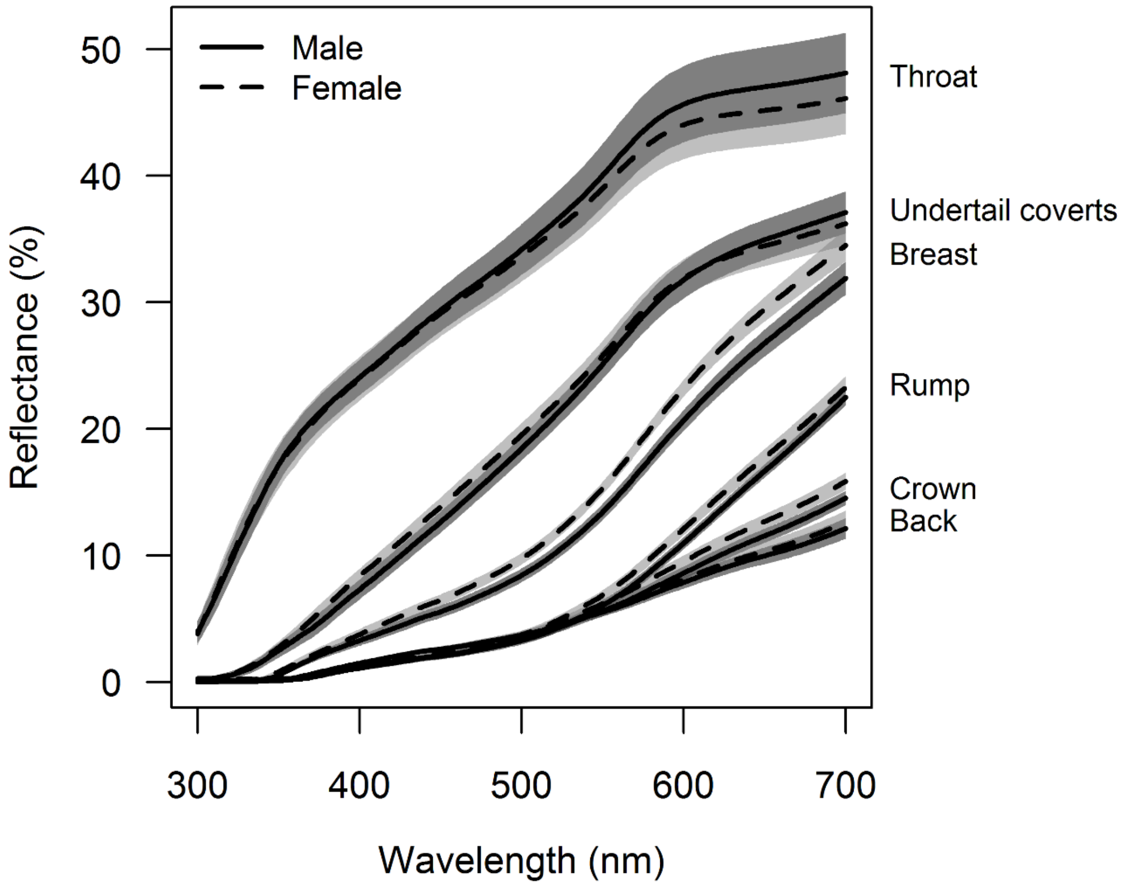
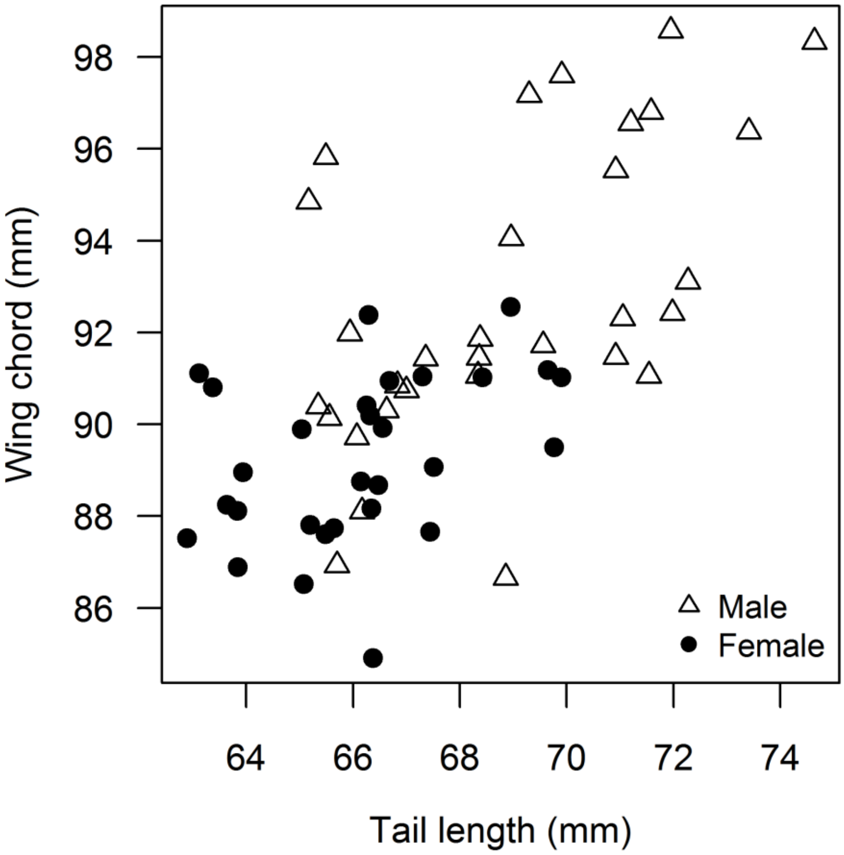

Male
Female
Throat
Undertail coverts
Breast
Rump
Crown
Back
Reflectance (%)
0
10
20
30
40
50
Wavelength (nm)
300
400
500
600
700

Figure 5-3


Wing chord (mm)
98
96
94
92
90
88
86
Tail length (mm)
64
66
68
70
72
74
△ Male
● Female

SUPPLEMENTARY MATERIAL

Table 5-2s. Post-hoc contrasts between inter-sexual and intra-sexual chromatic distances of perceived plumage colour in Rufous Horneros

Post-hoc comparisons were made between inter-sexual (IS) and intra-sexual (IM, males; IF, females) chromatic distances (log-transformed). We show the estimates of least-squares means and *t* ratios. *P* values in bold indicate non-significant results ($P < 0.05$) that were previously significant before controlling for false discovery rates (Benjamini and Hochberg 1995). *N*: 31 males and 30 females. U. coverts: undertail coverts. Degrees of freedom: 1,655.26 (IS – IF), 1,639.68 (IS – IM), 1,826 (IF – IM).

	Violet-sensitive system (VS)			Ultraviolet-sensitive system (UVS)		
	Estimate ± SE	*t* ratio	*P*	Estimate ± SE	*t* ratio (df)	*P*
IS – IF						
Back	–0.08 ± 0.19	–0.40	0.69	–0.09 ± 0.18	–0.50	0.62
Breast	–0.02 ± 0.17	–0.10	0.92	0.001 ± 0.20	–0.003	1.00
Crown	–0.50 ± 1.27	–0.40	0.69	–0.99 ± 2.57	–0.39	0.71
Rump	0.11 ± 0.23	0.46	0.65	0.04 ± 0.21	0.20	0.85
Throat	–0.07 ± 0.11	–0.64	0.52	–0.05 ± 0.11	–0.48	0.63
U. coverts	0.36 ± 0.16	2.26	**0.028**	0.28 ± 0.17	1.68	0.097
IS – IM						
Back	–0.03 ± 0.19	–0.17	0.87	–0.05 ± 0.18	–0.27	0.79
Breast	–0.10 ± 0.17	–0.59	0.56	–0.12 ± 0.20	–0.63	0.53
Crown	0.28 ± 1.24	0.23	0.82	0.26 ± 2.47	0.10	0.92
Rump	–0.12 ± 0.20	–0.61	0.54	–0.03 ± 0.19	–0.16	0.87
Throat	–0.03 ± 0.11	–0.03	0.76	–0.04 ± 0.10	–0.40	0.69
U. coverts	–0.13 ± 0.10	–1.26	0.21	–0.17 ± 0.12	–1.38	0.17
IF – IM						
Back	0.04 ± 0.27	0.16	0.87	0.04 ± 0.25	0.18	0.86
Breast	–0.08 ± 0.24	–0.34	0.73	–0.12 ± 0.28	–0.44	0.66
Crown	0.78 ± 1.77	0.44	0.66	1.25 ± 3.54	0.35	0.72
Rump	–0.23 ± 0.30	–0.76	0.45	–0.07 ± 0.28	–0.25	0.80
Throat	0.04 ± 0.15	0.25	0.80	0.01 ± 0.15	0.08	0.93
U. coverts	–0.49 ± 0.19	–2.63	**0.010**	–0.45 ± 0.20	–2.21	**0.030**

Table 5-3s. Sexual differences in plumage colour of adult Rufous Horneros

We show the magnitude of mean differences and *t*-test results. Sample sizes: 31 males and 30 females, except for paired *t*-test results (*N* = 23 breeding pairs, df =22). U. coverts: undertail coverts.

	Mean (%) ± SE			*t*-test		Paired *t*-test	
	Male	Female	Cohen's *d* (CI)	*t* (df)	*P*	*t*	*P*
Mean reflectance							
Back	4.94 ± 0.32	5.43 ± 0.29	–0.29 (–0.82, 0.23)	–1.14 (58.61)	0.26	–0.34	0.74
Breast	11.82 ± 0.48	13.23 ± 0.36	–0.60 (–1.13, –0.06)	–2.34 (55.25)	0.02	–1.90	0.07
Crown	4.67 ± 0.30	4.77 ± 0.34	–0.06 (–0.58, 0.46)	–0.22 (57.66)	0.82	0.47	0.64
Rump	6.41 ± 0.27	6.94 ± 0.29	–0.34 (–0.87, 0.18)	–1.34 (58.26)	0.19	–0.86	0.40
Throat	33.04 ± 1.94	32.25 ± 1.92	0.07 (–0.45, 0.60)	0.29 (59.00)	0.77	0.50	0.62
U. coverts	18.90 ± 0.85	19.34 ± 0.81	–0.10 (–0.62, 0.43)	–0.38 (58.95)	0.71	–0.44	0.66
Contrast							
Back	14.59 ± 0.56	15.86 ± 0.67	–0.38 (–0.90, 0.15)	–1.47 (56.83)	0.15	–0.60	0.55
Breast	32.00 ± 1.32	34.62 ± 1.27	–0.37 (–0.89, 0.16)	–1.59 (58.63)	0.12	–0.99	0.33
Crown	12.15 ± 0.80	12.69 ± 0.90	–0.12 (–0.64, 0.41)	–0.45 (58.03)	0.65	0.47	0.65
Rump	22.55 ± 0.65	22.81 ± 0.75	–0.07 (–0.60, 0.46)	–0.27 (56.15)	0.79	–0.71	0.48
Throat	44.49 ± 3.37	42.58 ± 3.02	0.11 (–0.41, 0.63)	0.42 (58.50)	0.68	0.33	0.74
U. coverts	37.11 ± 1.65	36.14 ± 1.73	0.10 (–0.42, 0.63)	0.40 (58.76)	0.69	–0.01	0.99
Red chroma							
Back	59.24 ± 1.49	58.02 ± 1.44	0.15 (–0.37, 0.67)	0.59 (58.97)	0.56	0.28	0.78
Breast	55.21 ± 1.39	53.65 ± 1.23	0.21 (–0.31, 0.74)	0.84 (58.38)	0.41	0.70	0.49
Crown	52.83 ± 1.24	53.25 ± 1.30	–0.06 (–0.59, 0.47)	–0.23 (57.56)	0.82	–0.53	0.60
Rump	65.00 ± 1.68	63.27 ± 1.52	0.19 (–0.33, 0.72)	0.76 (58.63)	0.45	0.42	0.68
Throat	33.82 ± 0.67	33.71 ± 0.73	0.03 (–0.49, 0.55)	0.10 (58.31)	0.92	–0.34	0.74
U. coverts	44.59 ± 0.93	42.71 ± 0.78	0.40 (–0.13, 0.92)	1.55 (57.59)	0.13	0.92	0.37

Table 5-4s. Multivariate correlation in plumage coloration between paired individuals of the Rufous Hornero

Mantel tests of dissimilarity matrices based on Euclidean distances and the variables´ mean reflectance, contrast and red chroma. *P* values were obtained based on 999 permutations. Sample size: 23 breeding pairs. U. coverts: undertail coverts.

Body region	Mantel *r*	*P*
Back	–0.03	0.53
Breast	0.01	0.42
Crown	–0.02	0.52
Rump	–0.05	0.63
Throat	0.13	0.14
U. coverts	–0.10	0.80

Table 5-5s. Correlation of isolated colour measurements between paired individuals of the Rufous Hornero

N = 23 breeding pairs. U. coverts: undertail coverts.

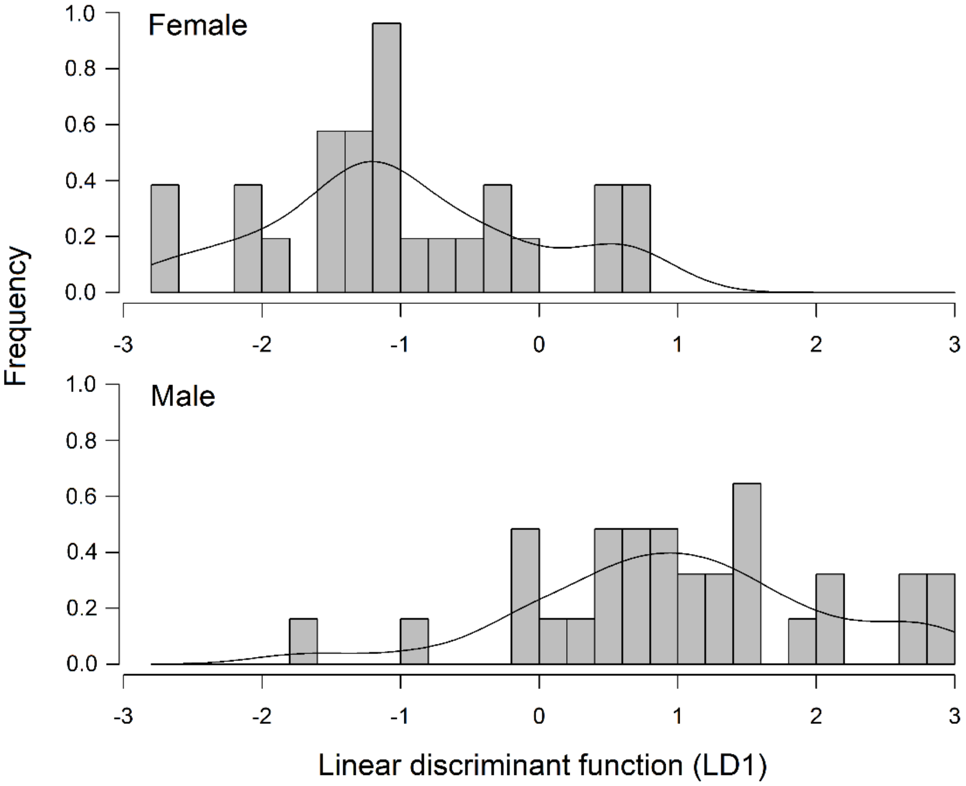
	Pearson correlation r_p (*P*)		
Body region/ colour trait	Mean reflectance	Contrast	Red chroma
Back	–0.23 (0.28)	–0.17 (0.43)	–0.21 (0.34)
Breast	–0.05 (0.81)	–0.20 (0.36)	0.06 (0.78)
Crown	–0.08 (0.71)	0.17 (0.43)	0.13 (0.56)
Rump	–0.36 (0.10)	–0.14 (0.53)	–0.28 (0.19)
Throat	0.08 (0.70)	0.009 (0.97)	–0.11 (0.61)
U. coverts	0.09 (0.68)	–0.009 (0.97)	0.05 (0.81)

Table 5-6s. Correlation of isolated size measurements between paired individuals of the Rufous Hornero

We show results of Pearson correlation tests. *N* = 23 breeding pairs.

Size variable (*N*)	r_p	*P*
Bill depth	0.35	0.10
Bill length	0.05	0.84
Bill width	–0.44	0.04
Mass	0.03	0.89
Tail	0.05	0.82
Tarsus	0.02	0.92
Wing chord	0.26	0.23

Figure 5-4s. Histogram generated by a Linear Discriminant Analysis showing discriminant function values for Rufous Hornero males and females. Positive LD1 values would indicate that an individual is a male, and negative LD1 values would indicate a female.



DISCUSSÃO GERAL

Exibições coletivas complexas envolvendo dois (duetos) ou mais indivíduos (coros) fascinam cientistas há décadas (Farabaugh, 1982; Levin, 1996; Hall, 2004) e representam uma das formas menos compreendidas de comunicação social encontradas na natureza (Tobias *et al*., 2016). O canto em dueto se destaca como uma das exibições coletivas mais estudadas, mas cuja natureza e função permanecem mal compreendidas (Hall, 2009; Logue e Krupp, 2016; Tobias *et al*., 2016). Por ser um comportamento coletivo, cada participante do dueto pode ter um interesse distinto e o dueto pode ter uma função diferente para cada participante (Hall, 2009; Logue e Krupp, 2016). Por outro lado, dois indivíduos podem ter interesses e benefícios em comum quando cantam em dueto (Hall, 2009; Logue e Krupp, 2016). Neste estudo, explorei detalhadamente os duetos do João-de-barro, relacionando as características, expressão e ocorrência do dueto com a biologia reprodutiva, territorialidade e a socialidade da espécie. Estudos intensivos em uma espécie modelo, como este estudo, podem contribuir significativamente para o entendimento da função do canto em aves, especialmente em espécies que cantam em dueto.

Primeiro, avaliei a relação entre as características do canto, sazonalidade reprodutiva e ocorrência de interações territoriais (capítulo 1). Nesse capítulo, testei e encontrei apoio para a ideia de que a função do dueto varia de acordo com o sexo, papel no canto (inicia o canto ou responde ao canto iniciado pelo parceiro) e nível de organização do dueto (individual ou do par reprodutor). Fêmeas iniciaram mais cantos no período não-reprodutivo, enquanto machos iniciaram mais cantos nos períodos pré-reprodutivo e pós-reprodutivo. Por outro lado, tanto machos quanto fêmeas responderam proporcionalmente mais e mais rapidamente aos cantos iniciados pelo parceiro (i.e. criando duetos) nos períodos pré-reprodutivo e fértil da fêmea. Os duetos ocorreram com maior frequência nos períodos pré-reprodutivo e pós reprodutivo. Por fim, a taxa de início de canto e a taxa de duetos, mas não a proporção de cantos do parceiro que foram respondidos para a criação de duetos, foram relacionadas à ocorrência de interações territoriais.

Os resultados do capítulo 1 sugerem que machos iniciam cantos para defenderem território, parceira, ou ambos, e para estimular a fisiologia reprodutiva da parceira (Capítulo 1: Tabela 1). Por outro lado, fêmeas parecem iniciar cantos para defenderem territórios, principalmente no período não reprodutivo. Os resultados sugerem que a resposta de canto (canto executado em resposta ao canto iniciado pelo parceiro) funciona como um mecanismo de guarda mútua de parceiro e estímulo hormonal para reprodução em ambos os sexos. Em conclusão, os resultados do capítulo 1 apoiam parcialmente a ideia de que o dueto a nível do par funciona na defesa de território e guarda mútua de parceiro, e sugerem fortemente que os cantos no João-de-barro se baseiam na cooperação entre parceiros reprodutores.

A avaliação da resposta comportamental ao *playback* de solos *versus* duetos é o método mais utilizado para se testar indiretamente a função de cantos em duetos (Douglas e Mennill, 2010). Esse método permite avaliar o nível relativo de ameaça gerado pela simulação da intrusão territorial por um indivíduo (solo) *versus* por dois indivíduos coordenando seus cantos (dueto) (Douglas e Mennill, 2010). De acordo com a hipótese de defesa conjunta de território, indivíduos devem responder mais ou tão intensamente aos *playbacks* de duetos em comparação com os *playbacks* de solos (Benedict, 2010; Douglas e Mennill, 2010). De acordo com a hipótese de guarda de parceiro unilateral, o *playback* de solos de indivíduos do mesmo sexo deve representar uma maior ameaça aos indivíduos focais em comparação com o *playback* de solos de indivíduos do sexo oposto (Rogers *et al*., 2006; Seddon e Tobias, 2006). Considerei que a presença de jovens no território dos pais poderia afetar o custo relativo dos pais em perderem o território *versus* o parceiro, pressupondo que a perda de território comprometeria a sobrevivência de ambos os adultos e jovens. Portanto, propus que a presença de jovens poderia afetar como os adultos responderiam ao *playback* de solos *versus* duetos.

No capítulo 2, expus grupos de João-de-barro ao *playback* de solo de fêmea, solo de macho, dueto e um controle heterospecífico, com o objetivo de avaliar duas questões: (1) adultos respondem diferentemente a *playbacks* de cantos solo de indivíduo do mesmo sexo, do sexo oposto, ou dueto? (2) a resposta diferencial ao *playback* de cantos solo de macho, solo de fêmea e duetos varia entre casais de João-de-barro com jovens *versus* casais de João-de-barro sem jovens? Mensurei a resposta física e vocal dos adultos focais ao *playback*, e também avaliei o grau de coordenação em que os parceiros respondiam ao *playback*.

Parceiros responderam equivalente e coordenadamente aos *playbacks* de cantos coespecíficos, especialmente duetos. Entretanto, casais com jovens diferiram de casais sem jovens na forma e intensidade em que responderam ao *playback* de solos *versus* duetos. Casais sem jovens responderam mais agressivamente ao *playback* de solos, enquanto casais com jovens responderam mais agressivamente ao *playback* de duetos e solos de fêmeas. Esses resultados sugerem que o dueto funcione principalmente no contexto de defesa conjunta de território, mas que a guarda mútua de parceiro também tenha um papel importante no dueto em casais sem jovens (Capítulo 2: Tabela 1).

Nos capítulos 1 e 2, explorei a função do dueto e de seus componentes individuais no João-de-barro, utilizando observações e experimentos. Mostrei que os cantos do João-de-barro estão mais relacionados à defesa conjunta de territórios e do vínculo social do par reprodutor. No capítulo 3, examinei alguns potenciais benefícios desses cantos. Existe ampla evidência na literatura de que o canto de aves reflete a qualidade de machos e afeta de várias maneiras a aptidão desses machos, através de vantagens competitivas em interações com outros machos ou na escolha de parceiros pelas fêmeas (Kroodsma e Byers, 1991; Gil e Gahr, 2002; Catchpole e Slater, 2008). Em contrapartida, pouco se sabe sobre as vantagens adaptativas do canto de fêmeas (Brunton *et al.*, 2016; Cain e Langmore, 2016) e dos duetos em aves (Hall, 2009; Dahlin e Benedict, 2013).

No capítulo 3, explorei se características do canto refletiriam o sucesso reprodutivo genético e a qualidade dos territórios no João-de-barro. Realizei análises de parentesco para descrever o sistema de acasalamento genético e as taxas de paternidade extrapar na população estudada. Amostrei a área de cada território bem como a disponibilidade de sítios para forrageamento e nidificação, como medidas para estimar a qualidade dos territórios. Por fim, estimei a quantidade e a sobrevivência de jovens que deixaram o ninho com sucesso. Considerando a natureza cooperativa do canto na defesa conjunta de territórios, demonstrada nos capítulos 1 e 2, esperava encontrar uma baixa taxa de paternidade extrapar na população, e uma relação positiva entre as características de canto, qualidade territorial e sucesso reprodutivo.

Como esperado, encontrei uma baixa taxa de paternidade extrapar na população de João-de-barro (<4% dos ninhegos). O sucesso reprodutivo aparente foi de 100%, ou seja, ao menos um filhote social de cada casal deixou o ninho com sucesso. Encontrei também uma relação positiva entre o investimento da fêmea em canto e a velocidade com a qual respondia ao canto do parceiro, com a qualidade de seu território, no que se refere à disponibilidade de manchas de recursos alimentares e tamanho do território. Similarmente, a duração do dueto foi relacionada positivamente ao tamanho do território do casal. No entanto, nenhuma característica do canto ou do território foi relacionada ao sucesso reprodutivo de fêmeas, machos ou casais. Em conclusão, os resultados do capítulo 3 sugerem que o canto em fêmeas e o dueto são utilizados na defesa de recursos alimentares dentro dos territórios (ver Cooney e Cockburn, 1995; Cain *et al*., 2015), ou permitem a aquisição de territórios de alta qualidade (ver Rosvall, 2011; Cain *et al*., 2015).

A coordenação temporal é um componente fundamental da definição de dueto e consiste na alternância (Rivera-Cáceres, 2015), sobreposição (Rehberg-Besler *et al*., 2016) ou associação rítmica entre elementos acústicos (Laje e Mindlin, 2003; Benichov *et al*., 2016). A coordenação temporal do dueto varia amplamente entre espécies (Hall, 2009). Em algumas espécies, como em *Cantorchilus modestus zeledoni*, machos e fêmeas intercalam elementos acústicos com pouca ou nenhuma sobreposição espectro-temporal (Rivera-Cáceres, 2015). Em outras espécies, como o João-de-barro, os elementos acústicos de macho e fêmea se sobrepõem parcialmente em frequência e tempo (Laje e Mindlin, 2003), e estudos prévios revelaram a existência de associação rítmica entre as frases de fêmea e macho no João-de-barro (Laje and Mindlin 2003; Diniz, dados não publicados).

Do ponto de vista do emissor do sinal, o nível de coordenação do dueto pode ter um papel no aumento de sua propagação espacial (Rehberg-Besler *et al*., 2016), refletir a interferência de um sinal sobre outro (Seddon e Tobias, 2006; Dahlin e Wright, 2007; Hall, 2009; Tobias e Seddon, 2009), ou sinalizar a motivação ou habilidade de um casal em lutar por um recurso em comum (hipótese da qualidade da coalisão) (Hall e Magrath, 2007). Do ponto de visto do receptor do sinal, sabe-se muito pouco sobre o que faz com que um dueto desencadeie uma resposta mais agressiva do que um canto solo: a vantagem numérica do dueto (Molles e Waas, 2006; Douglas e Mennill, 2010; Kovach *et* *al.*, 2014) ou propriedades da coordenação dos cantos (hipótese da qualidade da coalisão) (Hall e Magrath, 2007; Kovach *et al.*, 2014).

No capítulo 4, testei a hipótese da qualidade da coalisão (Hall e Magrath, 2007) no João-de-barro por meio de um experimento de *playback* que consistiu na apresentação de duetos com diferentes níveis de coordenação e sobreposição temporal de frases. Embora machos e fêmeas aparentemente identificaram diferenças no nível de coordenação temporal do dueto, eles responderam com o mesmo nível de agressividade a todos os *playbacks* de duetos coespecíficos, independentemente do nível de coordenação temporal desses duetos. Portanto, os resultados do capítulo 4 refutam a hipótese de qualidade da coalisão (Hall e Magrath, 2007) como uma possível explicação para o papel do nível de coordenação temporal do dueto no João-de-barro. Sugiro que o nível de coordenação temporal de frases nos duetos do João-de-barro tenha um papel no incremento da propagação espacial do sinal acústico (Rehberg-Besler *et al.*, 2016) ou na comunicação entre parceiros (Boucaud *et al.*, 2016).

Nos primeiros quatro capítulos, estudei em detalhes o papel do canto nas interações sociais e no sucesso reprodutivo no João-de-barro. Porém, ainda é possível que existam outros sinais sexuais ou sociais, além do sinal acústico, que tenham um papel importante na aptidão dos adultos nessa espécie (Guindre-Parker *et al.*, 2012). Explorei essa possibilidade por meio do estudo do componente sensorial visual. Presume-se que a maioria das espécies de aves da família Furnariidae é sexualmente monocromática, mas esse pressuposto advém da percepção humana de coloração das aves (Seddon *et al.*, 2010). No capítulo 5, investiguei a existência de dimorfismo sexual em relação ao tamanho dos indivíduos e à coloração da plumagem no João-de-barro. Também testei se fêmeas e machos se pareavam por associação de caracteres morfológicos: e.g. fêmeas com coloração mais brilhante se associariam com machos com coloração mais brilhante, ou fêmeas menores com machos menores.

Machos tiveram comprimentos de asa e cauda um pouco (~4%) maiores do que fêmeas, mas os sexos não diferiram em peso e medidas de tarso e bico. Não encontrei dicromatismo sexual. Modelos visuais indicam que, em média, indivíduos diferem na coloração da plumagem, independentemente do sexo. Também não encontrei evidência de pareamento associativo no tamanho ou coloração da plumagem. Os resultados do capítulo 5 sugerem que no João-de-barro a coloração da plumagem e o tamanho do corpo têm papel irrelevante nas interações sexuais, e não estão sob pressão de seleção sexual. Adicionalmente, esses resultados reforçam a convergência de papéis sexuais na espécie.

CONCLUSÕES

1. A maioria dos cantos do João-de-barro é coordenada em duetos, que são iniciados principalmente por machos.
2. O canto do João-de-barro tem múltiplas funções, que dependem do sexo, papel no dueto (início ou resposta de canto), nível de organização do dueto (individual ou casal) e estrutura do grupo social.
3. Em geral, fêmea e macho pareados cooperam quando cantam em dueto para defenderem um território em comum e o próprio vínculo social do casal.
4. Fêmea e macho pareados são altamente coordenados nas interações territoriais direcionadas a intrusos.
5. O sistema de acasalamento é socialmente monogâmico com baixa taxa de paternidade extrapar e alto sucesso reprodutivo.
6. O canto da fêmea e o dueto do casal refletem a qualidade do território, mas não o sucesso reprodutivo do casal.
7. O nível de coordenação de cantos no dueto não sinaliza a qualidade ou motivação do casal em competir por recursos territoriais.
8. Machos e fêmeas são praticamente idênticos no que se refere à coloração e à morfologia, indicando ausência de seleção sexual sobre esses caracteres.

Em suma, nossos resultados sugerem que machos e fêmeas de João-de-barro cooperam quando cantam em dueto na defesa de um território comum e do vínculo social do casal, e que essa cooperação se reflete em seu sistema de acasalamento socialmente monogâmico com baixas taxas de paternidade extrapar.

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