Universidade de Brasília
Instituto de Ciências Biológicas
Departamento de Ecologia

Biologia reprodutiva e socialidade no pica-pau-do-campo (*Colaptes campestris campestris*)

Raphael Igor da Silva Corrêa Dias

Orientadora: Regina H. F. Macedo

Tese apresentada ao Departamento de Ecologia da Universidade de Brasília, como requisito parcial à obtenção do grau de Doutor em Ecologia

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Tese aprovada junto ao Programa de Pós Graduação em Ecologia da Universidade de Brasília, como requisito parcial para obtenção do título de Doutor em Ecologia

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Brasília, março de 2011.

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Reprodução cooperativa é observada em aproximadamente 9% das aves e é definida como sendo um sistema em que mais de dois indivíduos auxiliam na criação dos filhotes em uma única tentativa reprodutiva. Esses sistemas são geralmente compostos por pares socialmente monogâmicos que são auxiliados por filhotes produzidos em estações anteriores. Nesse estudo, o sistema social e de acasalamento do pica-pau-do-campo (*Colaptes campestris campestris*) foi avaliado, utilizando-se técnicas tradicionais de marcação e observação, assim como, análises genéticas e modelagem estatística. O estudo revelou que o pica-pau-do-campo é uma espécie cooperativa facultativa, que apresenta um sistema reprodutivo variável, incluindo casais monogâmicos e grupos com poliginia simultânea. Adicionalmente, foi detectada a ocorrência de estratégias reprodutivas alternativas como são os casos da reprodução comunitária (i.e. mais de uma fêmea pondo ovos no mesmo ninho) em alguns grupos polignínicos e do parasitismo de ninho intra-específico. Algumas evidências sugerem que restrições ecológicas, especialmente em termos de dispersão, juntamente com traços de história de vida, devem ter favorecido a evolução da reprodução cooperativa na espécie, embora a seleção de parentesco aparente ter um papel importante no sistema. O estudo revelou também que a produtividade na espécie é diretamente afetada pela presença de auxiliares no grupo, mas que fatores climáticos podem ser determinantes para a qualidade dos filhotes produzidos. Em relação à influência dos auxiliares no investimento parental, os resultados sugerem que a espécie apresenta tanto uma redução compensatória quanto um efeito aditivo. Entretanto, foi observado que os auxiliares apresentam um investimento superior em filhotes mais aparentados com os mesmos. Esse estudo revelou que o pica-pau-do-campo apresenta componentes comportamentais de alta complexidade que faz dele um modelo para futuros estudos testando diferentes hipóteses relacionadas com a evolução da reprodução cooperativa.

Palavras-chave: reprodução cooperativa; reprodução comunitária; poliginia; monogamia; parasitismo de ninho; ajudantes

Abstract

Cooperative breeding is found in approximately 9% of bird species and is defined as a system wherein more than two individuals help to rear the offspring during a single breeding attempt. These systems are generally composed by socially monogamous pairs assisted by offspring produced in previous years. In this study, the social and mating systems of the campo flicker (*Colaptes campestris campestris*) were evaluated, using traditional techniques of banding and observation, as well as through genetic analysis and statistical modeling. The study revealed that the campo flicker is a facultative cooperative breeder with a variable mating system that includes monogamous pairs and groups with simultaneous polygyny. Additionally, the occurrence of alternative mating strategies was detected and included cases of joint-nesting (i.e. more than one female laying eggs in the same nest) in some polygynous groups and intra-specific nest parasitism. Some evidence suggests that ecological constraints, especially in terms of dispersal, in addition to life-history traits, must have favored the evolution of cooperative breeding in the species, although kin selected benefits must have an important role in the system. Results showed that the productivity in the species is directly affected by the presence of auxiliaries in the group, but that climatic factors may be crucial in determining the quality of fledglings. Relative to the influence of auxiliaries upon parental investment, results suggest that the species presents both a compensatory reduction as well as an additive effect. However, auxiliaries invested more in offspring to which they were genetically related. This study revealed that the campo flicker presents behavioral components of high complexity, which suggests that this species is a good model for future studies testing different hypotheses related to the evolution of cooperative breeding.

Key-words: cooperative breeding; joint-nesting; polygyny; monogamy; nest parasitism; helpers

Resumo expandido

Introdução

Sabe-se que grande parte do conhecimento ecológico foi construído com base em dados coletados no hemisfério norte, e na ornitologia não é diferente (Stutchbury e Morton 2001). Um bom exemplo desse padrão são os estudos realizados com as espécies da família Picidae, popularmente conhecidos como pica-paus. Durante o período entre 1985-2004, apenas 3% dos artigos publicados com Picideos foram realizados na America Latina, apesar da região apresentar mais da metade das espécies existentes. Enquanto isso, a America do Norte e Europa, que possuem apenas 7% de todas as espécies de pica-pau, foram responsáveis por 70% de todas as publicações realizadas durante o período (Mikusiński 2006). O mesmo padrão pode ser observado para teorias inteiras na área da ecologia comportamental, onde assume-se que conceitos derivados de estudos da avifauna de regiões temperadas aplicam-se igualmente à avifauna tropical. No entanto, já existem críticas a esse paradigma, devido ao fato de diversas espécies da região tropical não se enquadrarem no padrão estabelecido para aves de regiões temperadas (Koenig e Dickinson 2004; Macedo et al. 2008). Com base nesse retrospecto, percebe-se que é indispensável a realização de pesquisas de história natural em espécies tropicais, possibilitando assim a realização de trabalhos com hipóteses bem definidas e sustentadas em conhecimento prévio.

Uma espécie que traz consigo algumas dessas características é o pica-pau-do-campo (*Colaptes campestris campestris*), que apresenta um reduzido número de informações acerca de sua biologia reprodutiva e comportamento social. Apesar disso, as poucas informações disponíveis revelam que a espécie apresenta características sociais marcantes, como um amplo leque de vocalizações utilizadas em diferentes contextos (Goedert *em preparação*) e forrageamento social, incluindo forrageamento inter-específico (Short 1972).Além disso, pouco se sabe sobre os aspectos reprodutivos e outras interações sociais, embora tenha já sido sugerida a possibilidade da espécie estar enquadrada entre os 9% (Cockburn 2006) de espécies classificadas como reprodutoras cooperativas (Winkler et al. 1995).

Sistemas de reprodução cooperativa são caracterizados pela presença de indivíduos denominados auxiliares ou ajudantes, que provêem cuidados à prole produzida por outros indivíduos (Brown 1987). Inicialmente esse comportamento foi considerado como um grande enigma para a teoria da seleção natural (Darwin 1859). Embora, posteriormente o comportamento tenha sido parcialmente compreendido com a divulgação da teoria de seleção de parentesco (Hamilton 1964), onde se percebeu que grande parte dos auxiliares é composta por filhotes da estação reprodutiva anterior que adiaram sua dispersão, permaneceram no território natal e decidiram por ajudar na criação dos irmãos (Ekman et al. 2004). No mesmo artigo, Hamilton (1964) propôs uma regra que ficou conhecida como regra de Hamilton. Ela sugere que a seleção de parentesco deve ser favorecida quando o custo do comportamento de ajuda é compensado pelo benefício indireto da ajuda.

Nesse contexto, é evidente que os padrões de dispersão têm forte influência no aparecimento da reprodução cooperativa, na estrutura dos grupos e função dos seus membros. De forma geral, em aves, quem dispersa são as fêmeas, enquanto os machos permanecem no território natal e se tornam auxiliares (Greewood 1980), embora hoje se saiba que há variação nesse padrão (Berg et al. 2009; Eikenaar et al. 2010). Alguns fatores são determinantes na decisão dos filhotes em adiar a reprodução individual, permanecer no território natal e se tornar um ajudante. Dentre eles estão os associados a restrições ecológicas, que podem impedir ou dificultar a reprodução individual (Brown 1974; Emlen 1982). Outras possibilidades que não são mutuamente excludentes com a hipótese das restrições ecológicas são as hipóteses de história de vida (Russell 1989; Arnold e Owens 1998) e a dos benefícios da filopatria (Stacey e Ligon 1987, 1991), embora questões filogenéticas também devam ser levadas em consideração.

Ambas as hipóteses (história de vida e benefícios da filopatria) foram formuladas a partir de ideias simples e lógicas. O principio fundamental da hipótese da história de vida é a constatação de que as espécies cooperativas compartilham características comuns que as tornam diferentes de outras espécies, como residência anual, baixas taxas de mudança de território, alta sobrevivência de adultos e presença em ambientes com variabilidade climáticas (Ligon e Burt 2004). Entretanto, essas características também são encontradas em espécies que não são cooperativas, levando-nos a questionar por que essas características afetariam algumas espécies e não outras. Os benefícios associados à permanência no território natal, como a herança do território em questão, é um dos benefícios da filopatria, embora existam também alguns custos associados, como a possibilidade de acasalamentos consanguíneos (Koenig e Haydock 2004).

As pressões seletivas não necessariamente levarão ao aparecimento de um comportamento cooperativo, pelo contrário, um filhote pode adiar a reprodução individual, permanecer no território natal e não ajudar na criação dos seus irmãos (Ekman et al. 2004). Contudo, existem outros fatores favorecendo o comportamento cooperativo desses indivíduos, e estes podem ser diferenciados em benefícios diretos e indiretos. Dentre os benefícios diretos estão o aumento na possibilidade de sobrevivência (Brown 1987) e aquisição de habilidade para futura reprodução individual (Lingon e Lingon 1978). Por outro lado, os benefícios indiretos, como já mencionados anteriormente, estão baseados em seleção de parentesco. A ideia fundamental é de que os auxiliares devem ser aparentados aos indivíduos que eles estão ajudando (Hamilton 1964), embora se saiba que, de forma geral, os auxiliares obtêm menores valores adaptativos ajudando, do que ganhariam reproduzindo (Dickinson e Hatchwell 2004).

A mera presença de auxiliares no grupo pode influenciar diversos parâmetros reprodutivos e traços de história de vida dos reprodutores que estão sendo auxiliados. Dentre os principais efeitos da presença de auxiliares no grupo estão o aumento na produtividade (Emlen e Wrege 1991; Conner et al. 2004), na qualidade dos filhotes produzidos (Hatchwell 1999) e na sobrevivência dos reprodutores (Reyer 1984), sendo que o último pode ser observado especialmente quando os pais podem reduzir parcialmente sua carga de trabalho. Uma característica considerada como altamente influenciada pela presença de auxiliares no ninho é o grau de investimento que os pais depositam na prole (Heinsohn 2004). Os pais ajustam o seu investimento na presença de auxiliares através da: redução do esforço parental (Hatchwell e Russell 1996); manutenção do mesmo padrão de esforço (Emlen e Wrege 1991); ou até mesmo aumentando seu esforço, embora tal possibilidade tenha recebido pouco apoio empírico (Valencia et al. 2006). De forma geral, os pais tendem a reduzir o investimento, sendo compensados pelo investimento gerado pelos auxiliares, mantendo assim o mesmo nível de investimento total no ninho - ‘efeito compensatório’ (Khan e Walters 2002; Russell et al. 2008). Por outro lado, quando os pais mantém ou aumentam o nível de investimento, o provisionamento dos mesmos é somado ao realizado pelos auxiliares, gerando um ‘efeito aditivo’, representado pelo aumento total no investimento do ninho (Magrath e Yezerinac 1997; Cockburn 1998).

Em espécies que apresentam grande variabilidade no grau de parentesco dentro do grupo, a capacidade de reconhecimento de parentes pode ter evoluído ao longo do tempo, tanto maximizando os ganhos relacionados com a seleção de parentesco quanto desfavorecendo o cuidado indiscriminado por parte dos auxiliares (Griffin e West 2003; Cornwallis et al. 2009). Em diversas espécies, foi demonstrado que os auxiliares estão mais propensos a ajudar indivíduos aparentados, ou ajudar esses indivíduos numa taxa mais elevada, do que indivíduos não aparentados (Komdeur 1994; Dickinson et al. 1996; Russell e Hatchwell 2001; Richardson et al. 2003).

Um componente que está incluído no sistema social é o sistema de acasalamento. Os reprodutores cooperativos são, em sua maioria, formados por um casal socialmente monogâmico auxiliado por filhotes da estação reprodutiva anterior (Brown 1987). Entretanto, outros tipos menos comuns de sistema de acasalamento são encontrados em espécies cooperativas, incluindo poliginia, poliandria e poliginandria (Cockburn 2004). Além disso, embora raras, estão as estratégias reprodutivas alternativas, como ocorrência de paternidade extra-par, parasitismo de ninho intra-específico (Griffith et al. 2002; Lyon e Eadie 2008) e a presença de reprodução comunitária (i.e. mais de uma fêmea contribuindo com ovos no mesmo ninho; Brown 1987). Com o avanço de técnicas moleculares ficou mais fácil compreender os sistemas de acasalamento e suas variações. Adicionalmente, o conceito inicial que tínhamos acerca do sistema de acasalamento em diversas espécies teve de ser reconstruído, sendo incorporado um novo conhecimento baseado na realidade biológica e não meramente em observações. Para entender a evolução de sistemas cooperativos e a função de seus membros no grupo é essencial o conhecimento do que está ocorrendo em termos de reprodução e quais são os ganhos de cada indivíduo do grupo.

Os objetivos desse trabalho foram: (1) gerar conhecimento acerca da biologia reprodutiva e dos comportamentos sociais e de acasalamento do pica-pau-do-campo (*Colaptes campestris campestris*); e (2) avaliar a possibilidade de ocorrência de reprodução cooperativa na espécie. Para tanto, foram utilizados dados observacionais, genéticos e modelagem estatística para testar diversas hipóteses que avaliaram os efeitos dos ajudantes sobre parâmetros reprodutivos e o investimento parental do pica-pau-do-campo.

Metodologia

Espécie em estudo

O gênero *Colaptes* apresenta algumas características atípicas para pica-paus, representadas particularmente pela ocorrência de socialidade e hábitos terrestres (Short 1972). O gênero está distribuído ao longo das Américas e apresenta algumas espécies geograficamente separadas em sub-espécies, com claras zonas de hibridização (Short 1972). Como exemplo desse padrão pode-se mencionar o próprio pica-pau-do-campo, onde duas sub-espécies são reconhecidas: o *Colaptes campestris campestris* e o *C. campestris campestroides*. A única diferença observável entre as duas sub-espécies está na coloração da garganta, que é negra no *campestris* e branca no *campestroides* (Short 1972).

O pica-pau-do-campo (*Colaptes campestris campestris*) tem tamanho médio, apresenta um leve dimorfismo sexual e ocupa grande parte da América do Sul, desde o Paraguai até o Nordeste brasileiro (Short 1972), incluindo manchas isoladas de Cerrado na Amazônia (Silva et al. 1997). Apesar dessa ampla distribuição, pouco se sabe sobre os aspectos de sua história natural, ecologia e biologia reprodutiva, assim como, detalhes do seu sistema social e de acasalamento.

Área de estudo e procedimentos gerais

O estudo foi realizado na Fazenda Água Limpa (FAL; 15°56'S, 47°55'W) durante o período de outubro de 2006 à dezembro de 2009. A FAL está localizada na região central do Brasil, mais especificamente no Distrito Federal, ocupando uma área de 4,500 ha que apresenta diferentes fitofisionomias típicas de Cerrado, como áreas de campo limpo, campo sujo, cerrado *sensu stricto* e matas de galeria (Oliveira-Filho e Ratter 2002). Os dados climáticos utilizados no presente estudo foram obtidos diretamente de uma estação meteorológica presente na área de estudo.

Ao longo do projeto foram realizadas buscas em toda área de estudo por grupos de pica-pau-do-campo, utilizando-se também *playback* de vocalizações da espécie para facilitar o avistamento. A posição dos grupos encontrados foi registrada em GPS (Garmin eTrex®) e posteriormente os membros do grupo foram capturados com redes de neblina tipo bandeira. Durante a captura, os indivíduos receberam uma combinação única de quatro anilhas, sendo três anilhas plásticas coloridas e uma anilha metálica numerada do CEMAVE-IBAMA. Adicionalmente, os indivíduos foram medidos (tarso, asa, narina e cauda) com paquímetro (precisão 0.02mm) e pesados em balança de mola (Pesola). Adicionalmente, foram coletadas amostras de sangue (100µl) da veia braquial de todos os indivíduos capturados para posterior análise genética.

Durante a estação reprodutiva, foram realizadas buscas em cada território por cavidades em construção ou finalizadas, sendo todas registradas em GPS. Depois de encontradas, as cavidades foram periodicamente checadas com auxílio de um espelho acoplado a um arame e de uma lanterna. Ninhos ativos foram monitorados a cada 2-3 dias. Durante as checagens de ninho, foi registrada a presença ou não de piolhos de pena e carrapatos no interior da cavidade. Foi definido como sucesso de eclosão o percentual de ovos em relação ao tamanho total da ninhada que eclodiu. A predação de ninho foi considerada a causa de um ninho falhar quando todos os ovos ou filhotes desapareceram e o ninho apresentou sinais de destruição na entrada da cavidade e/ou penas ou sangue ao redor do ninho. Foram atribuídas como perdas por inanição as situações em que os filhotes foram encontrados mortos dentro do ninho e uma redução de ninhada foi observada em dias sequenciais, enquanto que perdas por parasitismo foram consideradas quando os mesmos sinais foram observados, mas com a presença maciça de parasitas de ninho. Um ninho foi classificado como tendo tido sucesso se os filhotes dispersaram do ninho dentro de um período de cinco dias da data esperada para a sua saída, sem sinais de predação (mais detalhes no Capítulo 1). Quando estava próximo dos filhotes saírem do ninho (25 dias), eles foram medidos, pesados, tiveram uma amostra de sangue coletada através da veia braquial, sendo marcados com anilhas da mesma forma que os adultos.

Os grupos sociais foram definidos como sendo qualquer agregação de dois ou mais indivíduos que mostraram uma forte associação, como deslocamentos juntos em uma específica porção da área de estudo, por um período de pelo menos seis meses. A composição dos grupos e interações sociais foram registradas ao longo dos anos do estudo. Os grupos foram inicialmente classificados como pares socialmente monogâmicos (apenas dois adultos) e grupos cooperativos (mais de dois adultos). Por definição, foram considerados como reprodutores dominantes aqueles indivíduos que produziram uma maior proporção de filhotes por tentativa reprodutiva, sendo essa determinação feita geneticamente. Os reprodutores subordinados ou auxiliares secundários consistiram basicamente de indivíduos auxiliares fêmeas que conseguiram produzir algum filhote durante a reprodução do grupo. Por fim, os auxiliares primários foram definidos como sendo indivíduos que investiram durante a reprodução na incubação ou alimentação da prole, mas que não produziram nenhum filhote.

Atividade parental e aloparental

Foram realizadas observações focais a partir de um esconderijo (aproximadamente 20-30m de distância) usando binóculo ou luneta. Algumas observações foram realizadas usando câmeras de vídeo (Sony DCR-HC52). Durante a incubação, a atividade no ninho foi registrada por uma hora no quinto e no décimo dia após a ninhada ter sido completada. Esses focais foram realizados para os anos de 2008 e 2009. Durante o período de ninhego, a atividade do ninho foi registrada em cinco dias diferentes (4, 10, 16, 22 e 28 após a eclosão). Foram registradas basicamente a identidade do adulto visitante, o número de visitas realizadas e o tempo em que o indivíduo permaneceu dentro da cavidade (mais detalhes nos capítulos 1 e 4).

Área de vida e território

A localização dos grupos monitorados na área de estudo ao longo dos anos foi registrada em GPS através de duas técnicas de busca. Os grupos foram encontrados por busca ativa em seu território ou, alternativamente, através da frequência de rádiotransmissores que foram colocados em indivíduos de doze grupos sociais. O cálculo da área de vida foi realizado no programa Home Ranges 1.5 (Hovey 1999) (maiores detalhes no Capítulo 1).

Análises genéticas

Procedimentos gerais

Para interpretar os dados genéticos foram utilizadas todas as informações obtidas ao longo dos anos referentes à estrutura dos grupos durante os períodos reprodutivo e não reprodutivo. Foram incluídos nas análises os dados de alguns grupos para os quais, embora tenham sido obtidas amostras de sangue, foram registradas poucas informações comportamentais ou reprodutivas. Foi considerado que essa informação adicional poderia ser útil para entender a estrutura dos grupos. Informações sobre mudanças na composição dos grupos e utilização de dados dos mesmos grupos ou indivíduos em estações reprodutivas diferentes foram controlados estatisticamente nas análises (informações detalhadas podem ser encontradas em cada capítulo).

Classificaram-se como reproduções consanguíneas aquelas entre indivíduos que compartilham um ancestral comum e que apresentem um coeficiente de parentesco entre 0,0625-0,5 (Koenig e Haydock 2004). Foi calculada a razão sexual da população com os dados de todos os indivíduos observados na área de estudo, inclusive os que não foram capturados. Tal discriminação entre os sexos foi possível uma vez que existe um leve dicromatismo sexual e os grupos são estáveis. O sucesso de nidificação foi definido como sendo uma variável binária, representando a produção ou não de filhotes em uma dada estação reprodutiva.

Extração do DNA e análises

As amostras de sangue foram armazenadas em uma solução tampão em temperatura ambiente até a extração do DNA. Foram examinados 12 loci originalmente isolados do pica-pau-mosqueado (*Colaptes auratus*, Kuhn et al. 2009) para serem avaliados quanto a amplificação e polimorfismo no pica-pau-do-campo, sendo, no fim, possível a utilização de 10 microsatélites polimórficos. Foram realizadas reações de polimerase em cadeia (PCR's), posteriormente diluídas para otimizar a visualização do produto no sequenciador (ABI 3100). Os eletroferogramas foram analisados utilizando o programa GeneMapper® (4.1; Applied Biosystems) (maiores informações no Capítulo 2).

Os seguintes parâmetros genéticos foram avaliados: paternidade, parentesco e relação genealógica. O programa Genepop 4.0 (Raymond e Rousset 1995) foi utilizado para determinar o nível de heterozigosidade observada e esperada, probabilidade de exclusão parental e frequência de alelos nulos (Tabela 1; Capítulo 2). As análises de paternidade foram realizadas no programa Cervus versão 3.0.3 (Marshall et al. 1998; Kalinowski et al. 2007). O poder de exclusão de todos os loci combinados foi alto, sendo 0.9913 quando assumido que nenhum dos pais era conhecido; e, 0.9996 assumindo que um dos pais era conhecido. Foram aceitas as determinações do programa quando o trio "fêmea/filhote/macho candidato" apresentou zero ou uma falta de combinação, e estava de acordo com os dados comportamentais. As análises de parentesco foram realizadas no programa SPAGeDi (Hardy e Vekemans 2002) e as análises das relações genealógicas no programa ML-RELATE, sendo possível diferenciar entre quatro categorias de relações de parentesco: não aparentado, meio-irmão/meia-irmã, irmão/irmã e pai-mãe/filhote (Kalinowski et al. 2006) (maiores informações no Capítulo 2).

De modo a estimar o valor adaptativo indireto dos auxiliares secundários/ reprodutores subordinados, foi calculada a regra de Hamilton (Hamilton 1964). Para a análise utilizaram-se apenas grupos com dados completos, incluindo a identificação de todos os membros do grupo com amostra de sangue e para os quais se obteve sucesso nas análises genéticas (maiores informações no Capítulo 3).

Análises estatísticas

As análises empregadas variaram no grau de complexidade de acordo com as hipóteses que foram testadas. De forma geral, foram utilizados testes univariados paramétricos e não paramétricos respeitando o tipo de dado e as premissas de cada análise empregada. Problemas com pseudoreplicação foram solucionados de maneiras diferentes de acordo com as exigências do problema e tipo de teste estatístico. Em alguns casos foi realizada simplesmente a média de medidas repetidas das mesmas unidades amostrais, em outros casos o problema foi controlado estatisticamente com a inclusão de variáreis aleatórias em modelos mistos. Todas as análises foram realizadas no pacote estatístico gratuito R (R Development Core Team 2010). Os testes foram bi-caudais e os resultados foram apresentados como média±EP.

Resultados

Resultados gerais

Foram capturados e marcados 160 indivíduos, sendo 72 adultos e 88 ninhegos, embora tenham sido coletadas amostras de sangue de outros dois indivíduos (ninhegos) que não foram marcados. No total, incluindo grupos em que poucos dados comportamentais foram coletados, foram localizados 36 grupos, sendo 21 pares socialmente monogâmicos e 15 grupos cooperativos. Um total de 57 ninhos foi encontrado durante os três anos de estudo. A composição dos grupos foi geralmente estável ao longo de todo o ano. Fora da estação reprodutiva, o tamanho dos grupos variou de 2-7 indivíduos, mas durante a estação reprodutiva o tamanho máximo chegou apenas a cinco indivíduos. Em geral, grupos cooperativos apresentaram mais fêmeas do que machos, entretanto, a razão sexual da população não diferiu da razão esperada 1:1 ($\chi^2_1 = 0.01$; $P = 0.91$).

O pica-pau-do-campo é uma espécie residente que defende seu território vigorosamente durante todo o ano. Os territórios foram encontrados especialmente em áreas que apresentam fitofisionomias mais abertas, como são os casos do campo sujo, campo rupestre, cerrado *sensu stricto* e áreas de pastagem. Os territórios de todos os grupos encontrados faziam contato com pelo menos um território vizinho, dando a impressão de um habitat saturado. A substituição de donos de territórios que morreram ou dispersaram foi rápida, sendo observada, em média, entre 5 a 12 dias (N = 3), estando essa característica geralmente associada a situações de saturação de habitat. O tamanho médio dos territórios foi de 48,50±3,93 ha, variando de 20,84 a 81,21ha.

Reprodução

Foram observadas cópulas no final de julho e início de agosto, período em que a maior parte das escavações de ninho ocorreu. A nidificação foi observada entre os meses de julho e novembro, com um pico de postura em setembro (Fig. 1; Capítulo 1). O início da estação reprodutiva parece ser influenciado por sinais ambientais, uma vez que houve variação na data da postura ao longo dos anos e essa coincidiu com o período imediatamente anterior ao inicio das chuvas na região (Fig. 1; Capítulo 1). Além disso, todos os ninhos que começaram tarde na estação de chuvas (outubro e novembro) falharam devido a alguma causa não associada com a predação (N = 5).

A grande maioria dos ninhos (84%) foi escavada em cupinzeiros terrestres, enquanto que o restante (16%) foi encontrado em cavidades escavadas em árvores. Entretanto, para os casos das cavidades em árvores, o ninho estava localizado em uma área com baixa densidade ou inexistência de cupinzeiros grandes. Apenas as cavidades localizadas em árvores foram reutilizadas ao longo dos anos (44% de todas as cavidades em árvores utilizadas). O processo de escavação do ninho tem duração de uma a quatro semanas (21,66±3,71 dias; N = 9) e todos os membros dos grupos parecem ajudar na escavação e vigilância.

O pica-pau-do-campo produz apenas uma ninhada por estação, embora possa realizar várias tentativas no caso de falha (foram observadas até três tentativas para o mesmo casal). Os ovos são elipticos e inteiramente brancos. O tamanho de ninhada variou de 3 a 9 ovos, sendo em média menor nos pares socialmente monogâmicos do que nos grupos cooperativos (Mann-Whitney *U*-test, $U = 78,00$; $P = 0,01$). Surpreendentemente, a taxa de eclosão tendeu a ser maior para pares do que para grupos cooperativos, ainda que a diferença não tenha sido estatisticamente significativa ($U = 74,50$; $P = 0,06$). Apesar disso, o sucesso de nidificação foi aparentemente maior em grupos cooperativos (72%) do que em pares socialmente monogâmicos (59%), embora a diferença mais uma vez não tenha sido significativa ($\chi^2_1 = 0.91$, $P > 0.10$). Perdas totais de ninhos devido à inanição ou parasitismo foram apenas encontradas para pares socialmente monogâmicos. Da mesma forma, perdas parciais foram observadas em 18% dos ninhos dos pares e em apenas 5% dos ninhos de grupos cooperativos.

Sistema de acasalamento

Foram realizadas análises genéticas para 32 ninhos. Observou-se que nenhum dos 20 ninhos dos pares socialmente monogâmicos apresentou casos de paternidade extra-par (PEP). A análise genética revelou que 90% das ninhadas e 90% (N = 51) dos ninhegos pertenciam a pares geneticamente monogâmicos. As únicas exceções foram dois casos de parasitismo de ninho intra-específico (PNI), que representou 6,25% de todas as ninhadas e 5,5% de todos os filhotes. Em ambos os casos os filhotes eram aparentados ao pai social, mas não à mãe social, um fenômeno conhecido como quasi-parasitismo ou maternidade extra-par. Em um dos casos a mãe genética pertencia a um grupo de um território adjacente.

Para os ninhos dos grupos cooperativos (N = 12), os dados genéticos revelaram que os filhotes de 50% dos ninhos foram produzidos por casais geneticamente monogâmicos amparados por auxiliares, enquanto que outros grupos exibiram poliginia simultânea (N = 5) ou poliginandria (N = 1). Similarmente aos pares socialmente monogâmicos, não foram observados casos de PEP (i.e., filhotes produzidos por machos de outros grupos). Foi confirmada a ocorrência de reprodução comunitária e esta esteve associada com a presença de machos poligínicos.

Estrutura de parentesco dos grupos

O grau de parentesco entre indivíduos do mesmo sexo localizados no mesmo grupo foi significativamente maior do que quando os mesmo indivíduos foram comparados a indivíduos do mesmo sexo localizados em outros grupos (ANOVA Fatorial de medidas repetidas; $F_{1,10} = 22.39$; $P < 0.001$; Fig 1; Capítulo 2). O mesmo foi observado para indivíduos do sexo oposto, considerando machos (Teste-*t* pariado: $t_6 = 3.07$; $P = 0.022$) ou fêmeas ($t_6 = 3.210$; $P = 0.018$; Fig. 1; Capítulo 2). O grau de parentesco entre os reprodutores (macho x fêmea) de grupos cooperativos não diferiu quando comparado ao dos pares socialmente monogâmicos ($t_{17} = 0.913$; $P = 0.374$; Fig. 2; Capítulo 2), embora os pares de grupos cooperativos sejam em média duas vezes mais aparentados do que pares socialmente monogâmicos. Adicionalmente, as análises sobre as relações genealógicas revelaram que 70% dos reprodutores foram classificados como não aparentados, porém, os resultados das relações genealógicas dos auxiliares com os reprodutores revelaram um variado nível de associações. No caso dos auxiliares machos, foi possível classificá-los estatisticamente como apresentando uma relação quanto ao macho reprodutor de pai-filhote (40%), irmão (20%) ou não aparentado (20%). Já em relação à fêmea reprodutora, as relações com os machos auxiliares foram classificadas estatisticamente como mãe-filhote (40%), irmã (20%), meia-irmã (20%) ou não aparentada (20%). Por outro lado, as fêmeas auxiliares foram classificadas estatisticamente como tendo uma relação com o macho reprodutor de pai-filhote (38%) ou não aparentadas (50%). Já considerando a fêmea reprodutora, foi observado que as fêmeas auxiliares apresentaram alguma relação genealógica em 78% dos casos. Entretanto, nos casos de reprodução comunitária, as fêmeas auxiliares que reproduziram eram geralmente não aparentadas à fêmea reprodutora principal (57%) (Fig 3; maiores detalhes no Capítulo 2).

Produtividade e outros parâmetros reprodutivos

A produtividade dos grupos foi diretamente influenciada pela presença de auxiliares no grupo (GLMM; χ^2_1 = 5,29; P = 0,021; Fig. 1; Capítulo 3), sendo que grupos ajudados por auxiliares produziram mais filhotes do que grupos sem auxiliares. Entretanto, não foi observado nenhum efeito do tamanho do grupo, da condição dos reprodutores, da qualidade do território ou ano. Além disso, a presença ou não de auxiliares não influenciou o sucesso de nidificação (χ^2_1 = 0.50; P = 0,478). Quando foram avaliados os efeitos das variáveis medidas sobre a condição dos filhotes observou-se que apenas o ano (χ^2_1 = 12,30; P = 0,002; Fig. 2; Capítulo 3) influenciou a massa corporal dos filhotes, mais especificamente o ano de 2007, que apresentou um efeito positivo.

Quando avaliados os efeitos na data de postura, observou-se que tanto a presença de auxiliares (χ^2_1 = 5,54; P = 0,018; Fig. 3; Capítulo 3) quanto o ano (χ^2_1 = 13,89; P < 0,001 Fig. 4; Capítulo 3) influenciaram o início da data de postura. A postura em 2007 começou em média 22,23±6,86 dias atrasada quando comparado aos anos de 2008 e 2009. Grupos com auxiliares também iniciaram mais cedo a postura. Foi observada uma tendência da condição dos reprodutores na razão sexual dos filhotes (χ^2_1 = 3,45; P = 0,06), com indivíduos em melhor condição tendendo a produzir mais fêmeas.

Apesar dos benefícios observados com o aumento na produtividade devido à presença de auxiliares, o cálculo da regra de Hamilton revelou que seleção de parentesco pelo acúmulo de benefícios indiretos de valor adaptativo talvez não seja a única explicação para a evolução da reprodução cooperativa no pica-pau-do-campo. Tanto para auxiliares machos, quanto para auxiliares fêmeas, o benefício em termos de produção adicional de filhotes por indivíduo aparentado não foi superior ao custo do adiamento da reprodução individual (maiores detalhes no Capítulo 3).

Efeitos no investimento parental e aloparental

Quando avaliado o investimento parental dos reprodutores, foi observado que durante a incubação o sexo do incubador influenciou o tempo investido na atividade de incubação (χ^2_1 = 7,25; P = 0,007), mas, por outro lado, características como a condição do reprodutor (χ^2_1 = 2.01; P = 0.155) e a presença de auxiliares (χ^2_1 = 2.37; P = 0.123) não afetaram o investimento dos pais. Fêmeas permaneceram dentro do ninho por um período mais longo que os machos. Por outro lado, durante o período de ninhego, observou-se que tanto a condição dos reprodutores (χ^2_1 = 5,51; P = 0,018; Fig. 1; Capítulo 4) quanto o número de ninhegos (χ^2_1 = 14,63; $P < 0,001$) afetaram a taxa de visitação dos pais. Similarmente, quando avaliado o tempo gasto no ninho durante o período de ninhego observou-se uma tendência para um efeito da condição dos reprodutores (χ^2_1 = 3,58; P = 0,058), assim como um forte efeito do número de ninhegos (χ^2_1 = 9,13; P = 0,002) sobre o investimento dos reprodutores. Apesar da falta de efeito da presença de auxiliares no investimento dos reprodutores, notou-se que os reprodutores sem auxiliares realizaram 35% mais visitas ao ninho e permaneceram 25% mais tempo dentro do ninho do que os reprodutores auxiliados (Fig. 2; Capítulo 4). Interessantemente, quando a análise foi restrita apenas a reprodutores cooperativos, observou-se que o número de visitas por hora feito pelos reprodutores durante o período de ninhego foi diretamente influenciado pelo número de visitas realizados pelos auxiliares (χ^2_1 = 10,82; P = 0,001; Fig. 3).

Considerando o investimento feito pelos auxiliares, observou-se que o número de visitas ao ninho destes foi afetado pelo grau de parentesco que eles possuíam com a prole (χ^2_1 = 4,79; P = 0,028; Fig. 4; Capítulo 4), assim como, com o número de ninhegos (χ^2_1 = 5,81; P = 0,016). Entretanto, o tipo de auxiliar (i.e., primário ou secundário; χ^2_1 = 0,05; P = 0,822), sua condição (χ^2_1 = 0,08; P = 0,775) ou sexo (χ^2_1 = 0,03; P = 0,851) não parecem ser relevantes para o investimento dos mesmos.

Com relação ao investimento total, observou-se que tanto a condição média dos membros do grupo (χ^2_1 = 0,58; P = 0,443), quanto o número de visitas realizadas ao ninho pelo grupo (χ^2_1 =2,75; P = 0,09), assim como o tempo total gasto no ninho (χ^2_1 = 0,48; P = 0,486), não afetaram o número de filhotes produzidos. O mesmo padrão foi observado quando avaliados os efeitos no sucesso de nidificação. Observou-se que a condição média dos membros do grupo afetou a massa corporal dos filhotes ao deixar o ninho ($\chi^2_1 = 11,80$; $P < 0,001$; Fig. 5; Capítulo 4), mas a mesma não foi influenciada pelo número de visitas ao ninho ($\chi^2_1 = 1,27$; $P = 0,258$) e nem pelo tempo gasto no ninho ($\chi^2_1 = 0,43$; $P = 0,512$). Por fim, não foram observados efeitos da presença de auxiliares no número de visitais totais realizadas ao ninho ($\chi^2_1 = 0,27$; $P = 0,598$), assim como, no tempo total de permanência no ninho ($\chi^2_1 = 0,87$; $P = 0,349$).

Discussão

O pica-pau-do-campo (*C. c. campestris*) mantém grupos estáveis que defendem amplos territórios ao longo de todo ano, comparáveis aos observados em outras espécies de pica-paus (Winkler e Christie 2002). Os habitats adequados na área de estudo aparentam estar saturados e são prontamente tomados em caso de aparecimento de territórios disponíveis. Essas características são tipicamente encontradas em espécies cooperativas (Ligon e Burt 2004). Junto com a aparente alta sobrevivência dos adultos do pica-pau-do-campo (observação pessoal), essas características podem promover a saturação de habitat, favorecendo a filopatria (Baglione et al. 2005). O cenário em questão pode diminuir as chances de dispersão dos filhotes, tornando a decisão de permanecer no território natal a melhor opção. Alguns indivíduos, no entanto, aparentemente conseguem dispersar do território natal para outro território, ocupando uma vaga de ajudante no grupo e podendo eventualmente obter cópulas com o reprodutor dominante.

Reprodução

O pica-pau-do-campo reproduz preferencialmente em cavidades escavadas em cupinzeiros terrestres, embora também utilizem cavidades em árvores quando há pouca densidade ou ausência de cupinzeiros disponíveis. O padrão encontrado difere do descrito para a sub-espécie *campestroides*, que exibe maior preferência por cavidades em árvores (Short 1972).

A estação reprodutiva do pica-pau-do-campo inicia no final da estação seca e os filhotes geralmente saem do ninho no começo da estação chuvosa, sugerindo uma forte associação ente pluviosidade e reprodução nessa espécie. É notória a influência de características climáticas no desencadeamento da estação reprodutiva de aves de ambientes tropicais sazonais (Hau 2001; Monadgem e Bamford 2009). Um fator paralelo que pode desencadear o início da estação reprodutiva para o pica-pau-do-campo é a disponibilidade de cupins e formigas. É provável que exista uma sincronização do período reprodutivo com o pico de disponibilidade de alimento.

A biologia reprodutiva do pica-pau-do-campo é similar à maioria dos membros da família Picidae (Winkler e Christie 2002). Entretanto, o tamanho de ninhada (5,05±0,43 ovos) é menor quando comparado com o de espécie congênere da região temperada (7,61±1,52 ovos; pica-pau-mosqueado, *C. auratus*; Wiebe e Swift 2001). A taxa de predação é similar, sendo 17% para o pica-pau-do-campo e 20% para o pica-pau-mosqueado, mas a taxa de eclosão parece ser ligeiramente maior para o pica-pau-do-campo (77%) em comparação ao pica-pau-mosqueado (66%, Wiebe e Swift 2001).

Os dados comportamentais revelaram pela primeira vez a presença de reprodução cooperativa no pica-pau-do-campo, que foi posteriormente corroborado com os dados moleculares. Aproximadamente metade dos grupos reprodutivos apresentou pelo menos algum auxiliar em adição ao par reprodutivo, com grupos cooperativos apresentando entre três a cinco indivíduos responsáveis pela criação dos filhotes. Os auxiliares foram inicialmente classificados de duas formas: 1) auxiliares primários – para os casos em que tanto auxiliares machos quanto fêmeas eram oriundos de prole do casal reprodutor na estação reprodutiva anterior; ou 2) auxiliares secundários – para aqueles casos em que adultos, de forma geral fêmeas, se uniram a um grupo pré-existente. Posteriormente, essas definições foram atualizadas e ampliadas com a confirmação dos dados moleculares, revelando que os auxiliares primários são em geral indivíduos altamente aparentados aos reprodutores do grupo, apresentando comumente uma relação de pai-mãe/filhote, e os auxiliares secundários são, em sua maioria, indivíduos não aparentados aos reprodutores do sexo oposto, embora possam apresentar alguma relação de parentesco com as reprodutoras, no caso das fêmeas. Esses auxiliares secundários geralmente são capazes de obter alguma cópula com o macho dominante e produzir algum filhote de forma comunitária.

A diferença no tamanho de ninhada entre grupos cooperativos e pares socialmente monogâmicos sustentou a hipótese inicial de que múltiplas fêmeas estavam pondo ovos no mesmo ninho, o que acabou sendo confirmado com os dados moleculares. Esse comportamento é relativamente raro, sendo encontrado em cerca de 14 espécies (Vehrencamp e Quinn 2004) incluindo um pica-pau (Mumme et al. 1988). Entretanto, a presença de auxiliares no grupo parece gerar um custo em termos de taxa de eclosão dos ovos. Os resultados sugerem que a taxa de eclosão foi menor para grupos cooperativos do que para pares monogâmicos, sugerindo uma possível restrição na efetividade de incubação para ninhadas maiores (mas veja Wiebe e Swift 2001). Uma outra alternativa seria a possibilidade de que os ovos que não eclodiram possam não ter sido fertilizados pelo macho dominante.

Sistema de acasalamento

O sistema de acasalamento genético do pica-pau-do-campo pode ser classificado como predominantemente monogâmico, considerando ambos os tipos de grupos sociais: os pares socialmente monogâmicos e os grupos cooperativos. Além dessa predominância de casais monogâmicos, a poliginia simultânea, um sistema de acasalamento pouco observado em pica-paus (Wiktander et al. 2000), foi encontrado em alguns grupos cooperativos. Uma das explicações para a baixa frequência desse sistema de acasalamento em aves seria a necessidade de cuidado biparental para produzir filhotes com sucesso (Winkler e Christie 2002). No pica-pau-do-campo esse sistema parece ser viável devido à existência de reprodução comunitária na espécie. Surpreendentemente, apesar do número elevado de grupos poligínicos, a razão sexual da população não estava enviesada para as fêmeas.

Nenhum caso de PEP foi observado, considerando tantos os pares sociais quanto os grupos cooperativos; tal comportamento parece ser raro para pica-paus (Pechacek et al. 2005). Entretanto, foram detectados dois casos de parasitismo intra-específico (PIN) de ninho, estratégia alternativa que também já foi descrita para outras espécies de pica-paus (Wiktander et al. 2000; Bower e Ingold 2004; Pechacek et al. 2005; Li et al. 2009). Os dados genéticos sugerem que os casos de PIN são na verdade casos de quasi-parasitismo (maternidade extra-par), uma vez que os machos sociais eram os pais dos filhotes, mas as mães sociais não eram as mães biológicas.

Estrutura de parentesco dos grupos

Grupos cooperativos são formados por indivíduos que são mais aparentados entre si do que a membros de outros grupos, criando um cenário ideal para o surgimento de benefícios associados à seleção de parentesco. Apesar disso, os resultados moleculares sugerem a possibilidade de custos associados ao alto nível de parentesco (e.g. acasalamentos consanguíneos, Koenig e Haydock 2004) uma vez que os reprodutores dos grupos cooperativos são duas vezes mais aparentados do que os pares socialmente monogâmicos, embora não tenha havido diferença significativa em termos de grau de parentesco.

Quando detalhada a estrutura de parentesco nos grupos cooperativos, observou-se que os auxiliares apresentaram um variado nível de relação de parentesco como os reprodutores, influenciado diretamente pelo sexo do auxiliar. Quando o auxiliar era macho, este era geralmente muito aparentado aos reprodutores. Entretanto, quando o auxiliar era fêmea, esta tendeu a ser menos aparentada ao macho do que à fêmea reprodutora, sugerindo que essas fêmeas têm o potencial para acasalar com o macho dominante. Considerando apenas as fêmeas que reproduziram em grupos poligínicos, estas eram, em sua maioria, não aparentadas entre si. Os dados permitem uma possível inferência sobre os padrões de dispersão do pica-pau-do-campo, sugerindo que tanto machos quanto fêmeas podem permanecer no território natal e se tornar ajudantes ou podem dispersar sozinhos ou em coalizões (particularmente as fêmeas).

Produtividade e outros parâmetros reprodutivos

De forma geral, o território e a qualidade dos reprodutores não parecem afetar a produtividade do grupo. No entanto, conforme demonstrado em outros estudos (Woxvold e Magrath 2005; Doerr e Doerr 2007) a presença de auxiliares influenciou o número de filhotes produzidos, com grupos auxiliados produzindo mais filhotes do que grupos sem auxiliares. Entretanto, o mesmo efeito não pode ser observado quando analisado o sucesso de nidificação, sugerindo que grupos com ou sem auxiliares são capazes de produzir filhotes (Pruett-Jones 2004), embora grupos sem auxiliares produzam em uma taxa menor. Tal resultado vai ligeiramente contra a expectativa inicial baseada nos dados preliminares, que mostraram que grupos cooperativos, diferentemente dos casais socialmente monogâmicos, não apresentaram perda total da prole por inanição e que apresentaram também menores percentuais de perdas parciais (Capítulo 1).

Apesar da presença ou não de auxiliares não ter afetado a condição dos filhotes ao deixar o ninho, o ano parece ter sido determinante para a massa corporal dos filhotes. A despeito do extenso período de seca ocorrido no primeiro ano do estudo (2007), os filhotes produzidos apresentaram uma massa corporal superior aos filhotes produzidos em outros anos. Esse resultado pode ser visto como um efeito indireto da presença dos auxiliares em anos considerados mais adversos.

Adicionalmente, quando considerados os efeitos na data de postura, observou-se que tanto a presença de auxiliares quanto o ano afetaram o início da postura dos ovos. Grupos com auxiliares iniciaram a postura mais cedo do que grupos sem auxiliares. Por outro lado, a postura no ano de 2007 começou mais tardiamente, provavelmente devido ao período de seca mais prolongada observado naquele ano (Capítulo1). Interessantemente, alguns estudos sugerem que a influência dos auxiliares pode ser observada em alguns anos, mas não em outros, sendo mais forte em anos de condições adversas (Hatchwell 1999; Magrath 2001). Considerar o ano de 2007 como sendo adverso, o atraso na postura pode ter favorecido uma sincronização na disponibilidade de insetos, que também são dependentes da chuva para sua reprodução. Apesar disso, não houve diferença na frequência de grupos cooperativos entre os anos, diferente de um estudo que propôs que ninhos com auxiliares são mais comuns em anos adversos (Canário et al. 2004).

Apesar do claro benefício adquirido em decorrência da presença dos auxiliares, notado especialmente pelo aumento na produtividade, quando aplicada a regra de Hamilton, o custo da ajuda para os auxiliares foi superior aos benefícios que os mesmos poderiam adquirir reproduzindo individualmente, indicando que a reprodução cooperativa no pica-pau-do-campo pode ter evoluído considerando-se outros benefícios, além da seleção de parentesco. Para maioria das espécies cooperativas em que a regra de Hamilton foi calculada, os resultados indicam que a evolução da cooperação não parece depender unicamente dos benefícios da seleção de parentesco (Lucas et al. 1996). Consequentemente, pode-se esperar que a reprodução cooperativa deva ocorrer apenas quando a reprodução independente não seja possível (Du-Plessis et al. 1995). Conforme mencionado anteriormente, algumas características podem restringir indivíduos do pica-pau-do-campo a ficar no território natal e desempenhar uma atividade sub-ótima de forma a não perder completamente qualquer benefício genético (aumento da representação de seus genes numa próxima geração), executando o que poderia ser classificado em tradução livre como “o-melhor-de-uma-situação-ruim” (Dickinson et al. 1996).

Efeitos no investimento parental e aloparental

Os resultados demonstraram que durante a incubação as fêmeas reprodutoras permanecem por mais tempo no ninho do que machos reprodutores. Durante a fase de ninhego, por outro lado, nenhuma diferença no investimento do reprodutor foi observada em relação ao sexo. Entretanto, a condição dos pais foi fundamental para determinar a taxa de visitação destes ao ninho, independente de estarem sendo auxiliados ou não. A condição parental é reconhecida na literatura como um fator determinante para a sobrevivência da prole, sua razão sexual (Nager et al. 2000) e condição dos filhotes (Parker 2002).

De acordo com vários estudos, a presença de auxiliares no grupo pode ou não afetar o provisionamento dos reprodutores aos filhotes. Se os reprodutores mantiverem o mesmo investimento, o provisionamento total refletirá o investimento adicional dos auxiliares (Emlen e Wrege 1991; Magrath e Yezerinac 1997). Por outro lado, os reprodutores podem, em condições específicas, reduzir o próprio investimento, sendo compensados pelo investimento obtido através dos auxiliares, mantendo assim o mesmo provisionamento esperado se não tivessem a ajuda dos auxiliares (Brown et al. 1978; Legge 2000; Khan e Walters 2002). Tal cenário é particularmente esperado em espécies em que o cuidado parental é muito custoso. Adicionalmente, alguns estudos revelaram a ocorrência de ambos os efeitos na mesma espécie (Kingma et al. 2010; Meade et al. 2010). Esse também parece ser o caso do pica-pau-do-campo, uma vez que os reprodutores, na presença de auxiliares, reduziram seu investimento em cerca de 25-35% em comparação aos reprodutores sem auxiliares, similarmente ao observado por Kingma et al. (2010), apesar da diferença não ser significativa. Além disso, o investimento dos auxiliares foi alto, sendo comparável com o apresentado pelos reprodutores, especialmente as fêmeas. Entretanto, apesar de notadamente superior, o investimento total em ninhos com auxiliares também não diferiu daqueles ninhos sem auxiliares. Curiosamente, quando analisado o efeito que o investimento dos auxiliares teve no investimento dos reprodutores, foi observada uma relação direta entre as taxas de visitação ao ninho de ambas as classes de indivíduos. Esse resultado apenas reforça a conclusão de que os reprodutores devem tentar aumentar o investimento total, e talvez a produtividade, em situações favoráveis, quando o investimento dos auxiliares for alto. Outro ponto importante que deve ser mencionado é a substancial taxa de perda de ninho por inanição (Capítulo1). Estudos revelam que espécies com altas taxas de inanição de filhotes apresentam um aumento no provisionamento total – efeito adicional dos auxiliares, como uma forma de minimizar as perdas (Hatchwell 1999).

Os resultados revelaram que o investimento dos auxiliares, medido como taxa de visitação, esteve diretamente relacionado ao nível de parentesco dos mesmos com a prole, assim como com o número de filhotes. Como pode ser observado no capítulo 2 dessa tese, os indivíduos de grupos cooperativos são em geral mais aparentados entre si do que quando comparados com membros de outros grupos, embora essa alta relação de parentesco seja bem variável dentro de cada grupo e entre os grupos. Essa situação favorece benefícios advindos de seleção de parentesco. Em algumas espécies está claro que os auxiliares conseguem modular o investimento próprio baseados em diferenças mínimas no nível de parentesco que os mesmos têm em relação à prole (Nam et al. 2010; Wright et al. 2010). Sabe-se que auxiliares são capazes de favorecer parentes (Griffin e West 2003) e que a discriminação de quem é um parente é de provável ocorrência em espécies com parentesco variável dentro do grupo (Cornwallis et al. 2009), como é o caso do pica-pau-do-campo.

Notou-se que o investimento total por ninho não afetou a produtividade ou o sucesso de nidificação. Todavia, os resultados do Capítulo 3 dessa tese revelam que a presença dos auxiliares aumentou a produção de filhotes. Esse resultado sugere que o efeito na produtividade não deve estar relacionado apenas à mera presença dos auxiliares no grupo, e que outros fatores, além da taxa de provisionamento dos auxiliares aos filhotes ou do aumento não significativo na taxa de provisionamento total, devem influenciar de alguma forma a produção dos filhotes. A massa corporal dos filhotes foi influenciada pela condição média dos membros do grupo, mas não pelo investimento total no ninho. Esse resultado sugere que a condição dos filhotes está diretamente associada à condição dos indivíduos responsáveis pela sua criação.

Esse estudo mostrou que o sistema de acasalamento e o sistema social de espécies estudadas através de métodos tradicionais de captura, marcação e observação, podem esconder muitos detalhes que só podem ser desvendados através de técnicas moleculares que determinam parentesco. O pica-pau-do-campo é uma espécie que apresenta uma variedade enorme de componentes comportamentais de alta complexidade e o completo entendimento do cenário ainda levará anos de pesquisa. No momento, sabe-se da ocorrência de um sistema de acasalamento variável com estratégias reprodutivas alternativas, porém não se sabe a causa de tal variação. Também não estão claros os fatores que levaram à evolução da socialidade na espécie na região do estudo. Ainda não é possível saber com precisão quais os fatores estão favorecendo o comportamento social dos ajudantes e se a ajuda é realmente efetiva a longo prazo, em relação à sobrevivência dos filhotes que dispersaram.

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Breeding ecology and social behavior of the campo flicker, *Colaptes campestris campestris*, a cooperative neotropical woodpecker

Introduction

In cooperative breeding systems, auxiliaries ("helpers") provide parental care to offspring of a brood produced by a breeding pair, thereby helping to rear offspring that are not their own (Brown 1987). Cooperative breeding has been recorded in about 9% of bird species (Cockburn 2006), and in many cases helpers are non-breeding young that remain at the natal site and help to care for their own siblings (Skutch 1961). In other cases, auxiliaries are unrelated individuals that may produce offspring within the group, either by (for auxiliary males) copulating with the group breeding female, or (for auxiliary females) contributing eggs to the nest ("joint-nesting") (Brown 1987).

In general, cooperative breeding appears to be strongly associated with tropical regions (Rowley 1968, 1976). For example, long-term studies (at least 10 years) of cooperative breeding species have been conducted for about 20 birds (reviewed in Stacey and Koenig 1990 and Koenig and Dickinson 2004), of which all but three are subtropical or tropical species, and 50% of which are exclusively tropical. It is thus expected that many more species of cooperative breeders will be described in the tropics as more of its avifauna are intensively studied, leading to a greater understanding of the ecological and phylogenetic basis for the evolution of sociality.

Among the traits associated with cooperation, the most prominent include delayed dispersal of juveniles, postponement of reproduction, and the development of parental behaviors to help rear non-descendent offspring (Ekman et al. 2004). Several non-mutually-exclusive hypotheses have been proposed to explain cooperative breeding. First, cooperative breeding may be promoted by ecological constraints (Brown 1974; Emlen 1982) that can limit independent reproduction by young individuals ('Ecological Constraints Hypothesis'). Alternatively, some life-history characteristics may limit the opportunities for independent breeding and promote cooperation, such as low adult mortality and year-round residence ('Life-history Hypothesis'; Russell 1989; Arnold and Owens 1998). Finally, remaining on the natal territory may itself bring some benefits, such as the possibility of territory inheritance ('Benefits-of-philopatry Hypothesis'; Stacey and Ligon 1987, 1991) and indirect fitness benefits, if helpers can increase the productivity of their parents' nest (Ekman et al. 2004).

Studies on woodpeckers have provided a substantial contribution to our understanding of the evolution of social behavior. However, cooperative breeding has been well studied in only a few temperate zone species, such as the Acorn Woodpecker (*Melanerpes formicivorus*, Koenig and Pitelka 1979; Koenig 1981; Haydock and Koenig 2003; Koenig et al. 2009) and the Red-Cockaded Woodpecker (*Picoides borealis*, Ligon 1970; Walters et al. 1988; Khan and Walters 1997; Malueg et al. 2009). Indeed, studies of North American and European woodpecker species, which represent 7% of the Picidae family, account for approximately 70% of the papers on breeding ecology in woodpeckers published between 1985-2004; only 3% of these studies were conducted in Latin America, where more than half of the woodpecker species occur (Mikusiński 2006). It therefore remains unclear whether the pattern of association between the tropics and cooperative breeding also holds for woodpeckers, or whether insights from studies of temperate woodpeckers can be generalized to tropical species. However, new descriptions of cooperative breeding in previously described non-cooperative tropical species have been reported recently, for example the Great Slaty Woodpecker (*Mulleripicus pulverulentus*; Lammertink 2004).

Within the complex social structure exhibited by cooperative breeders, the social mating system is expected both to affect and to be affected by the levels of parental and alloparental behavior. Levels of parental investment by males and females are frequently associated to variations in mating system (Clutton-Brock 1991). Further understanding of the link between mating systems and parental investment relies upon descriptions of individual and gender differences in offspring investment. This issue seems especially relevant in woodpeckers in which bi-parental care seems to be essential for breeding success (Winkler et al. 1995).

Campo Flickers (*Colaptes campestris campestris*) are moderately social, medium-sized woodpeckers with a wide distribution across South America from Paraguay to northeastern

Brazil (Short 1972), and on isolated patches of Amazonian upland savanna (Silva et al. 1997). Although abundant and widely distributed, most aspects of the natural history of the Campo Flicker are poorly known, and basic information is also lacking about all aspects of its ecology, breeding and social and mating systems. Our goal in this study was to describe the breeding biology of the Campo Flicker, including its social system and mating behavior. We also investigated the effects of weather on timing of breeding and the effects of social organization upon incubation and provisioning. Finally, we discuss possible forces driving the evolution of cooperative breeding in this species, which we describe for the first time.

Methods

Study species

The genus *Colaptes* is widely distributed throughout the New World (Short 1972), and includes species known to have well developed social behavior and that are also distinguished from most other woodpeckers by their terrestrial (i.e., on the ground) foraging habits (Short 1972). Campo Flickers are divided into two subspecies: *Colaptes campestris campestris*, distributed from northeastern Brazil to central Paraguay; and *C. campestris campestroides*, found from southern Paraguay and Brazil to northeastern Argentina (Short 1972). The sub-species differ only in throat coloration, which is black on *campestris* and white on *campestroides* (Short 1972). The studied subspecies, *C. campestris campestris*, exhibits yellow coloration on the sides of the head, neck and breast, with black crown and throat and brown dorsal plumage barred with dull white. Sexual dimorphism is very subtle, with males showing a red stripe on the malar region, which is black in the females.

The limited data on social behavior and breeding ecology for Campo Flickers of the sub-species *C. campestris campestroides* suggest they usually occur in small groups of three to four individuals during the breeding season, and up to eight during foraging activities at a single site (Short 1972). Breeding activity seems to start in late August and clutch size is typically from four to five eggs (Short 1972).

Study area and general procedures

We conducted field work at Fazenda Água Limpa (FAL; 15°56'S, 47°55'W), an area of 4,500 ha in central Brazil, Brasília, from October 2006 to December 2009. The area is within the Cerrado (tropical savanna) biome and comprises several vegetation types, including open grassland (*campo limpo*), grassland dotted with shrubs (*campo sujo*), scrub forest (*cerrado sensu stricto*) and gallery forests. We obtained weather data from a meteorological station within the study area. The climate is strongly seasonal with a marked rainy season from October through March.

We searched for social groups by surveying the study site, using playbacks of vocalizations and sightings to initially detect groups. Whenever we discovered a social group, we mist-netted and banded all individuals with a unique combination of three color bands and a numbered metal band from the Brazilian regulatory agency (IBAMA) for individual recognition. At the time of capture, individuals were measured (tarsus, wing, beak and tail length) with calipers (to nearest 0.02 mm) and weighed (to nearest gram).

During the breeding season we checked the contents of potential nesting cavities within the study area using a mirror and flashlight attached to a pole, and recorded the location (GPS coordinates) of all active nests, which were subsequently monitored every 2-3 days. During nest checks we recorded whether or not nest parasites (mites and ticks) occurred. We defined hatching success as the percentage of eggs from the total clutch that hatched; eggs that disappeared within five days of hatching were counted as unhatched, rather than depredated, because adults sometimes remove unhatched eggs from the nest. We considered predation as the cause of nest failure when eggs/nestlings disappeared and the nest entrance was destroyed and/or there was blood and feathers in the vicinity of the nest. Nest losses attributed to starvation occurred when nestlings were found dead within the nest cavity and brood loss occurred in sequential days, while parasitism was determined as the probable cause of nest loss when these same characteristics were associated with a very large number of mites and ticks in the nest. We considered a nest to have successfully fledged young whenever a nestling disappeared from the nest within five days of the expected fledging date with no sign of predation (e.g. destroyed nest entrance, presence of blood or large number of feathers).

We defined a social group as any aggregation of two or more individuals that showed a strong association, such as ranging together within a specific area on the study site, over an extended period of at least six months. Social groups were classified as either socially monogamous pairs (only two adults) or cooperative groups (more than two adults).

Breeding synchrony

A breeding synchrony index was calculated as the average percentage of females that were fertile per day during the breeding season (Kempenaers 1993). The female fertile period was defined as the period starting five days before the start of egg laying until the penultimate egg was laid (Kempenaers 1993).

Parental activity

We recorded adult activity during one hour of observation at each nest twice during the incubation period (years two and three of the study): on the fifth and tenth days after the last egg of the clutch was laid. After hatching, we recorded adult behavior (from 1h40 min h to 2 h) at each nest on five days during the nestling period (days 4, 10, 16, 22 and 28). We conducted focal observations of 2 h for most nests from a blind approximately 20-30 m from the nest cavity (to avoid disturbing the birds). Some additional observations were obtained with video cameras (Sony DCR-HC52), where tapes lasted 1h40min. During all observation periods we recorded the number of visits by each adult and how long each spent inside the nest cavity. Near the end of the nestling period (25th day after hatching), we measured and banded nestlings, similarly to adults.

Home range and territory

At least three times per week throughout the study we monitored individuals and used GPS to map their locations, as well as locations of all aggressive interactions with neighbors and of territorial displays. Usually no more than three points were recorded per day for each social group. To facilitate the location of groups we fitted one individual from each of twelve different social groups in 2008 with backpack transmitters that weighed 4.0-5.0 g, approximately 2-3% of adult weight (Sopb-2190 HWSC; Wildlife Materials, Inc.). We did not observe adverse effects of the transmitters on birds, and all radio tagged individuals survived to the next year (see also Vukovich and Kilgo 2009). We calculated home range size as the 95% fixed kernel contour (Seaman and Powell 1996) with the software Home Ranger 1.5 (Hovey 1999). Because kernel home range estimates are known to be influenced by sample size (Seaman et al. 1999), for these analyses we only included social groups with more than 40 sampled points.

Statistical analyses

We compared pairs with cooperative groups relative to clutch size and hatching success using Mann-Whitney *U*-tests, and also relative to nesting success, using a chi-square test. We used factorial ANOVA to evaluate nesting behavior (visitation frequency and time spent at the nest) relative to two factors, sex and social organization (pairs versus cooperative groups), and also their interaction. To avoid pseudoreplication, we used average values for individuals that bred more than once across years. Values are presented as mean ± standard error. For all analyses we used the software R 2.7.2 (R Development Core Team, 2009). Statistical tests were two-tailed and the null hypothesis was rejected at $P < 0.05$.

Results

General results

We captured and banded 160 individuals (71 adults and 89 nestlings), monitored 26 social groups, and found 57 nests during the three-year study period. Each social group was classified unambiguously as either a socially monogamous pair (58%; $n = 15$) or a cooperative group (42%; $n = 11$). Group composition (i.e., individual membership) generally persisted through the entire year. Outside the breeding season, social unit size ranged from 2-7 individuals, but a few weeks before the observation of the first reproductive behaviors (e.g. cavity excavation and copulations) some individuals of larger groups disappeared from the area, and may have dispersed to unidentified areas. During the breeding season social unit size varied from 2-5 individuals. Most cooperative groups (7 of 11, or 64%) had more females than males, with group composition varying from one male and three females to two males and three females. In two (18%) groups there were multiple males (2-3 males) with a single female, and the remaining two groups (18%) contained two males and two females. Socially monogamous pairs were very stable, persisting throughout the year, with some pairs (20%; *n* = 3) maintaining the bond for the entire duration of the study.

Home range and territoriality

Campo Flickers are year-round residents and defend their territory intensively throughout the year, executing territorial displays 1-12 times per hour, with a seemingly higher incidence of aggressive behaviors during the breeding season. Territories were found in areas composed of different landscapes such as grasslands with scattered shrubs and trees (*campo sujo*), rocky grasslands, floodplain grasslands with earth-mounds, *cerrado sensu stricto* (dominated by trees and shrubs often 3-8 m tall), gallery forest borders and grazed pastures. Despite some changes in the composition of groups (socially monogamous pairs or cooperative groups), these were found in the same sites across two (*n* = 4), three (*n* = 6) or four (*n* = 2) years of the study. However, some groups were found before others, so the persistence of groups in territories may be underestimated. Territory owners (pairs or whole groups) approached territorial intruders and attempted to repel them with a conspicuous wing flicking display and vocalizations (*n* = 39 observations). In cases where the intruder was persistent, territory owners sometimes attacked and bill poked the intruder (*n* = 7). Physical combats between individuals were rarely observed, but sometimes occurred at territory borders (*n* = 3). We never observed non-group members within another group's territory, and it appears that Campo Flicker territories completely overlie their home ranges, since individuals defended all areas where they were ever observed. For all social units, territory borders made contact with at least one neighbor, appearing to saturate the available suitable habitat. In those cases where one of the breeding adults in a group died, it was replaced in 5-12 days (*n* = 3). Territories were on average 48.50±3.93 ha, ranging from 20.84-81.21 ha.

Breeding period and reproduction

Observations of copulation were rare and occurred in late July and August (*n* = 6), during the period when most nest excavation took place. Nesting behavior was observed from July through November, with egg laying peaking in September, when 45% of the nests received their first eggs (Fig. 1.1). Breeding synchrony ranged from 11% in 2009 to 20% in 2008. The beginning of the breeding season seemed to be influenced by weather cues, since laying date varied between years and coincided with the first rains in the region (Fig. 1.1). The dry season in 2007 was exceptionally long, with the first significant rains starting in September (0.5mm) together with the first nests (Fig. 1.1). In contrast, in 2009 the dry season was very short, restricted to the month of July, and egg laying started in early August (Fig. 1.1). Nevertheless, the duration of the breeding season seems to be constrained, since all nests starting late in the season (October and November) failed due to a cause other than predation (*n* = 5). Of the 57 nests found, 84% were excavated on termite mounds while 16% were found in tree cavities. The latter nests occurred in areas of the study site that lacked large termitaria, suggesting that tree cavities were less preferred as nest sites. Only nests located in tree cavities were reused across years (44% of all tree cavity nests). Even when a tree cavity was reused, adults always excavated to increase cavity depth prior to egg laying. Parasites such as mites and ticks were found in approximately 12% of the nests. Reuse of the same termitaria mound was always associated with the excavation of a new cavity, since termites often closed nest cavities after the young fledged (*n* = 6). Nest excavation lasted for up to four weeks, but sometimes occurred swiftly in one week (mean = 21.66±3.71 days; *n* = 9). Males and females both excavated nests, and all members of cooperative groups helped with cavity excavation and vigilance.

Eggs were plain white and elliptical in shape with an average length of 30.41±0.25mm and width of 21.51±0.15mm. Clutch size ranged from 3-9 eggs, and average clutch size for pairs (4.10±0.11 eggs, range = 3-5, *n* = 15) was significantly smaller than that for cooperative groups (6.00±0.64 eggs, range = 4-9, *n* = 11; Mann-Whitney *U*-test, *U* = 78.00; *P* = 0.01). Typically, one egg was laid daily until clutch completion, but there were three cases of a two-day laying interval between sequential eggs.

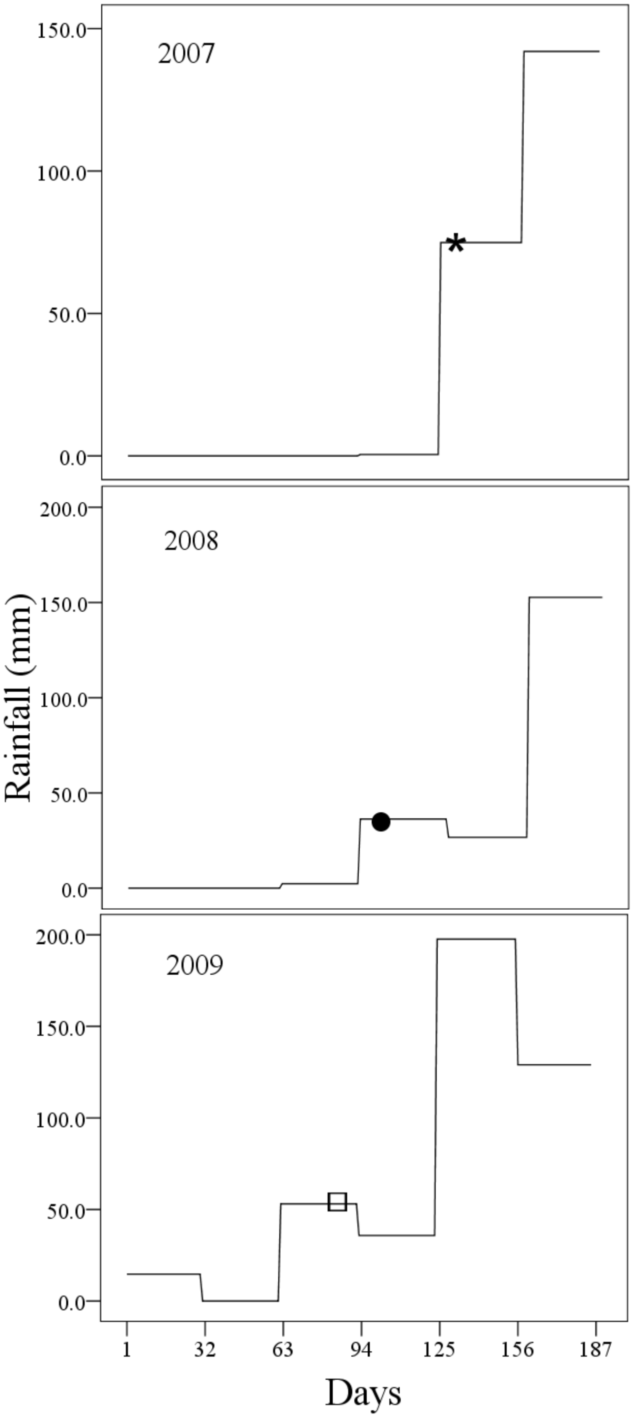

Figure 1.1 – Reproduction of Campo Flickers in three breeding seasons in central Brazil. Lines show monthly level of rainfall (mm) during the different breeding seasons. Solid, dotted and dashed lines represent, respectively, the years 2007, 2008 and 2009. The ‘Days’ axis starts on June 1st (day 0) and ends on November 30th (day 183). The average laying date for each year is marked with a symbol on the respective year line.

Campo Flickers appear to be single-brooded, as we never observed more than one brood per group fledging successfully within a season, although we did observe up to three nesting attempts within a breeding season after previous nest failure. Hatching success averaged 75.89%, and was higher for pairs (84.54%, $n = 15$) than for cooperative groups (67.24%, $n = 11$), although this difference was not quite significant (Mann-Whitney *U*-test, $U = 74.50$; $P = 0.06$). Incubation and nestling periods lasted 15.67 ± 0.33 days ($n = 15$) and 29.11 ± 0.35 days ($n = 18$), respectively. Overall, nesting success was low (65.5%), but cooperative groups had a higher nesting success rate (72%) than did pairs (59%), although again this difference was not statistically significant ($\chi^2_1 = 0.91$, $P > 0.10$). Total nest losses attributed to predation events account for 17% of the failures. Total nest losses apparently caused by starvation or parasitism were found only for pairs and represented 9% of all nest losses (20% of pair losses). Partial losses were observed in 18% of pair nests and 5% of cooperative group nests. Interestingly, we observed cases of all eggs in a clutch vanishing without any sign of predation in some nests of cooperative groups ($n = 6$), followed by re-nesting in the same nest cavity. Re-nesting in the same cavity never occurred when the eggs disappeared due to predation ($n = 8$).

Parental and alloparental care

Behavioral observations revealed that this species is a facultative cooperative breeder, with more than two individuals involved in parental duties in all cooperative groups ($n = 11$). Banding records show that auxiliaries assisting at nests could be male ($n = 2$ groups) or female ($n = 1$ group) nestlings from previous years. In all cases where an adult auxiliary joined a group and was not an offspring from a previous season, the auxiliary was a female ($n = 3$ groups). In two of these three cases (66.67%) we observed the breeding group female aggressively interacting with the immigrant female, probably attempting to drive the potential auxiliary female away. During incubation, total visitation frequency did not differ between the sexes (factorial ANOVA; $F_{1,26} = 0.69$, $P = 0.41$) or between type of social organization (pairs versus groups; $F_{1,26} = 0.00$, $P = 0.93$), nor was there a significant interaction between these factors ($F_{1,26} = 0.80$, $P = 0.37$). However, individual members of cooperative groups spent less time at the nest, per capita, than did individuals in pairs, regardless of sex ($F_{1,26} = 5.68$, $P = 0.02$; Fig. 1.2). These results are equivalent to results when we conducted the same analysis and controlled for the number of eggs in the clutch.

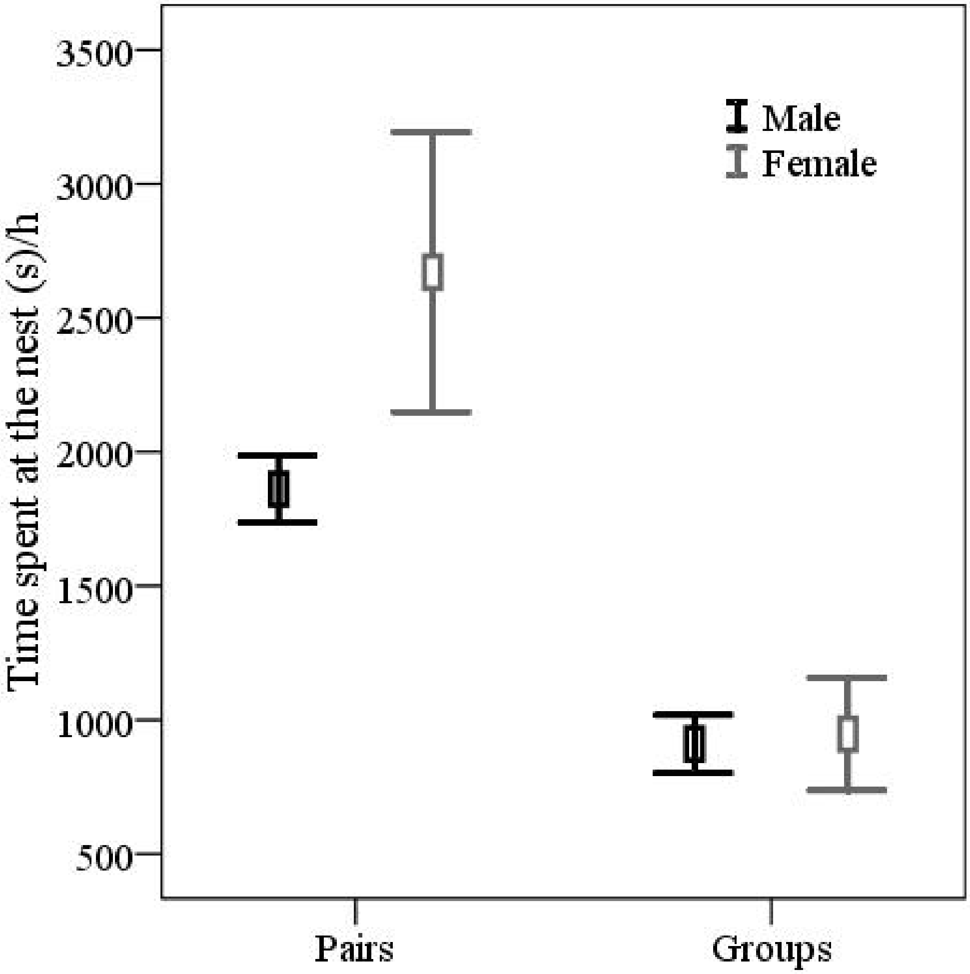

Figure 1.2 – Effects of sex and social organization on time spent at the nest during the incubation period of Campo Flickers in central Brazil.

During the nestling phase, visitation frequency differed significantly between type of social organization, and males in cooperative groups, but not females, visited the nest less frequently when compared to males of socially monogamous pairs ($F_{1,46} = 8.22$, $P = 0.006$; Fig. 1.3). The interaction between social organization and sex was also significant ($F_{1,46} = 5.31$, P =

0.025; Fig. 1.3). These results suggest that social condition affects male visitation rates but has little influence upon females. We also observed a marginally nonsignificant trend for an effect of social organization on time spent at the nest during the nestling period ($F_{1,46} = 3.76$, $P = 0.058$; Fig. 1.4), with individuals of groups spending less time at the nest compared with those in pairs.

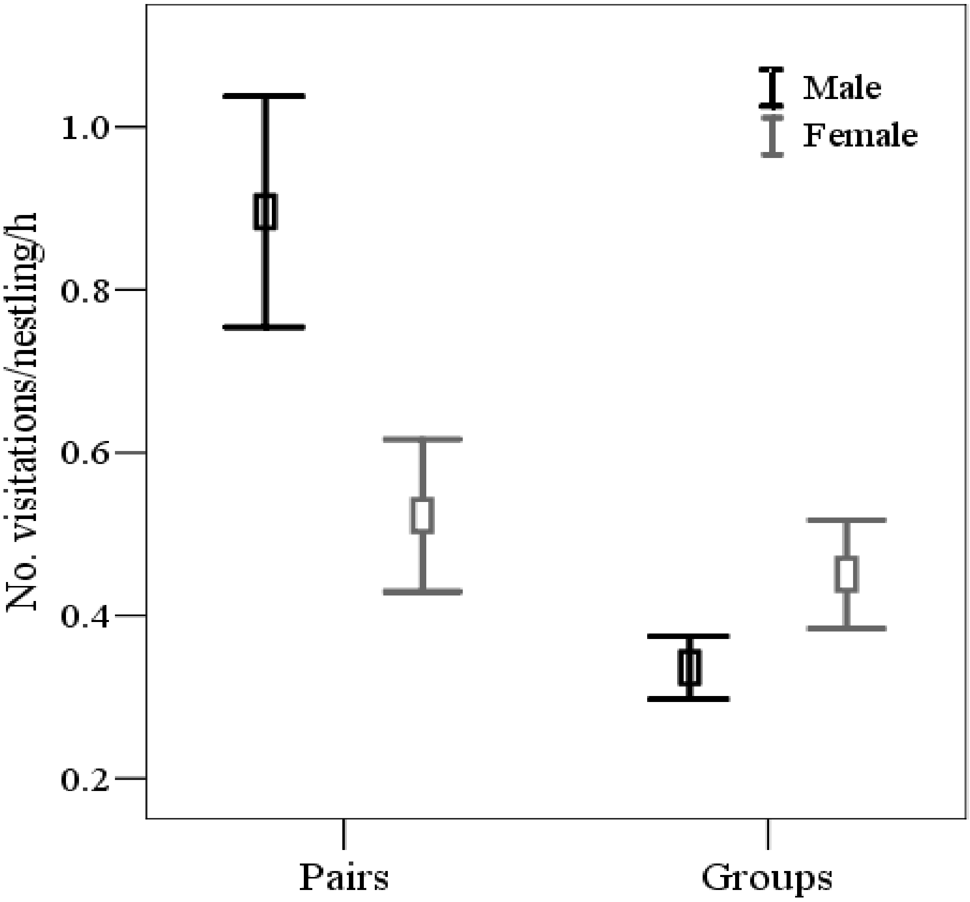

Figure 1.3 – Effects of sex and social organization on visiting frequency during the nestling period of Campo Flickers in central Brazil.

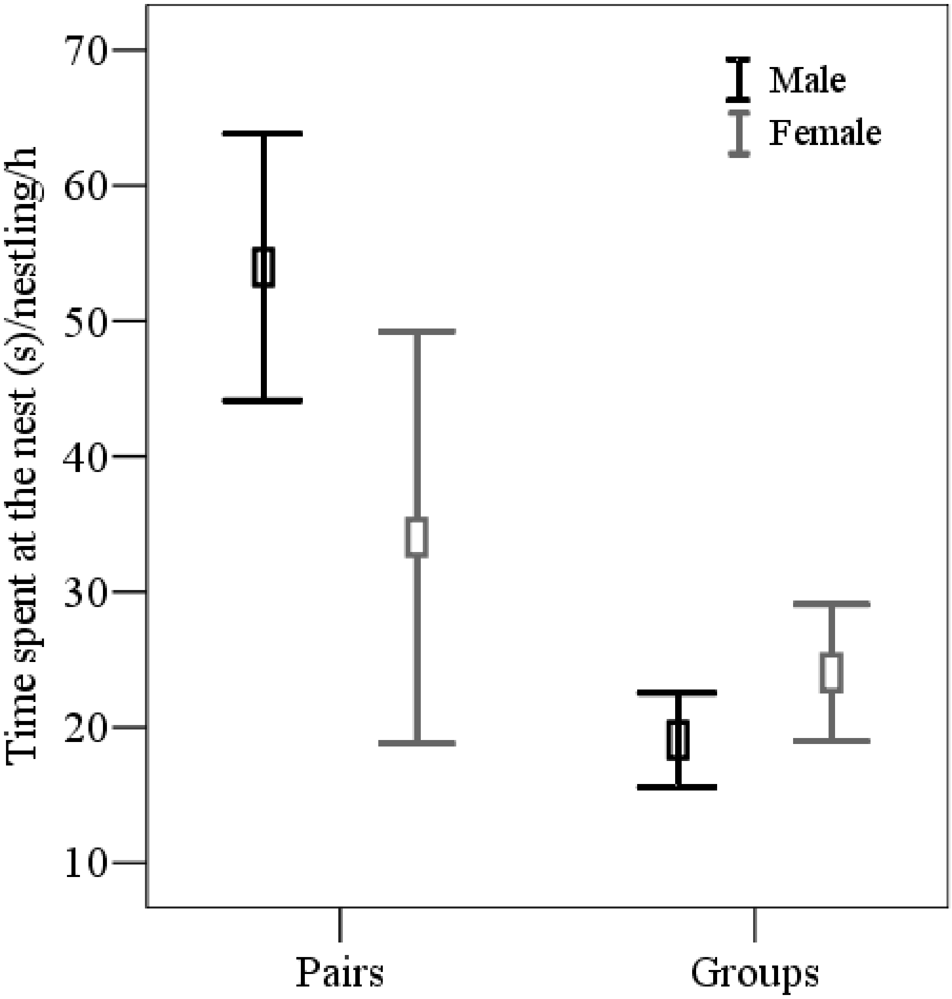

Figure 1.4 – Effects of sex and social organization on time spent at the nest during the nestling period of Campo Flickers in central Brazil.

Discussion

Breeding ecology and nesting behavior

Campo Flicker groups maintained a stable social organization and defended territories year-round, in some cases for several years. Suitable habitats in the study area seem to be saturated, with territories abutting each other. Territory sizes are comparable to those observed for other woodpecker species (del Hoyo et al. 2002).

Campo Flickers in this study (subspecies *campestris*) preferred nesting in termite mounds and used tree cavities only when there were few or no termitaria available. This pattern differs from that observed for the *campestroides* subspecies, which exhibits a preference for tree cavities (Short 1972). Cavity reuse among years was observed in tree cavities but did not occur for cavities in termitaria, as Campo Flickers generally open a new cavity each year, possibly to avoid the accumulation of ectoparasites (Short 1979). However, cavity reuse observed in Campo Flickers (10%) is much lower than that reported for the Northern Flicker (63%; Wiebe et al. 2006), a species clearly affected by ectoparasites (Wiebe 2009). Although some Campo Flicker nests were found infested with mites, especially those initiated late in the season, it is unclear whether the mites were in fact detrimental to nestling survival, although some nestling mortality at these nests was observed. Another possible explanation for why Campo Flickers excavate a new nest cavity is termite mound reconstruction behavior. After the woodpecker chicks fledge the termites usually close the cavities, often within a few days, and in one unusual event, termites sealed the cavity entrance with live 22-day-old nestlings within (R. I. Dias pers. obs.). This general pattern of low cavity reuse and annual cavity excavation differs from the observed behavior of the temperate zone congener species, the Northern Flicker (*C. auratus*), which lives in areas without termitaria and nests exclusively in trees (Wiebe 2001).

Termites of the *Cornitermes* genus build very large termitaria that are used as nesting substrate by several birds, and have a key role in the ecology of the Brazilian savanna (Cerrado) (Redford 1984). A phylogeny-based study of parrots and trogons suggests that nestling predation favored the transition from nesting in tree cavities to nesting in termite mounds (Brightsmith 2005a), and this may apply to woodpeckers such as Campo Flickers as well. Moreover, an experimental study reinforced the hypothesis that predation, more than nest competition, may be a major influence in the evolution of life history traits of many cavity-nesters (Brightsmith 2005b). Direct benefits for Campo Flickers nesting on termite mounds in comparison to tree cavities still have to be evaluated.

Campo Flickers began breeding at the end of the dry season and nestlings usually fledged at the beginning of the wet season, suggesting that rainfall is intrinsically linked to reproduction in this species. Other studies have demonstrated an effect of rainfall and other weather conditions on the timing of breeding for tropical birds in seasonal climates (Hau 2001; Monadgem and Bamford 2009). Among woodpeckers, such an association has been found for the Middle Spotted Woodpecker (*Dendrocopus medius*), which begins incubation earlier in years with warmer spring conditions (Pasinelli 2001). In the case of the Campo Flickers, higher availability of termites and ants during the rainy season may be the ultimate cause of breeding at the end of the dry season. It is likely that synchronization of the nestling period with the peak of higher food abundance would lead to higher reproductive success. On the other hand, the beginning of laying appears to be constrained to a specific period in the breeding season, since all nests initiated in October or November failed.

Breeding ecology of Campo Flickers is similar to that of most members of the family Picidae (del Hoyo et al. 2002), but detailed comparisons to other congeners is difficult due to a lack of information (but see Wiebe 2001; Wiebe and Swift 2001). Campo Flicker clutch size adheres to the general pattern of smaller clutch sizes typical for tropical birds, with an average clutch size (5.05±0.43 eggs) that is smaller than that of Northern Flickers (7.61±1.52 eggs; Wiebe and Swift 2001). Predation of eggs and nestlings of Campo Flickers (17%) was similar to that observed for Northern Flickers (20%), but general hatching success appears to be somewhat higher for the former (77%) when compared to its northern counterpart (66%, Wiebe and Swift 2001).

Cooperative breeding

Here we present the first description of facultative cooperative breeding in Campo Flickers. In this study nearly half of all breeding groups had auxiliary adults in addition to the breeding pair, with cooperative groups having three to five individuals helping to rear the offspring. Auxiliaries were of two types: in some cases they were known to be male or female offspring of the breeding pair from the previous breeding season, hereafter called “primary auxiliaries”, whereas in other cases they were adult females that joined the group, hereafter denominated “secondary auxiliaries”. In the latter case the females immigrated from outside the study site and were of unknown origin, but were likely unrelated to the breeding pair.

Year-round territoriality and high adult survival in Campo Flickers may lead to habitat saturation, which may be an important factor favoring philopatry and low territory turnover (see Baglione et al. 2005). Habitat saturation is also supported by observations of the fast replacement that followed the death of some paired individuals of socially monogamous pairs (unpubl. data), suggesting that the population contains adults waiting for a breeding opportunity. Our results therefore suggest that primary auxiliaries are probably constrained in terms of dispersal opportunities, such that the best option in most cases may be to remain on the natal territory. It is also likely that, similarly to other cooperative breeders, there are several potential benefits for primary auxiliaries that delay dispersal, including possible inheritance of the natal territory or abutting areas (Stacey and Ligon 1991), as well as indirect fitness benefits (Hamilton 1964).

In contrast, secondary auxiliaries are not previous young constrained from natal dispersal, but instead appear to be dispersing females that may be constrained in their ability to find a mate or breeding resources (e.g. food, nesting sites, territory). These females may join existing groups where they could benefit by directly contributing eggs to the nest. Clutch size variation between social groups and socially monogamous pairs supports this hypothesis and suggests that the Campo Flicker belongs to a small group of birds that are joint-nesting cooperative breeders, i.e., multiple females laying eggs in the same nest. This is a relatively rare breeding system that has been documented for only about 14 species (Vehrencamp and Quinn 2004), and includes only one woodpecker (the Acorn Woodpecker, Mumme et al. 1988).

Our results also suggest that there may be costs associated with the presence of secondary auxiliaries for Campo Flickers. Hatching success was lower for joint-nesting cooperative groups than for monogamous pairs, suggesting a possible constraint of cavity size in incubation effectiveness of large clutches (but see Wiebe and Swift 2001). An alternative possibility is that unhatched eggs were not fertilized by the breeding male. On the other hand, the absence of total nest loss due to starvation and the lower extent of partial nest loss in nests of cooperative groups suggest that auxiliaries, whether primary or secondary, have an important role in determining nest fate. Studies of several species, including woodpeckers, have demonstrated that the presence of auxiliaires has a positive effect on the number of young that fledge per nest (Conner et al. 2004). In Campo Flickers, there appears to be a relationship between the presence of helpers and fledging success as well as fledgling condition (Chapter 3).

The investment in incubation and feeding was shared by males and females of both social pairs and cooperative groups. Similar to other woodpecker species, males and females do not differ in their investments in incubation (Michalek and Winkler 2001), but individual members of groups spent less time at the nest than did those of pairs. On average, as observed in the Lesser Spotted Woodpecker (*Dendrocopos minor*; Wiktander et al. 2000), males do not differ from females in their provisioning, but taking in consideration only socially monogamous pairs, Campo Flicker males visited nests more frequently than did females. Furthermore, during the nestling phase there was also a tendency for individuals in cooperative groups to spend less time at the nest compared to those in pairs. We also found that members of cooperative groups paid fewer visits to the nest when compared with paired individuals, but the main difference occurred for males of cooperative groups. This reduction in offspring feeding rates fits a pattern that has been described as a compensatory reduction (Hatchwell 1999), usually observed for breeding males of cooperative species, which may reduce the costs of future breeding and survival. Another possibility to explain the lower investment found for males of social groups could be the enhanced uncertainty of paternity (Trivers 1972) due to the presence of other individuals at the nest, and possibly less time to guard multiple females. Conversely, the high level of paternal investment found in pairs may be associated with a high certainty of paternity (Birkhead and Møller 1996). High levels of paternal investment have also been found in other woodpecker species (e.g., Three-toed Woodpecker, Pechacek et al. 2005).

In general, the mating system of most woodpeckers is described as mainly socially monogamous with some cases of polyandry (Winkler et al. 1995; Willimont et al. 1991; Kotaka 1998; Weibe 2002; Pechacek et al. 2005). Furthermore, it is usually assumed that biparental care in woodpeckers is essential to rear a brood (Winkler et al. 1995; Wiktander et al. 2000). Results of our study suggest that Campo Flickers have a variable mating system, ranging from social monogamy to polygyny and some cases of joint nesting (see below). Polygyny is considered to be rare among woodpeckers; however, a study of the Lesser Spotted Woodpecker revealed that the species has a variable mating system that is usually socially monogamous, but also presents some cases of polyandry and polygyny (Wiktander et al. 2000). Sex-ratio bias has been suggested to explain these polygamous systems, with an excessive number of females leading to polygyny (Wiktander et al. 2000). However, sex-ratio bias does not seem to be the case for Campo Flickers, since adult sex-ratio appears to be balanced (Chapter 3).

Our results also suggests the occurrence of female joint-nesting in this species, as indicated by the atypically large clutch sizes of social groups with multiple females and the admittance of unrelated females to established groups. These cases of joint nesting may lead to competition as well as cooperation between females, particularly when the auxiliary female is unrelated to the primary breeder. Interestingly, cases of eggs vanishing from the nest without any sign of predation occurred only for cooperative groups with secondary auxiliary females, and when this happened the group re-nested in the same nest cavity within a few days (in contrast to cases of nest predation). This suggests that females may be destroying each others eggs as has been documented in other joint nesting species such as crotophagines and Acorn Woodpeckers (Vehrencamp 1977; Mumme et al. 1983; Macedo 1992). In addition, we observed aggressive behaviors among group females during nesting activities. In one case, the dominant female displaced a subordinate female every time the latter approached the nest (R. I. Dias pers. obs).

In woodpeckers, obtaining an additional mating partner may be constrained by time-demanding activities such as nest excavation. In the Campo Flicker, though, excavation investment made by males may be less arduous than in other woodpeckers because the substrate is a termite mound instead of a tree, which could provide extra time for males to acquire additional mates. However, as male investment in offspring is very high, it may still be impossible for males to divide their investment among multiple nests, favoring joint-nesting by females.

We believe that our study provides further understanding of a complex system that includes variability in both mating and social patterns, for a tropical species from the understudied woodpecker group. Our results relative to the life history and social traits of the Campo Flicker reveal the potential for conflict as well as cooperation in this species, and may contribute toward a more sophisticated interpretation of geographic variation in social and mating patterns of woodpeckers and other birds.

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Capítulo 2

Mixed mating strategies and patterns of kinship in the cooperatively breeding campo flicker

Introduction

Cooperative breeding is a social system in which more than two individuals help to care for offspring in a breeding event (Brown 1987), and has been described for both vertebrates and invertebrates. Particularly for birds, this social system has attracted a great deal of interest, generating both theoretical and empirical inquiries. In most of such systems, cooperative breeding is facultative, with some but not all breeding adults being assisted during breeding by other individuals, deemed auxiliaries (“helpers”). The identification of these auxiliaries and their relationship to the individuals being aided is crucial to understand the possible fitness gains that may be attained by both auxiliaries and the aided breeders. In general, for most cooperative bird species the auxiliaries are young from the previous year that delayed dispersal and stayed in the natal territory; auxiliaries are usually males, as females typically disperse from the natal territory (Greenwood 1980). However, variations to this pattern may occur, as demonstrated by the male-biased natal dispersal and presence of female helpers in white-throated magpie-jays (*Calocitta formosa*, Berg et al. 2009) and Seychelles warblers (*Acrocephalus sechellensis*, Eikenaar et al. 2010).

The possibility of inbreeding is an important issue for social species, especially when there are low levels of territory turnover, high philopatry, limited possibilities of dispersal and when the young remain at the natal site and try to attain breeding status by mating with relatives (Koenig and Haydock 2004). However, the identity of auxiliaries of several cooperative breeders and the ultimate mechanisms favoring them to stay or join a cooperative group and help rear young that are not their own, remain poorly understood.

Cooperative breeders have complex social and mating patterns that include strict monogamy, which is common, to rarer strategies such as polygynandry (Cockburn 2004). The mating system of a particular species is affected by factors such as population density, sex ratio and food supply, among others (Emlen and Oring 1977). Variation in reproductive strategies can be found intraspecifically, and may include extra-pair paternity (EPP) and intra-specific brood parasitism (IBP, egg dumping), for example. These alternative mating strategies occur on several groups (Griffith et al. 2002; Lyon and Eadie 2008), including woodpeckers (Li et al. 2009). Although EPP is not often observed among cooperative species (Cornwallis et al 2010), it has been described for a few species (Tarvin et al. 2005; Eimes et al. 2005; Berg 2005). It has been proposed that EPP may be a strategy to avoid costs related to inbreeding (Bensch et al. 1994; Blomqvist et al. 2002), or may function as insurance against infertile pairing or to increase genetic diversity or heterozygosity of the offspring (Double and Cockburn 2000; Rubenstein 2007). Cases of IBP, when females lay eggs in the nests of conspecifics and do not provide any care (Davies 2000) or even quasi-parasitism, when they perform this behavior facilitated by the male with which they copulated has also been observed, albeit rarely (Emlen and Wrege 1986; McKitrick 1990; Birkhead et al. 1990; Alves and Bryant 1998). Additionally, some systems may also present joint-nesting behavior (i.e. more than one female contributing eggs to a single nest, Brown 1987). This behavior may be associated to different types of mating systems ranging from groups composed of multiple monogamous pairs laying in the same nest to highly competitive scenarios wherein several females attempt to contribute eggs to the nest in polygynandrous groups (Vehrencamp and Quinn 2004).

The long-term pioneer studies of several cooperative species generated a substantial body of empirical knowledge concerning the evolution of their mating systems (Stacey and Koenig 1990). However, the more recent application of molecular techniques indicates that new concepts need to transcend the initial explanations generated for the evolution of reproductive strategies in such species, since they appear to be much more complex than originally proposed. The development of sophisticated molecular techniques to measure paternity, relatedness and the kinship structure among social groups have transformed the way researchers view social and reproductive behavior (Queller et al. 1993; Ross 2001, Blouin 2003). These techniques can also establish patterns of dispersal and philopatry (Beck et al 2008; Berg et al 2009). Molecular studies have revealed an astonishing diversity of avian mating systems and social structures relative to what was initially inferred from behavioral observations on social interactions, pair bonding and copulations. Clearly, understanding the development of helping behavior and the array of mating strategies exhibited by cooperative breeders demands the identification of the reproductive roles of all group members.

We studied the tropical campo flicker (*Colaptes campesteris campestris*), a facultative cooperative breeder (Chapter 1) endemic to South American savannas, to determine patterns of group composition, reproductive skew, mating system and within- and between-group relatedness. Natural history observations of this species suggest a high degree of social complexity in addition to a low rate of territory turnover and, apparently, low levels of dispersal (Chapter 1). Thus, genetic analyses not only will reveal the genetic identity and relationships among individuals within groups, but also shed light on the degree of inbreeding and patterns of dispersal of the species. By understanding genetic kinship patterns and the social mating system of this species we hope to provide further insights on the evolution of sociality in birds.

Methods

Study species

Campo flickers are conspicuous, sexually dimorphic, medium-sized woodpeckers that, despite their extensive distribution across the savannas of South America (Short 1972), are poorly known. In central Brazil where this study was conducted, groups defend large, year-round territories, usually start breeding in August, and males invest heavily in parental care (Chapter 1). Two subspecies are recognized: *Colaptes campestris campestris*, distributed from northeastern Brazil to central Paraguay; and *C. campestris campestroides*, found from southern Paraguay and Brazil to northeastern Argentina (Short 1972). Field observations suggested that this species breeds cooperatively and that breeding relationships are highly variable, with the possible occurrence of female joint-nesting as well as helpers-at-the-nest (Chapter 1), but molecular analyses have not been conducted to support these observations.

Study area and general procedures

The study site was a 4,500 ha area called Fazenda Água Limpa (FAL; 15°56’S, 47°55’W) located within the Brazilian savanna (Cerrado), a highly seasonal biome in central Brazil. Field work was conducted during four consecutive years (2006-2009) and comprises three breeding seasons. The study area is composed of different vegetation types including open grassland (*campo limpo*), grassland dotted with shrubs (*campo sujo*), scrub forest (*cerrado sensu stricto*), and gallery forests, among others.

We searched for and captured virtually all individuals of social groups that were found in the study area. We used playbacks and mist-nets to capture individuals, which were then uniquely identified with combinations of three colored plastic bands and a single numbered metal band from the Brazilian regulatory agency (IBAMA). For each individual captured we measured body mass (to nearest gram) and lengths of the tarsus, wing, beak and tail (all to nearest 0.02 mm), and collected approximately 100µl of blood from the brachial vein for genetic analyses.

During the breeding season we checked the contents of potential nesting cavities within the study area using a flashlight and mirror attached to a pole, and recorded the GPS coordinates of all active nests, which were subsequently monitored every 2-3 days. Group composition and nest visitation parameters recorded for a parallel study (Chapter 4) were used here to interpret molecular results. The variables recorded included, among others, the identification of group members and the identities of adults who brooded and provisioned young in the nest. Near the end of the nestling period (25th day after hatching), we measured and banded nestlings, similar to adults. Adults were considered to belong to the same social group if they showed long term social affiliation and occupied the same territory (Chapter 1). We used data from both breeding and non-breeding seasons to evaluate the structure of social groups, but only classified as cooperative groups those wherein all members helped to rear the young. We also include data from some social groups for which we had blood samples but little behavioral or breeding information, because this added information could still contribute to the understanding of group structure.

When group composition changed, we classified the newly formed group as a new group; these events resulted from both dispersal of some original group members and immigration of new individuals into the group (N = 1). Similarly, when there was a substitution of one of the paired members of a socially monogamous pair we also then classified it as a new pair (N = 4). Groups that changed from cooperative groups to socially monogamous pairs through the loss of all auxiliaries, but without the replacement of either of the previous socially paired individuals, were treated as non-independent samples. Despite this, behavioral information and group composition were taken into consideration during paternity analyses. We considered as an inbred mating those matings between individuals that shared a recent common ancestor and that had a coefficient of relatedness between 0.0625 to 0.5 (Koenig and Haydock 2004). We determined the adult population sex-ratio based on the complete data set from the entire study because virtually all group members were banded, and we could sex the few unbanded birds using the slight sexual plumage dichromatism. Moreover, adult survival was high in our study population and group composition was fairly stable.

DNA extraction and analysis

Blood samples were stored in of lysis buffer (100 mM Tris, pH 8.0, 100 mM EDTA, 2% SDS) at room temperature and the DNA was extracted using Qiagen DNeasy Blood and Tissue Kit® (QIAGEN). We initially screened 12 loci, all originally isolated for the northern flicker (*Colaptes auratus*, Kuhn et al. 2009), for amplification and polymorphism in campo flickers, but in the end used only 10 polymorphic microsatellite markers (see below). We applied a multiplex polymerase chain reaction (PCR) in 10µl reactions with 1µl of template DNA, 0.1µl of JumpStart™ *Taq* DNA polymerase (Sigma-Aldrich), 1µl of 10x PCR buffer, 3.25mM of $MgCl_2$, 0.2mM of dNTP and 0.10-0.45µM of each primer. The PCR profile was as follows: initial incubation at 94ºC for 1 min; 30 cycles of amplification at 94ºC for 1 min, 52-57ºC (depending on locus) for 1 min, and 72ºC for 1 min; and a final extension step at 72ºC for 5 min. One primer within each pair was labeled at the 5’ end with a fluorescent dye (VIC, NED, 6-FAM or PET). After PCR reaction the amplification products were diluted to optimize the product signal and visualized on the Applied Biosystems (ABI) 3100 automated capillary sequencer, using the

GS500 LIZ^{TM} size standard (Applied Biosystems). Electropherograms were analyzed using the GeneMapper® (version 4.1; Applied Biosystems).

Parentage, relatedness and relationship analyses

We applied different methods to analyze: 1) parentage, which is basically the search for the most likely parents among different candidates for a specific offspring; 2) relatedness (r), defined as the proportion of alleles identically shared between individuals through descent from a common ancestor; and 3) relationship, considered as a specific category of genealogical relation, such as parent-offspring or full sibs (Blouin 2003). For each locus used in these analyses, we used Genepop 4.0 (Raymond and Rousset 1995) to determine observed and expected heterozygosities, parental exclusion probabilities, and null allele frequency (Table 2.1).

Table 2.1. Summary statistics from loci used for paternity and relatedness analysis of Campo Flickers. The data include number of alleles (*k*), range of allele size in base pairs (bp), observed (H_O) and expected (H_E) heterozygosity, non-exclusion probability assuming no parents known (NEP_{1p}) and one parent known (NEP_{2p}), and estimated frequency of null-alleles (F_{Null}).

Locus	*k*	Size (bp)	H_O	H_E	NEP_{1p}	NEP_{2p}	F_{Null}
Cau1	10	199-233	0.686	0.765	0.626	0.448	0.0535
Cau2	7	148-163	0.757	0.779	0.618	0.441	0.0128
Cau3	6	177-222	0.700	0.728	0.687	0.513	0.0161
Cau5	7	145-170	0.886	0.775	0.619	0.439	-0.0761
Cau8	16	235-274	0.914	0.868	0.436	0.277	-0.0321
Cau9	18	225-264	0.800	0.914	0.313	0.185	0.0644
Cau10	9	108-140	0.643	0.629	0.758	0.570	-0.0014
Cau11	11	241-290	0.786	0.695	0.705	0.524	-0.0746
Cau12	8	170-191	0.743	0.630	0.772	0.596	-0.0957
Cau13	3	212-215	0.314	0.345	0.941	0.848	0.0385

Parentage analyses were conducted with the program CERVUS version 3.0.3, which uses a likelihood approach to assign parentage (Marshall et al. 1998; Kalinowski et al. 2007). We entered genotype data from all genotyped chicks and all adults, along with population allelic frequencies from all adults genotyped, into CERVUS. The sampling of parents in the study area was exhaustive, and we estimated that approximately 90% of the adults were sampled. As a result of the high observed polymorphism, the exclusionary power of the combined loci was 0.9913 assuming neither parent was known, and 0.9996 assuming one parent was known (Table 2.1). Assignments were carried out at a strict level of 95%. We ran the parentage analyses with all known males and females as potential parents and allowed CERVUS to assign the pair that had the highest likelihood of being the true parents of the offspring. We accepted the CERVUS assignment if the trio "female/chick/candidate male" had zero or one mismatch and if it agreed with the behavioral data.

We used the software package SPAGeDi (Hardy and Vekemans 2002) to estimate the coefficient of relatedness (r) for all possible combinations of pairwise individuals within and among-groups, based on Queller and Goodnight's (1989) formula. Subsequently, to avoid pseudoreplication, we randomly selected one individual of each sex from all groups and compared their average relatedness within their group and in relation to the members of other groups for all dyad combinations: female-female, male-male and male-female. We used paired *t*-tests to evaluate differences within and among groups.

We used the program ML-RELATE to discriminate among four common pedigree relationships: unrelated, half-sibs, full-sibs and parent-offspring (Kalinowski et al. 2006). This program can accommodate null alleles by using a maximum likelihood estimate of the frequency of null alleles in all calculations. We calculated the likelihood of the relationship for each pair of adult individuals. Subsequently, we used the program function confidence sets, and whenever more than one relationship was consistent with the genotypes of the individuals, we performed likelihood ratio tests using the *a priori* suspected relationship based on behavioral data, rejecting the alternative hypothesis when the *p*-value of the test was below 0.05.

Statistical analyses

We used a two-way repeated measures ANOVA to evaluate differences in the level of relatedness in relation to the sex of the dyads and group (within and among groups). Analyses were conducted using the software package R (2.11.1; R Development Core Team 2010). All tests were two-tailed and the results are presented as mean±SE.

Results

Social organization, paternity and mating system

We collected data from 36 social groups that ranged from two to seven individuals (more details in Chapter 1). Fifty-seven nests were located within the study site, and from 32 we were able to obtain samples for genetic analyses from 32 of these. We genotyped 162 individuals, 72 of which were adults (36 males, 36 females) and 90 were nestlings (45 males, 44 females). Social groups were classified as 21 socially monogamous pairs and 15 cooperative groups (i.e., groups with > 2 adults). The adult sex ratio of the study population did not differ from the expected ratio of 1:1 ($\chi^2_1 = 0.01$; $P = 0.91$).

In our study population, 20 nests were from socially monogamous pairs which did not exhibit any cases of EPP. Genetic data revealed that 90% of these pairs were genetically monogamous and accounted for 90% of the broods and 90% (N = 51) of the nestlings. The only exceptions were two cases of apparent intraspecific brood parasitism (IBP) representing 6.25% of all broods and 5.5% of all nestlings. For both cases of IBP the young were related to the social father but not to the social mother, a phenomenon known as quasi-parasitism. In our behavioral observations for nest attendance (around 10h per nest) and group activities before and after the breeding season (around 13h per group in 2007), only the social female was ever recorded within the group or visiting the nest to incubate and feed nestlings. In one case, the genetic mother of the IBP nestling was a female of an adjacent territory and in the other case the genetic mother was not identified. The IBP nestlings in each brood had an r of approximately 0.5 relative to other broodmates, indicating that only one female parasitized each clutch.

Twelve nests were from cooperative groups, and the molecular data revealed that some of these nests (N = 6) comprised a single monogamous breeding pair aided by auxilliaries, while others exhibited simultaneous polygyny (N = 5) or polygynandry (N = 1). Here as well there were no cases of EPP (i.e., offspring sired by males from other groups). Joint-nesting was associated with the presence of polygynous males. There was a skew in the number of nestlings produced by each joint-nesting female wherein the reproductively dominant female usually produced 50 to 75% of the nestlings. However, females of some groups seem to alternate dominance status across years (N = 2). There was no difference in the sex-ratio of cooperative groups with polygynous males (0.64±0.14 males/females) in comparison to groups with a single breeding pair (0.70±0.14 males/females; Student *t*-test: $t_7 = 0.332$; P = 0.75).

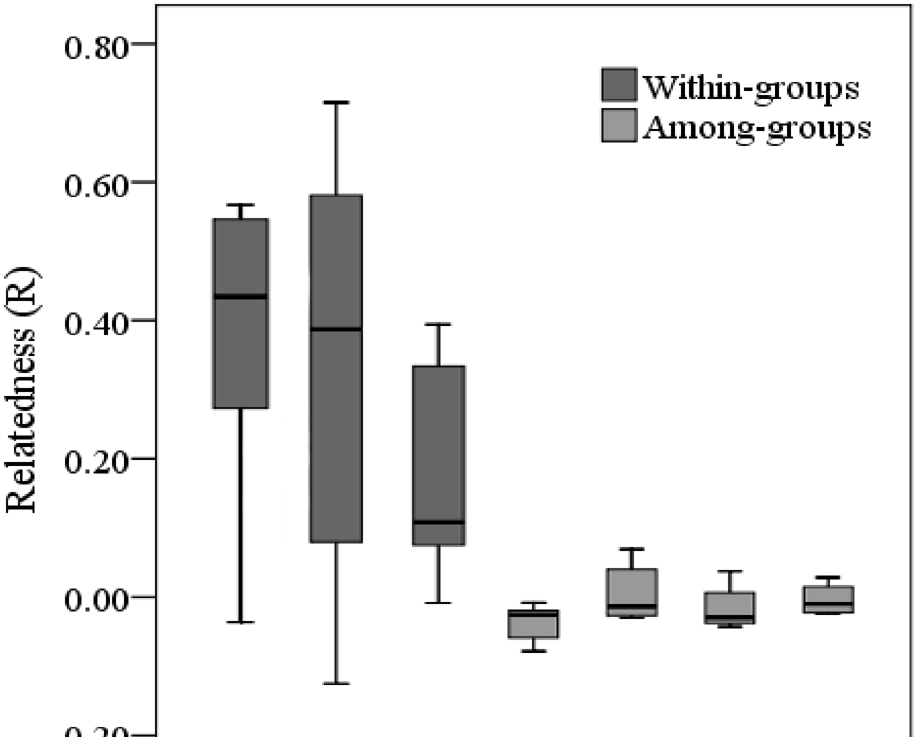

Figure 2.1 – Level of relatedness (R) between dyads of individuals of both sexes (male – M and female – F) within and among groups for campo flickers in central Brazil.

Relatedness and relationship

Mean relatedness among adults within cooperative groups was high for both dyads of males (0.34±0.13) and females (0.37±0.09; Fig 2.1). Relatedness between individuals of the same sex within groups was significantly higher than that observed among groups ($F_{1,10} = 22.39$; $P < 0.001$; Fig 2.1) and it was not different in relation to sex ($F_{1,10} = 0.00$; $P = 0.96$). The interaction of group treatment and sex was also not significant ($F_{1,10} = 0.21$; $P = 0.65$).

We also found that individuals of opposite sexes are more related within their groups (0.16±0.06) than when compared to the opposite sex of other groups, whether considering males (0.03±0.01; Paired *t*-test: $t_6 = 3.07$; $P = 0.022$) or females (0.02±0.01; Paired *t*-test: $t_6 = 3.210$; $P = 0.018$; Fig. 2.1). These results remained significant after the Bonferroni correction was applied.

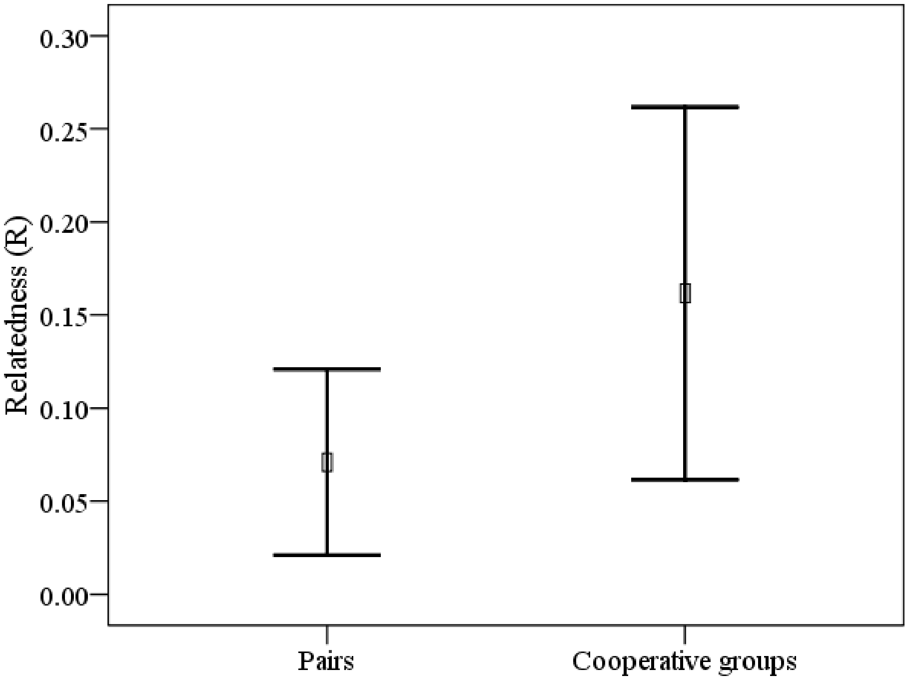

Figure 2.2 – Level of relatedness (R) between breeders of socially monogamous pairs and cooperative groups for campo flickers in central Brazil.

Breeders within cooperative groups did not differ in degree of relatedness to each other (0.16+0.10) when compared to r of socially monogamous pairs (0.07±0.04; t_{17} = 0.913; P = 0.374; Fig. 2.2), although they are on average twice as related to each other when compared to socially monogamous pairs.

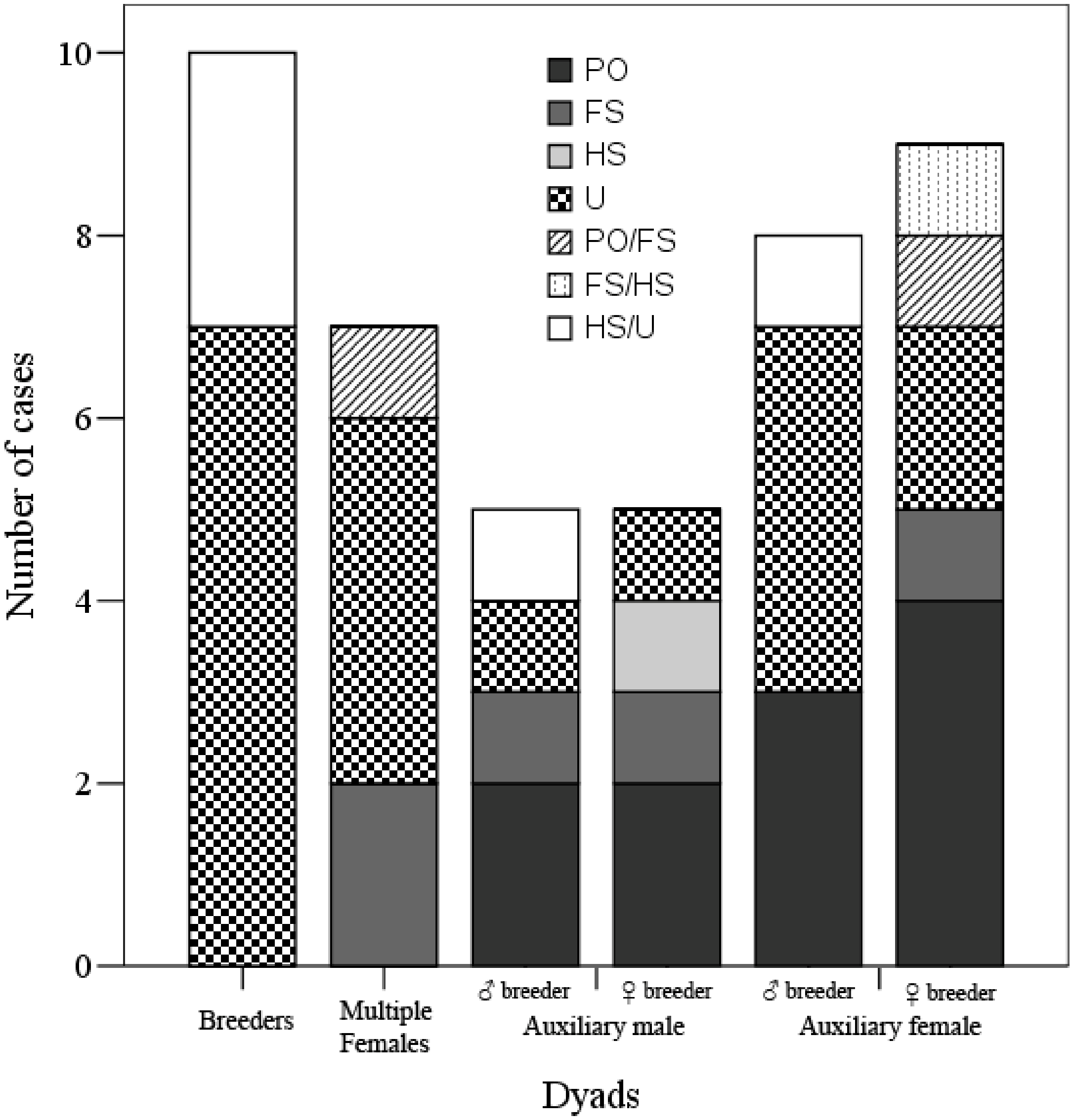

Figure 2.3 – Relationship between group members discriminated among four common pedigree relationships for campo flickers in central Brazil: unrelated (U), half-sibs (HS), full-sibs (FS) and parent-offspring (PO). When it was not possible to statistically differentiate between the pedigree relationships we presented both possible associations (e.g. U/HS, HS/FS or PO/FS).

The relationship analysis within ten cooperative groups revealed that breeders were mainly classified as unrelated individuals (70%). For the remaining cases, the genetic analyses also supported that the breeders were unrelated to each other, but it was not possible to statistically exclude the possibility that the breeders were related at the level of half-siblings. In contrast, analyses of the relationship of auxiliaries to the breeders indicated a highly mixed level of associations. Each male auxiliary (N = 5 groups) was classified unambiguously as either parent-offspring (40%), full-sibling (20%) or unrelated (20%) with respect to the breeding male, and as parent-offspring (40%), full-sibling (20%), half-sibling (20%) or unrelated (20%) with respect to the breeding female. Female auxiliaries (N = 8 groups), on the other hand, were either parent-offspring (38%) or unrelated (50%) to the breeding male. On the other hand, considering the relationship between breeding females and auxiliary females, some relationship occurred in 78% of the cases. In cases where multiple females bred jointly at a single nest they were usually unrelated (57%; Fig. 2.3).

Discussion

The genetic structure of groups in the Campo Flicker clearly determines both the mating system as well as social complexity and patterns of kinship. The genetic mating system of the Campo Flicker can be classified as mainly genetically monogamous considering both types of social organizations, cooperative groups and socially monogamous pairs. However, cases of simultaneous polygyny were also frequently observed in cooperative groups, although this is rarely found in woodpeckers (Wiktander et al. 2000). However, despite the existence of groups with polygynous arrangements, the sex-ratio of the adult population was not biased toward females, nor did the sex-ratio differ between polygynous and monogamous cooperative groups. In general, most woodpeckers have been described as genetically monogamous (Winkler et al. 1995), and the most likely explanation for the rarity of polygynous woodpeckers is their dependence upon biparental care to successfully fledge young (Winkler and Christie 2002). In Campo Flickers this requirement may have been met by the evolution of joint-nesting, allowing polygyny to take place.

No case of EPP was found. This agrees with results from studies of nonpasserines (Griffith et al. 2002; but see Huyvaert et al. 2000 and Mee et al. 2004), although EPP has already been reported for at least one other woodpecker, the Three-toed Woodpecker (*Picoides tridactylus*; Pechacek et al. 2005). Moreover, we observed low levels of IBP, which has also been detected in other woodpeckers (Wiktander et al; 2000; Bower and Ingold 2004; Pechacek et al. 2005; Li et al 2009). In our study, however, the cases of IBP were in fact examples of quasi-parasitism because the social males sired the nestlings, but the social females were not their biological mothers. Although one could argue that other hypothesis cannot be excluded since we do not have behavioral data evidencing egg-dumping for the parasitized nests (i.e. abnormally large clutches or appearance of several eggs at the same day; Griffith et al. 2004) we are pretty sure that errors in the molecular analysis are unlikely and our behavioral data strongly support against rapid-mate switching, since data on group composition was collected starting months before the breeding season. Both the absence of EPP and the low levels of IBP may also reflect some life history traits usually found in woodpeckers such as high levels of male investment during breeding, long bouts of incubating and brooding, and a demanding nest excavation process (Winkler and Christie 2002). In addition to these factors some other aspects observed for the Campo Flicker, such as large territories with year-round territoriality, high adult survival and constant proximity of group members, may constrain individuals from obtaining extra-pair copulations.

For cooperative groups, in addition to the polygynous matings, we also observed one case of polygynandry, where a subordinate male (son of the dominant male) copulated with one of the two females of the group (his mother´s sister) producing one nestling, while his father copulated with both group females producing three nestlings. All groups with multiple breeding females presented joint-nesting behavior, with one female usually producing more nestling than other females, and this reproductive skew favoring one individual may affect interactions among group members (Magrath et al. 2004). However, the number of nestlings produced by each laying female varied among years and breeding dominance by one female was not often maintained subsequently. Aggressive interactions among females within groups have been observed in Campo Flickers (Chapter 1). Despite the fact that one could expect that groups of polygynous males would present more females than groups of non-polygynous males, that was not the case, we did not observed any difference in the sex-ratio between those groups.

Individuals of the same sex within groups were more related among themselves when compared to members of other groups. The same pattern was observed between individuals of the opposite sexes, although the level of relatedness within group was lower. In addition, breeders of cooperative groups did not differ statistically in terms of relatedness in comparison to socially monogamous pairs, although they are twice as related than the latter. This scenario was expected given the kin selected benefits that can be produced due to group composition and structure (Brown 1987). However, data suggest that inbreeding can be a potential cost for this close genetic association among group members, since breeders of some cooperative groups are more closely related to each other. Although we cannot be conclusive about long-term deleterious effects due to high relatedness due to inbreeding, we have detected low hatchability in cooperative groups (Chapter 1).

Breeders were usually unrelated individuals. However, auxiliaries were found to vary in their degree of relatedness to breeders, and different types of associations occurred depending on whether the auxiliary was male or female. When the auxiliary was a male, it was usually highly related to the breeders of both sexes within the group, and was considered essentially as a primary auxiliary. However, auxiliary females tended to be less related to male than to female breeders, suggesting that these female auxiliaries may be potential breeders waiting for a breeding opportunity. Hence, we considered these females as secondary auxiliaries. Additionally, multiple breeding females in polygynous groups were mostly unrelated. These results provide clues that allow the inference of some dispersal patterns in the Campo Flicker, suggesting that there probably are several modes of dispersal. Data show that both males and females may stay at the natal site and help parents or they may disperse alone or in coalitions (especially females) to create a new group or join an existing one. Patterns of dispersal of social insects may have profound effects upon the distribution of genetic variation among groups (Blows and Schwarz 1991; Schwarz et al. 1996), and this study and others (e.g. Painter et al. 2000) may show similar patterns for vertebrates.

We have documented here an unusual system involving a facultative cooperative, joint-nesting breeder that presents alternative mating strategies that include a variable mating system ranging mainly between monogamy to polygyny with presence of IBP, as well. Group members usually have some degree of genetic relatedness although auxiliary females tend to be less related to members of the opposite sex, and when there are multiple breeding females, these are usually unrelated.

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Capítulo 3

Does helping enhance productivity in Campo Flicker (*Colaptes campestris campestris*) nests?

Introduction

The presence and behavior of "auxiliaries" in cooperative breeding systems, that is, non-breeding individuals that help to rear non-descendant young (Brown 1987), have been a prevailing topic in behavioral studies. In several species, auxiliaries are young from previous years that have delayed dispersal and stayed at the natal site helping rear their siblings (Ekman et al. 2004). Knowing what effects the presence of these auxiliaries have on group reproduction and why they help are key questions that still stimulate extensive debate (Emlen 1991; Dickinson and Hatchwell 2004). The presence of auxiliaries is generally thought to have a positive effect on breeder fitness, such as enhancing group productivity (Emlen and Wrege 1991; Conner et al. 2004) and the quality of young produced (Hatchwell 1999). A less obvious observed effect is an increase in breeders' survival (Reyer 1984). There are even special cases of species that are not able to breed without assistance, such as the white-winged chough (*Corcorax melanorhamphos*, Heinsohn 1992) and the apostlebird (*Struthidea cinerea*, Woxvold and Magrath 2005). However, some studies have found no effect – or even a negative effect – of the presence of auxiliaries within a group (Caffrey 2000; Eguchi et al. 2002; Cockburn et al. 2008).

Nevertheless, the outcome of having auxiliaries on productivity or breeding success may be confounded by other factors that are difficult to separate from the effects of auxiliaries, such as territorial or individual quality (Brown 1987; Emlen 1991; Cockburn 1998; Dickinson and Hatchwell 2004). In a study of the laughing kookaburras (*Dacelo novaeguineae*), for example, the quality of the breeding pair and of the territory strongly affected fledgling condition and survival, masking the effects of auxiliaries (Legge 2000).

Different approaches have been used to evaluate the effect of auxiliaries, although most present some flaws (Dickinson and Hatchwell 2004). Among the most common approaches are experiments with auxiliary removal. The first experiments using this method were conducted with the grey-crowned babbler (*Pomatostomus temporalis*) and showed that the presence of auxiliaries indeed increased reproductive success in the species (Brown et al. 1978). Comparisons between breeders with and without auxiliaries has also been frequently used, including repeated comparisons of the same pairs in years with and without assistantship (Caffrey 2000; Legge 2000; Eguchi et al. 2002). Finally, specific statistical methods have been applied to investigate auxiliaries' effect on group breeding parameters (Hatchwell et al. 2004).

A related set of questions often asked in studies of cooperative breeding attempt to elucidate why auxiliaries help (Dickinson and Hatchwell 2004). Both direct and indirect benefits have been proposed as explanations for the investment made by auxiliaries, although their relative roles in the evolution and maintenance of cooperation remain unclear (Cockburn 1998; Clutton-Brock 2002; Dickinson and Hatchwell 2004). Possible direct benefits to auxiliaries proposed in the literature include: enhanced survival (Gaston 1978; Brown 1987); increased chances of future breeding (Ligon and Ligon 1978; Heinsohn et al. 1988); increased probability of future breeding (Carlisle and Zahavi 1986); and acquisition of skills needed for independent breeding (Komdeur 1996, but see Khan and Walters 1997), but others studies observed no direct benefits of helping (Dickinson et al 1996). Another possibility is that helping behavior may generate no immediate gain for auxiliaries, but by providing help they are allowed to stay on the natal territory in a "pay to stay" situation (Mulder and Langmore 1993; Kokko et al. 2002).

The indirect benefits explanation for the evolution of cooperative breeding are based on kin selection and inclusive fitness (Lucas et al 1996; Oli 2003), which requires that auxiliaries be related to the individuals they are helping, so they can reap the benefits associated with producing relatives (Hamilton, 1964). However, it is generally established that auxiliaries accumulate lower fitness by helping than they would guarantee by breeding independently (Stacey and Koenig 1990), though in some cases it appears that auxiliaries really may gain kin-selected benefits by increasing the survival of related offspring (McGowan et al. 2003; Hatchwell 2009). One possibility is that auxiliaries are simply making the best-of-a-bad-job (Dickinson and Hatchwell 2004).

Kin selection should be favored when the the indirect benefit of helping outweighs the cost of helping (e.g. postponing independent breeding) (Hamilton 1964). For this purpose some assumptions have to be met: the helper and the individual being aided must be genetically related and there should be a measurable benefit to the individual being aided due to the behavior of the helper (Hamilton 1964; Lucas et al. 1996). Many empirial studies have used this theoretical framework to determine the possible costs and benefits for non-breeding auxiliaries in a diversity of species (Krakauer 2005). In some social and ecological contexts, helping behavior may be maladaptive to auxiliaries or may be only slightly helpful to breeders (Emlen 1982). Alternatively, as mentioned above, there are species that depend to a great extent on the help provided by auxiliaries. The questions relative to the nature and effect of helping are evolutionarily interesting especially when comparing systems and species exposed to different conditions

In this paper we investigate the factors that influence breeding parameters in the campo flicker (*Colaptes campestris campestris*), a facultative cooperative breeder, and ask whether helping behavior by auxiliaries enhances the productivity of breeders. Campo flickers are Neotropical woodpeckers endemic to savannas of South America, and thus subjected to seasonal conditions that restrict breeding to the end of the dryseason (see below). We use behavioral, demographic and genetic data to assess the effect of auxiliaries, breeding season, territory and breeders' quality on several breeding parameters such as nest success, productivity, fledgling quality, offspring sex-ratio and laying date. Additionally, we calculate Hamilton's rule using the degree of relatedness obtained through molecular analysis. Finally, we discuss possible benefits obtained by auxiliaries and their implications to cooperative breeding theory.

Methods

Study species

Campo Flickers are terrestrial, medium-sized woodpeckers with a subtle sexual dichromatism based on plumage ornaments (Short 1972). The species is widely distributed across South America and comprises two subspecies: *Colaptes campestris campestris* and *C.*

campestris campestroides (Short 1972). The subspecies under study, *C. campestris campestris*, presents a complex social and mating system and exhibits facultative cooperative breeding, associated with monogamy or simultaneous polygyny (Chapter 2). Group size ranges from two to five individuals during breeding and affects levels of provisioning to nestlings (Chapter 1). Auxiliaries are of both sexes, although auxiliary females tend to be more unrelated to the opposite sex breeder than are auxiliary males (Chapter 2).

Study area and general procedures

The study was conducted at Fazenda Água Limpa (FAL; 15°56'S, 47°55'W), an area of 4,500 ha in Central Brazil within the Cerrado biome (Brazilian Savanna). The region is characterized by a strong seasonality and vegetational landscapes ranging from open grasslands to gallery forests. Field work was performed through four years (2006-2009) that included three breeding seasons.

We used playbacks and mist nets to capture individuals that were then banded with a unique combination of three color bands and a numbered metal band from the Brazilian regulatory agency (IBAMA). We measured (nearest 0.02mm: tarsus, wing, beak and tail length) and weighed (nearest gram) each captured bird, and also collected a small blood sampled (~ 100µl) from the brachial vein for genetic analyses. Blood samples were initially stored in Lysis buffer and extracted using Quiagen DNeasy Blood and Tissue Kit®.

During the study we searched for nests in new cavities on both termite mounds and trees, checked the contents of pre-existing cavities and also used behavioral clues to identify potential nests. After finding an active nest or a nest under construction we started monitoring them systematically every 2-3 days. We used a flashlight and a mirror attached to a pole to check cavity contents. All cavity locations were recorded with a GPS. Nest visitation parameters such as identity of brooders and provisioners were recorded for a parallel study (Chapter 4) and were used here to help interpret results (more details below). When nestlings approached fledging (25th day after hatching), we measured, banded and took blood samples similarly to the protocol for adults. We defined laying date as the day when the first egg was laid in each nest, relative to a continuous succession of days, starting in June, which is prior to the occurrence of the first breeding activities (e.g copulations, cavity excavation).

We used the residuals of the regression between body mass and tarsus as an index of body condition for the genetic parents. We considered the number of termite mounds in the territory, estimated as the total number of mounds within 200m radius around the nest, as a measurement of territory quality since termite mounds are the main substrate used for nesting (Chapter 1) and termites are the most frequent item of the campo flicker diet (Dias et al in prep.).

In all but one case there was only a single male breeder in each of the groups, but several groups had contained multiple breeding females. We genetically defined as dominant breeders those individuals that produced a higher proportion of nestlings per breeding attempt. Primary auxiliaries were defined as those individuals of either sex that helped to brood or feed nestlings, but that did not directly produce any young. Subordinate breeders (secondary auxiliaries) were mostly females that attained some reproduction with the dominant male. In the analyses, we considered that groups of three individuals with two breeding females were assisted, since in these cases more than two individuals helped with parental care. We classified as a new group a single observed case of a drastic change in composition of an existing group with both dispersal of previous group members and immigration of new ones. We also considered as new groups those socially monogamous pairs in which one of the pair members was replaced. Groups that we sampled repeatedly over the years were considered non-independent samples. Nests that were depredated early in development (e.g. few days after laying) did not enter the analyses.

Molecular analysis

We used molecular analysis to determine parentage of nestlings and the degree of relatedness among group members, as detailed elsewhere (Chapter 2). In brief, we used 10 polymorphic microsatellite markers in a multiplex polymerase chain reaction (PCR), diluting the amplification products to optimize the product signal and visualize on the Applied Biosystems (ABI) 3100 automated capillary sequencer. Electropherograms were examined using the GeneMapper® (version 4.1; Applied Biosystems). For the parentage analysis we used the program CERVUS version 3.0.3 (Marshall et al 1998; Kalinowski et al 2007). We accepted the

CERVUS assignment if the trio “female/chick/candidate male” had zero or one mismatch and if it was in accordance to the behavioral data. Pairwise relatedness (r) was estimated on the software SPAGeDi (Hardy and Vekemans 2002), based on Queller and Goodnight’s (1989) formula.

We estimated the indirect fitness of auxiliaries/subordinate breeders by calculating Hamilton’s rule. Despite the fact that there are cases of more than one helper per group, we believe that this is a valuable simplification of a more complex scenario that can shed some light on the benefits of helping. For this calculation, we separately estimated the variables for both male and female helpers. We only used groups with complete data sets that included the identity of all members with blood samples and molecular analysis. Five groups had at least one auxiliary male and seven groups had at least one auxiliary/subordinate breeder female. The level of relatedness (*r*) was calculated between the helpers and the dominant breeders of both sexes. Campo flickers are known to be found in cooperative groups of related individuals (Chapter 2). The fitness of males and females differed mainly because subordinate males usually do not breed, while it is common to find multiple breeding females in some groups. The benefit of helping (*B*) was calculated as the difference between the mean fitness of the assisted breeder and the average fitness of non-assisted breeders (calculated for males and females separately). The cost of helping (*C*) for auxiliary/subordinate breeder was considered as the difference between the mean fitness of non-assisted individuals and the fitness of the auxiliary/subordinate breeder; again, the values were based on both males and females of socially monogamous pairs.

Statistical analysis

We used generalized linear mixed models (GLMM) for binomial and count response variables considering a Binomial and Poisson distribution, respectively. Linear mixed models for continuous response variables with Gaussian distribution were also used. Models were implemented using the “lmer” function in the “lme4” package (R 2.11.1. R Development Core Team 2010). We incorporated the random term “Group ID” to all models to avoid pseudoreplication because of the repeated sampling of some of the groups over the years of study. We evaluated the effects of the presence of auxiliaries, group size, breeders’ condition, territory quality and year on the number of fledglings produced, nest success, nestling body weight, laying date and nestling sex-ratio. The models were progressively simplified by removing variables starting with higher level interactions. We used likelihood ratio tests (LRT) using the change in deviance as a chi-square approximation. The model simplification was retained if the simpler model did not differ in terms of fit. The Tukey post-hoc test ("glht" in the R package "multcomp") was used to evaluate differences between levels of dummy variables. Residuals of the models were examined to check for the assumption of normality. All analyses were conducted in the free software R 2.11.1. (R Development Core Team 2010). Results are shown as mean±1standard error.

Results

We studied 27 groups during three breeding seasons. Of these groups 17 were monitored in only one year, six were monitored in two years and four were monitored in the three years of study. Group size averaged 2.46±0.16 (range: 2-5) and successful groups produced only a single brood per year. On average, 30% of the groups contained at least on auxiliary, but over the study period only two groups changed status from assisted to unassisted and two other groups changed in the opposite direction.

The number of fledglings produced per group was affected by the presence of auxiliaries ($\chi^2_1 = 5.29$; $P = 0.021$; Fig. 3.1), but nest productivity was not affected by the other measured parameters, which included group size ($\chi^2_1 = 0.05$; $P = 0.820$), breeders' condition ($\chi^2_1 = 0.20$; $P = 0.840$), territory quality ($\chi^2_1 = 0.03$; $P = 0.849$) or year ($\chi^2_1 = 0.39$; $P = 0.822$). Groups aided by auxiliaries, fledging more nestlings (3.14±0.35) than single pairs (2.03±0.27). However nest success was not affected by the presence of auxiliaries ($\chi^2_1 = 0.50$; $P = 0.478$), group size ($\chi^2_1 = 0.50$; $P = 0.478$), breeders' condition ($\chi^2_1 = 0.01$; $P = 0.899$), territory quality ($\chi^2_1 = 0.03$; $P = 0.850$), or year ($\chi^2_1 = 1.56$; $P = 0.457$). Surprisingly, we found that nestling body mass (near fledgling at 25 days) was strongly affected by year ($\chi^2_1 = 12.30$; $P = 0.002$; Fig. 3.2), but not by the presence of auxiliaries ($\chi^2_1 = 1.81$; $P = 0.177$), group size ($\chi^2_1 = 0.77$; $P = 0.377$), breeders' condition ($\chi^2_1 = 0.79$; $P = 0.361$) or territory quality ($\chi^2_1 = 0.24$; $P = 0.623$). More specifically, the main difference in nestling body mass occurred between 2007, when nestlings were much heavier (143.33±2.92g) and 2008 (132.08±4.78g; Tukey Post-hoc test; Z = -3.53; P = 0.001). No other difference was observed between other years (all *Z* < 2.04; P > 0.101).

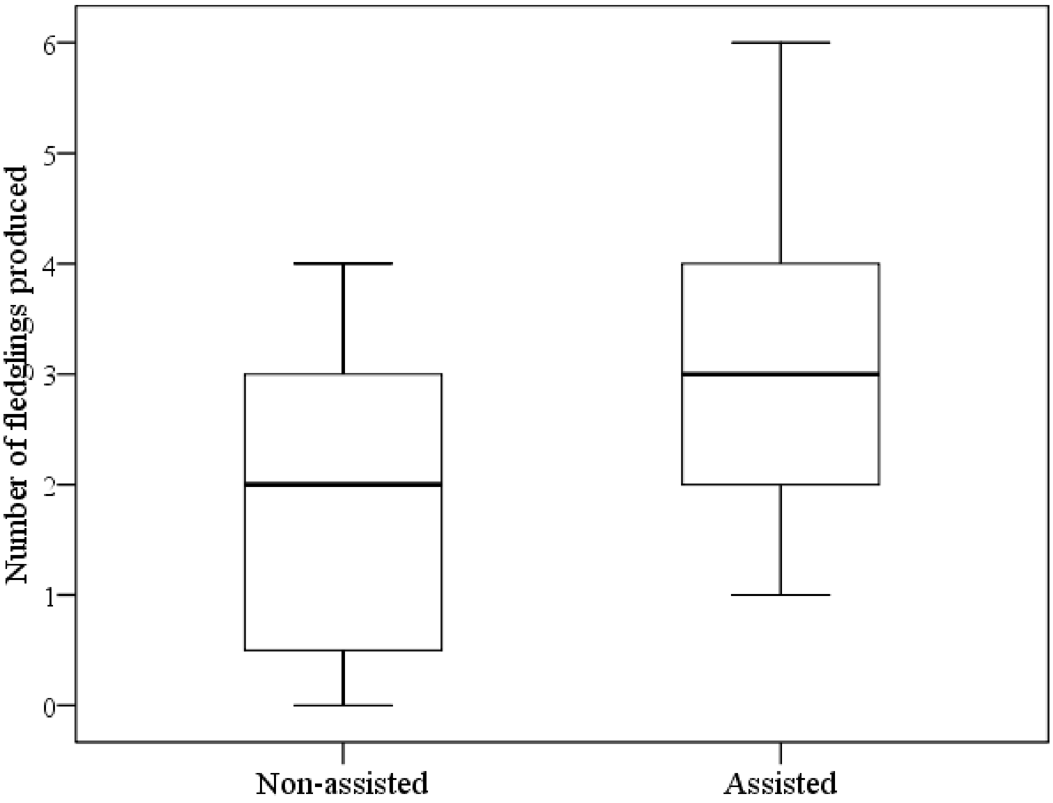

Figure 3.1 – Comparison of the number of fledglings produced between assisted and non-assisted groups of campo flickers (*Colaptes campestris campestris*) in Brazilian savanna.

The date for beginning of egg laying was affected by both the presence of auxiliaries (χ^2_1 = 5.54; P = 0.018; Fig. 3.2) and year (χ^2_1 = 13.89; P < 0.001 Fig. 3.4). However, laying date was not influenced by group size (χ^2_1 = 2.19; P = 0.138), breeders' condition (χ^2_1 = 0.61; P = 0.507) or territory quality (χ^2_1 = 0.36; P = 0.547). Laying date started on average 22.23±6.86 days later in 2007 when compared to 2008 (Tukey Post-hoc test; *Z* = -2.62; P = 0.023) and 2009 (*Z* = -4.15; P < 0.001). There was no significant difference between 2008 and 2009 (*Z* = -1.36; P = 0.359).

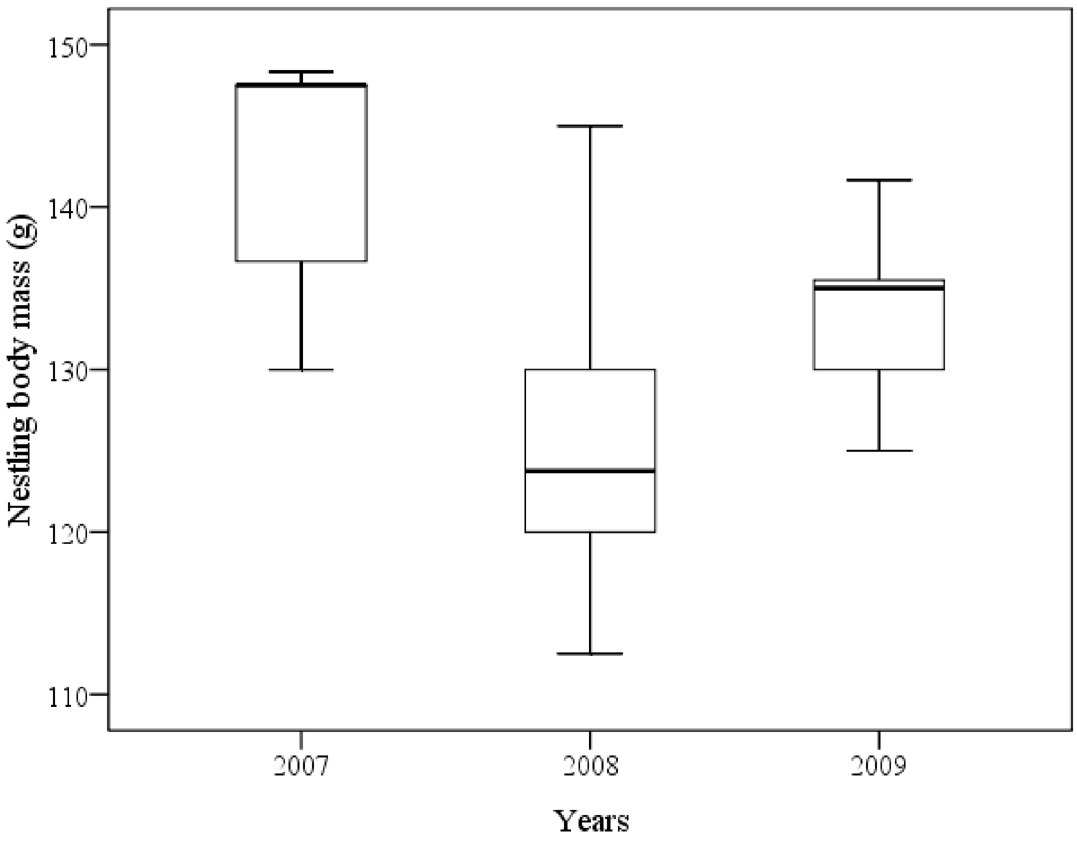

Figure 3.2 – Comparison of nestling body mass of campo flickers (*Colaptes campestris campestris*) between the study years in Brazilian savanna.

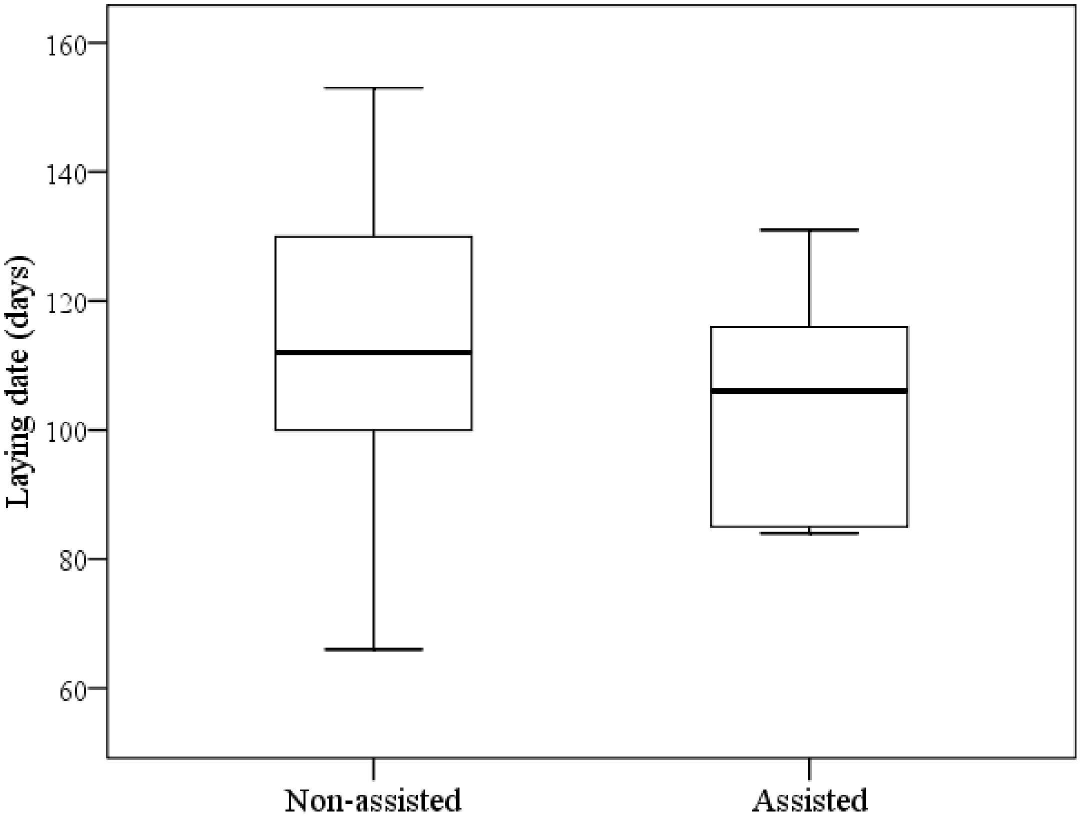

Figure 3.3 – Comparison of the laying date between assisted and unassisted groups of campo flickers (*Colaptes campestris campestris*) in Brazilian savanna.

None of the evaluated variables (presence of helpers, group size, territory quality and year) significantly affected the sex-ratio of the nestlings (all $\chi^2_1 < 0.42$; $P > 0.719$). However, we observed a non-significant trend for an effect of breeders' condition on nestling sex-ratio ($\chi^2_1 = 3.45$; $P = 0.06$). Despite the effects of year on some breeding parameters, there was no difference in the number of helpers between years (Chi-square test: $\chi^2_1 = 0.98$; $P = 0.611$).

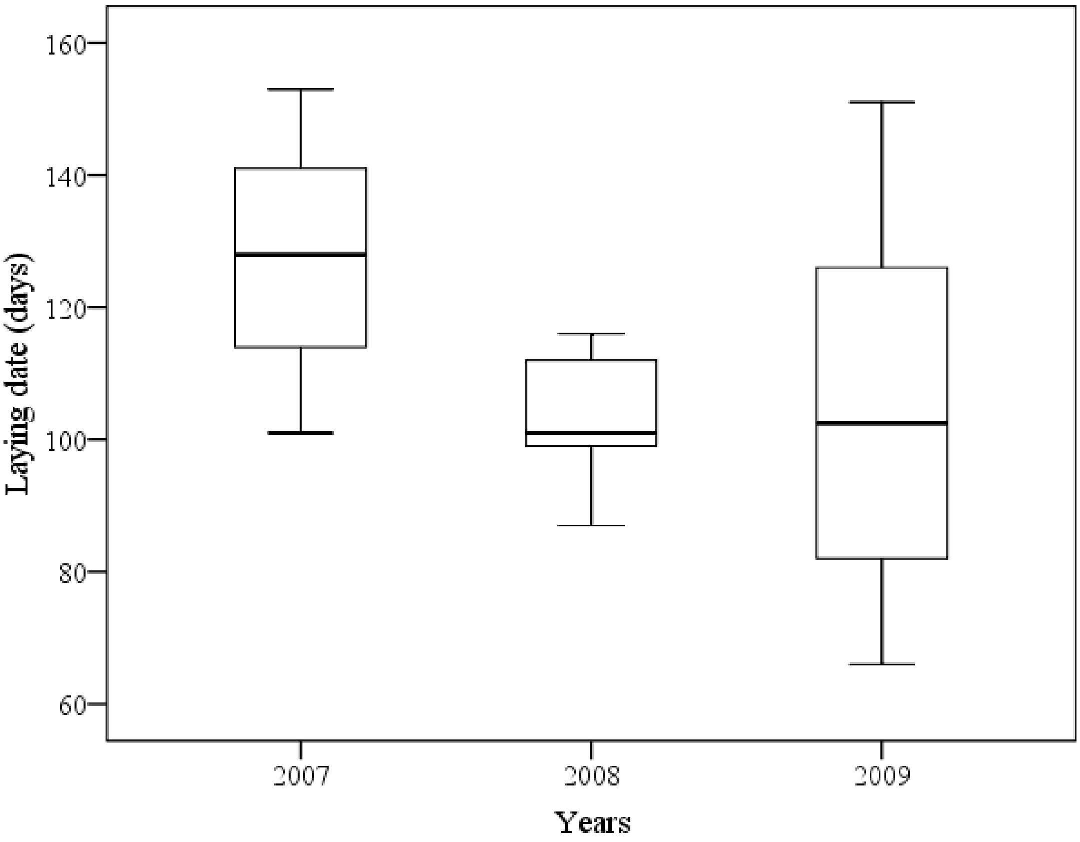

Figure 3.4 – Comparison of the laying date of campo flickers (*Colaptes campestris campestris*) between study years in Brazilian savanna.

Table 3.1 – Calculation of Hamilton's rule, rB – C > 0 for campo flickers in central Brazil.

Variable	Description	Value	
		Male	Female
r	Coefficient of relatedness	0.40	0.37
B	Benefit to dominant	1.5	0.5
C	Cost to subordinate	2.1	1.3
	rB – C	-1.5	-1.1

The calculation of Hamilton's rule revealed that the benefit (B) for male auxiliaries was estimated as 1.5 offspring per male and the cost C was equal to 2.1 offspring per male (Table 3.1). Thus, helping appears to be a costly behavior, with a net cost of helping estimated as -1.5 offspring per male. The estimated values were similar for female auxiliaries. For females, the cost was 1.3 offspring and the benefit (B) was estimated as 0.5 offspring, with a final net cost of -1.1 offspring per female (Table 1). The extra production of nestlings would have to be more than fourfold higher, on average, for the benefit of helping to outweigh the cost.

Discussion

In general, territorial and breeders' quality did not affect any of the evaluated factors associated with breeding productivity. Similar to other studies, results revealed that the presence of auxiliaries increased the number of fledglings produced, compared with unassisted groups (Doerr and Doerr 2007, but see Dunn et al 1995). However, the total number of auxiliaries did not seem to be important to assure offspring survival.

Nevertheless, when we evaluated nest success we observed that none of the evaluated factors could determine nest fate. Among other things, this means that similar to most cooperative breeders, unassisted pairs of campo flickers are able to successfully produce nestlings, although at a lower rate than assisted pairs (Pruett-Jones 2004). In the grey-crowned babbler (*Pomatostomus temporalis*), helpers effect was also associate to an increase in fledgling production but not survival (Blackmore and Heinsohn 2007).

Despite the positive effect of auxiliaries upon the number of fledglings, this does not extend to nestling condition at fledging. Auxiliaries in other species have been found to have both a positive effect on fledgling condition (e.g. white-fronted bee eaters: Emlen and Wrege 1991) as well as no effect (e.g. American crow: Caffrey 2000). Our data suggests that the additional offspring produced by assisted pairs may in fact be of comparable quality relative to the fewer offspring produced by unassisted pairs, which could be interpreted as a positive effect overall.

We found that the variable *year* had a strong effect on nestling body mass. Interestingly, a previous study at the same site indicated that 2007 was a harsh year, with a very long dry season (Chapter 1). But contrary to what could be expected in such conditions, nestlings produced in 2007 were in better condition than those of 2008, on average 11g heavier. Moreover, when evaluating laying patterns we observed that both the presence of auxiliaries and the year affected the date females started laying eggs. Assisted groups started laying earlier than unassisted groups, and laying started later in 2007 when compared to other years. Laying date in campo flickers is influenced by rainfall (Chapter 1), a link that also has been established for other birds dependent upon invertebrate prey. For example, a reduction in food availability due to a shift in the peak of insect abundance seemed to explain the onset of breeding in the cooperative azure-winged magpie (Canário et al. 2004). It is known that rainfall may directly influence invertebrate phenology (van Noordwijk et al. 1995) and abundance (Canário et al. 2002). A study in the Brazilian savanna revealed that termite abundance usually peaks in the first half of the wet season (Pinheiro et al. 2002), a pattern that could have been disrupted by the long dry season in 2007, influencing laying date and the amount of food available for the offspring. Studies of several cooperative breeders suggest that the auxiliaries' influence on reproductive success may be observed in some years but not in others, and is usually strongly noticed during harsh conditions (Hatchwell 1999; Magrath 2001). Despite considering 2007 a poor year due to the extended dry period (Chapter 1), the delay in the rains may have postponed the beginning of laying so that offspring production coincided with a higher abundance of insects later in the season, positively affecting nestling body condition. Regardless of this year's effect, we did not observe differences in the number of assisted groups between years, differently from azure-winged magpies that had more assisted nests in the poorer years (Canário et al 2004).

Several explanations have been suggested relative to possible variations in sex-ratio for offspring of cooperative breeders. Two proposals are that selection should favor the production of the helping sex, if auxiliary investment helps to repay their cost of production (Emlen et al. 1986); and that the sex-ratio of offspring should be based on the different reproductive values of male and female offspring (Clark 1978). A meta-analysis showed that a greater sex-ratio adjustment occurs in species where auxiliaries strongly influence fitness outcome (Griffin et al. 2005). Notwithstanding this, we observed that nestling sex-ratio in the campo flicker was not affected by any of the evaluated variables.

Despite the fact that auxiliaries increased productivity in campo flickers, the calculation of Hamilton's rule revealed that the cost of helping was still very high for both auxiliary sexes in comparison to the benefit, suggesting that auxiliaries may not be acquiring enough indirect benefits to support the evolution of cooperative breeding in the species based merely on kin selection. As predicted for most species (Lucas et al. 1996), the calculation of inclusive fitness was negative in campo flickers: the fitness benefits of helping are lower than the direct fitness obtained from independent breeding. Thus, breeding independently for campo flickers produces a considerable number of offspring and the presence of auxiliaries is not mandatory for successful breeding. Consequently, we would expect that cooperative breeding must occur when independent breeding is not possible (Du-Plessis et al 1995). Due to ecological constraints such as lack of territories or breeding positions, some fledglings of campo flickers may be have to stay in their natal territory and act as auxiliary, which must also be true for secondary auxiliaries that joined existing groups, both trapped in a "best-of-a-bad-job" situation (Dickinson et al. 1996). In addition to the low indirect benefits obtained by auxiliaries of campo flicker, other benefits may arise such as an increased survival (e.g. waiting at the natal site), chances of dispersal (Clutton-Brock 2002), acquisition of other skills necessary for independent survival or breeding (Komdeur 1996) or they may be signaling suitability as future breeders (Carlisle and Zahavi 1986). Alternatively, we cannot exclude the possibility that auxiliaries may be helping breeders while staying at the natal territory as a "rent payment" (Gaston 1978). Additionally, other variables associated to successful production of offspring were not evaluated in this study and we may have underestimated the benefits of indirect fitness. For instance, it was found that when the number of auxiliaries increased in the long-tailed tit (*Aegithalos caudatus*) there was elevated recruitment of offspring as breeders (Hatchwell et al. 2004).

In conclusion, we have demonstrated that the presence of auxiliaries increased reprodutive productivity in campo flickers, but that this does not compensate the direct fitness costs of not breeding. Consequently, we believe that helping behavior in campo flickers must be associated to ecological constraints (Emlen 1982) and that other traits associated to the condition of fledglings produced by either assisted and unassisted groups may be crucial in the decision of auxiliaries to disperse or help in the natal territory.

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Parental and alloparental investment in a neotropical woodpecker: effects of auxiliaries, condition and relatedness

Introduction

Parental care is a time demanding and energetically expensive behavior that involves a trade-off between the survival of the offspring and costs for the parents, such reduced fecundity and mating opportunities (Trivers 1972; Clutton-Brock 1991). The investment of parents is influenced by factors ranging from partner quality (Burley 1988; de Lope and Møller 1993; DeMory et al. 2010) to resource availability (Whittingham and Robertson 1994; Eikenaar et al 2003). Among cooperative breeders (i.e. a social system where more than a pair help rear young within a nesting attempt, Brown 1987), parental investment can be shared with other individuals, known as auxiliaries or helpers.

The investment made by auxiliaries can influence, among other factors, the provisioning of group breeders to the offspring (Heinsohn 2004). Parents can react to the presence of auxiliaries, adjusting their parental investment in at least two ways. They may reduce their parental effort, being compensated by the investment of auxiliaries and consequently maintaining the same overall provisioning rate ('compensatory effect'; Hatchwell and Russell 1996; Khan and Walters 2002; Russell et al 2008). Conversely, parents can maintain their original effort, accumulating a higher overall investment when the auxiliaries' effort is considered, but without increasing the costs associated to parental care ('additive effect'; Emlen and Wrege 1991; Magrath and Yezerinac 1997; Cockburn 1998). The latter pattern is expected in species with high chances of nest failure due to starvation where parents cannot compensate for the lack of assistantship (Hatchwell 1999, but see Legge 2000b). Another possibility is the occurrence of both patterns in the same species (Russell et al. 2008; Kingma et al. 2010; Meade et al. 2010).

However, different strategies generate different outcomes. For species in which compensatory effect occurs, a reduction in parental workload is expected (load-lightening), resulting in an increase in breeder survival (Russell and Rowley 1988; Khan and Walters 2002; Kingma et al. 2010), earlier initiation of nesting (Koenig and Stacey 1990) and reduced intervals between breeding attempts (Woxvold and Magrath 2005; Canestrari et al. 2008). On the other hand, additive care is expected to increase productivity and nestling survival rates in the current breeding attempt (Hatchwell 1999; Kingma et al. 2010; but see Legge 2000a).

Kin selection is among the most accepted explanations for the usual extensive investment of auxiliaries in rearing non-descendent young (Hamilton 1964). It is considered that helping behavior is widespread in cooperative breeders partly because auxiliaries acquire indirect fitness benefits helping kin, since most groups are formed by closely related individuals (Brown 1987; Emlen 1997; Dickinson and Hatchwell 2004). However, given the possibility that some of the offspring may not be closely related to the auxiliaries due to the mating system, extra-pair copulations or joint-nesting, auxiliaries sometimes are more prone to help relatives, or help them at higher rates, when compared to unrelated individuals (Komdeur 1994; Dickinson et al. 1996; Russell and Hatchwell 2001; Richardson et al. 2003). However, there are cases where auxiliaries may provide care regardless of their degree of relatedness to the offspring they are provisioning (Dunn et al. 1995). Thus, to maximize the benefits obtained by helping, auxiliaries are expected to adjust their investment according to their relatedness to the offspring whenever necessary. Kin discrimination has been shown to occur consistently in several species and is expected to be favored in species presenting a variable level of relatedness within the group (Griffin and West 2003; Cornwallis et al 2009). Interestingly, the mechanism leading to kin discrimination has been revealed for some species, such as the long-tailed tit (*Aegithalos caudatus*; Sharp et al. 2005).

We studied the tropical campo flicker (*Colaptes campestris campestris*), a facultative cooperative breeder with a complex social system. Cooperative groups are usually formed by kin-related individuals with a large variation in degree of relatedness among group members, especially relative to auxiliary females (Chapter 2). We asked whether breeder's care patterns reflected parameters of the group, measured using the following variables: presence or absence of auxiliaries, parents' condition, number of nestlings and the sex of nest attendants.

Additionally, we investigated how parents modulated their investment relative to the investment of auxiliaries. Finally, we evaluated if auxiliaries adjusted their investment in accordance to their relatedness to the offspring and analyzed the effect of the overall effort on group productivity and other breeding parameters.

Methods

Study species

Campo Flickers (*Colaptes campestris campestris*) are medium-sized woodpeckers widely distributed in South America (Short 1972). The subspecies is a facultative cooperative breeder with a variable mating system, with both monogamous and polygynous groups, and mixed mating strategies that include joint-nesting behavior and intra-specific brood parasitism (Chapter 2). Both parents and auxiliaries contribute to the nest during incubation and nestling feeding (Chapter 1).

Study area and general procedures

We conducted the study at Fazenda Água Limpa (FAL; 15°56'S, 47°55'W), an area of 4,500 ha belonging to the University of Brasília. The area is located in central Brazil within the Cerrado biome. The region is highly seasonal with distinct dry and wet seasons. Field work was conducted through four years (2006-2009), which included three breeding seasons.

We used playbacks and mist nets to attract and capture the woodpeckers, which were then banded, measured to the nearest 0.02mm (tarsus, wing, beak and tail length) and weighed to the nearest gram. Additionally, we sampled 100µl of blood from each captured individual. We monitored the groups year-round and identified potential nesting cavities within the study area, recording their location with a GPS. We checked cavity content using a flashlight and a mirror attached to a pole. Active nests were monitored every 2-3 days. Nestlings close to fledging (25th day after hatching) were measured, banded and blood sampled similarly to adults.

Group identity was assessed frequently through the study period. There was only one case where a group underwent a drastic change in composition with the dispersal of some previous members and immigration of new ones, and was subsequently classified as a new cooperative group. Alterations in socially monogamous pairs occurred more frequently (N = 4), whenever one of the pair members changed, and these were classified as new pairs. On the other hand, those groups that changed from cooperative groups to socially monogamous pairs but without changing composition (replacement of one of the previous members) were treated as not different groups (see statistical analysis section). We classified the auxiliaries as: (1) male or female primary auxiliaries, that were usually young from the previous breeding season and did not reproduce; and (2) secondary auxiliaries, which were subordinate females that copulated with the breeding males and contributed a few eggs to the nest.

Parental and alloparental behavior

During the incubation and nestling phases we carried out direct observations from a blind (approximately 20-30 m from nests) using binoculars or a spotting scope, but some observations were made using video cameras (Sony DCR-HC52). During incubation, we recorded nest activity for each nest during one hour (2008 and 2009) on the fifth and tenth days after clutch completion. During the nestling period, we recorded nest activity (from 1h40 min to 2 h) during five different days for each nest (days 4, 10, 16, 22 and 28 after hatching). For all observations, we recorded the identification of the visiting adult, the number of visits performed and the time spent inside the cavity.

Molecular analysis

The molecular analyses to determine parentage among group members are detailed elsewhere (Chapter 2). Succinctly, we stored blood samples in Lysis buffer and extracted the DNA using Quiagen DNeasy Blood and Tissue Kit®. We used 10 polymorphic microsatellite markers, originally isolated from the northern flicker (*Colaptes auratus*, Kuhn et al. 2009), in a multiplex polymerase chain reaction (PCR) and visualized the amplification products on the

Applied Biosystems (ABI) 3100 automated capillary sequencer. We used GeneMapper® (version 4.1; Applied Biosystems) to evaluate the electropherograms. For the parentage analysis we used the program CERVUS version 3.0.3 (Marshall et al. 1998; Kalinowski et al. 2007) and for the pairwise relatedness (r) estimates we used the software SPAGeDi (Hardy and Vekemans 2002), based on Queller and Goodnight's (1989) formula.

Statistical analysis

All statistical analyses were performed with the free software R and models were implemented using the "lmer" function in the "lme4" package (R 2.11.1; R Development Core Team 2010). GLMM models were fitted for binomial and count response variables considering binomial and Poisson distributions, respectively. Linear mixed models for continuous response variables with Gaussian distribution were also used. We fitted random factors in the model to correct for hierarchical repeated sampling. When considering investment during the nestling period, all models incorporated the random terms "Group ID", "Individual ID" and "Days after hatching". For the models concerning investment during incubation we only incorporated the random terms "Group ID" and "Individual ID" because we averaged the results of the two focal observations. When we evaluated the effect of provisioning on breeding parameters we only incorporated the random terms "Group ID" and "Days after hatching" because we considered the whole group investment in those analyses. For the analysis concerning the nestling period we used the number of nestlings as a factor to control for possible differences in investment for different clutch sizes. We used the residuals of the regression between body mass and tarsus as an index of body condition for the genetic parents. The models were progressively simplified by removing variables starting with higher level interactions. We used likelihood ratio tests (LRTs) using the change in deviance as a chi-square approximation. The model simplification was retained if the simpler model did not differ in terms of fit. Results are shown as mean ± standard error.

Results

During incubation the frequency of hourly nest visits by genetic parents was not affected by their own condition (χ^2_1 =0.93; P = 0.333), sex (χ^2_1 = 2.16; P = 0.141) or the presence of auxiliaries (χ^2_1 = 0.05; P = 0.827). Neither condition (χ^2_1 = 2.01; P = 0.155) nor the presence of auxiliaries (χ^2_1 = 2.37; P = 0.123) had an impact upon time spent by the genetic parents at the nest, but we found a significant effect of breeder sex upon time spent at the nest (χ^2_1 = 7.25; P = 0.007), with breeding females spending more time inside the nest during daily incubation than breeding males. On the other hand, during the nestling period, we observed that both parents' condition (χ^2_1 = 5.51; P = 0.018; Fig. 4.1) and the number of nestlings (χ^2_1 = 14.63; $P < 0.001$) significantly affected parental provisioning rate. Surprisingly, there was a negative relationship between frequency of nest visits and brood size, but we found no effect of the breeders's sex (χ^2_1 = 2.23; P = 0.135) or the presence of auxiliaries (χ^2_1 = 1.51; P = 0.218) upon parents provisioning. Regarding the time spent at the nest during provisioning we found a near significant effect of condition (χ^2_1 = 3.58; P = 0.058) and a strong effect for the number of nestlings (χ^2_1 = 9.13; P = 0.002). But similarly to the number of visits to the nest, we found no significant effect of breeders' sex (χ^2_1 = 0.00; P = 0.982) and presence of auxiliaries (χ^2_1 = 0.12; P = 0.720). Despite this lack of difference, non-assisted pairs made 35% more visits to the nest and spent 25% more time inside the nest than assisted pairs (Fig. 4.2).

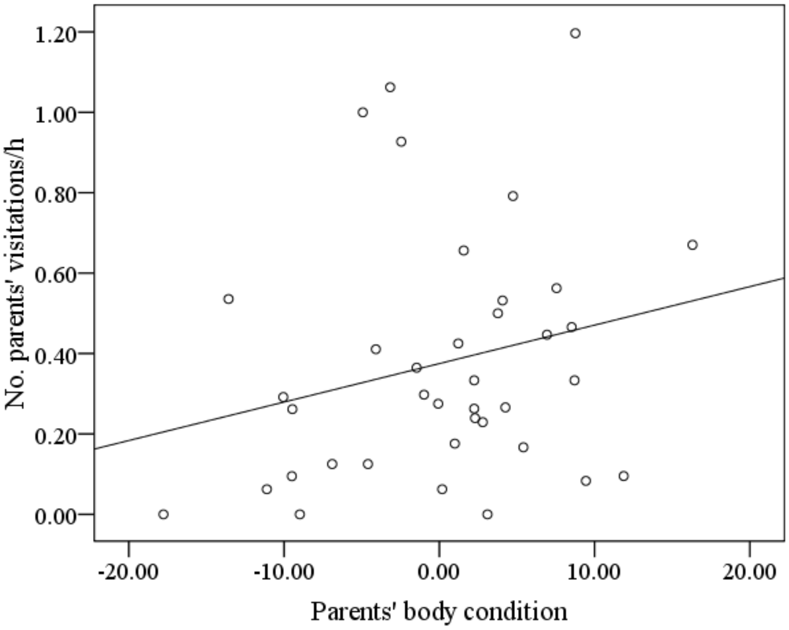

Figure 4.1 – Effect of body condition of breeders on nest visitation rate during the nestling period in campo flickers (*Colaptes campestris campestris*) in central Brazil.

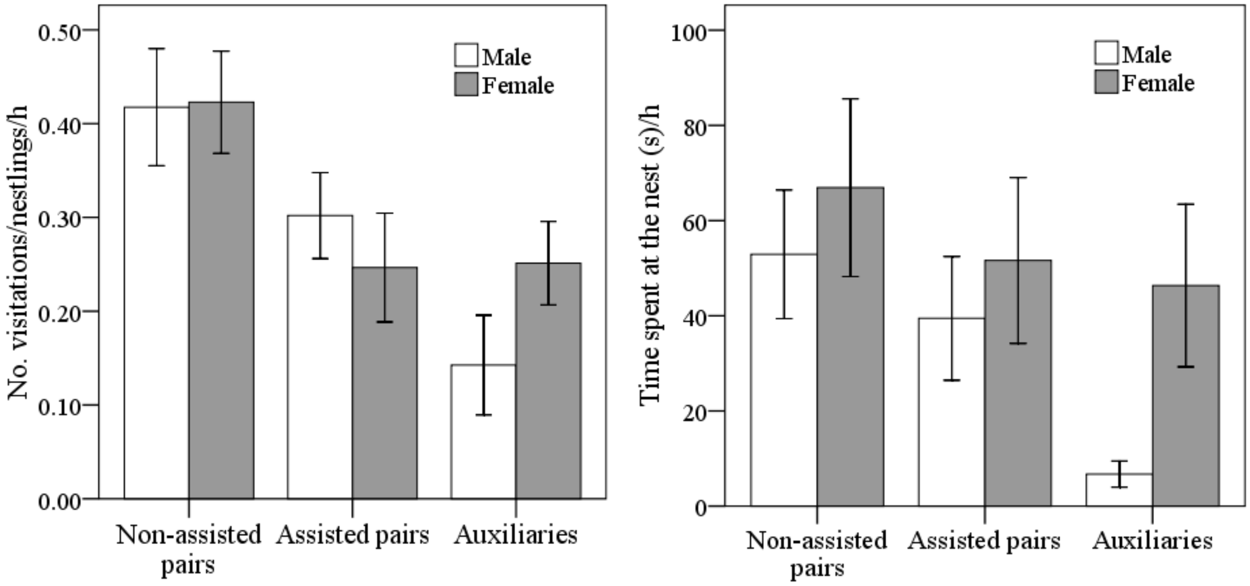

Figure 4.2 – Differences in nest visitation rate and time spent at the nest by breeders and auxiliaries in relation to sex and occurrence of helping behavior during the nestling period in campo flickers (*Colaptes campestris campestris*) in central Brazil.

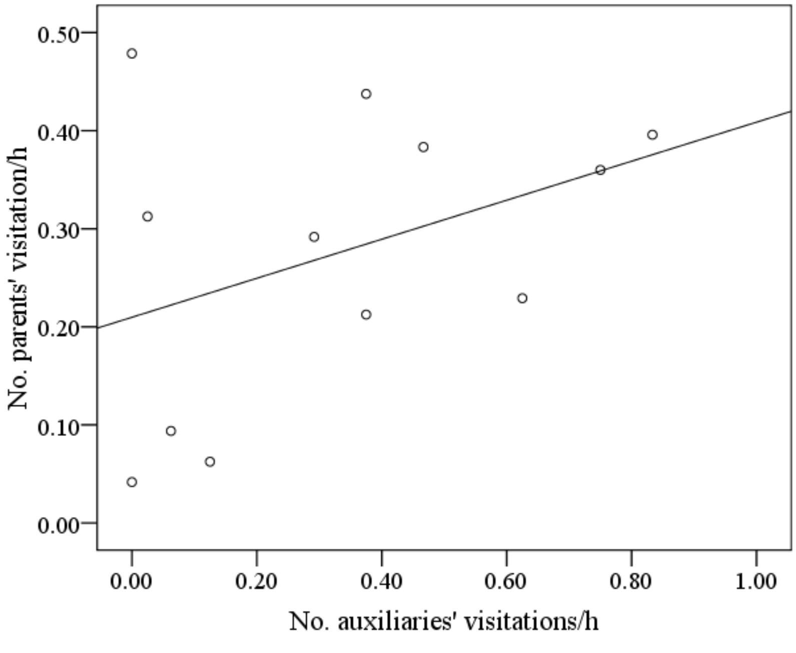

Figure 4.3 – Nest visitation rate by auxiliaries in relation to nest visitation rate of breeders during the nestling period in campo flickers (*Colaptes campestris campestris*) in central Brazil.

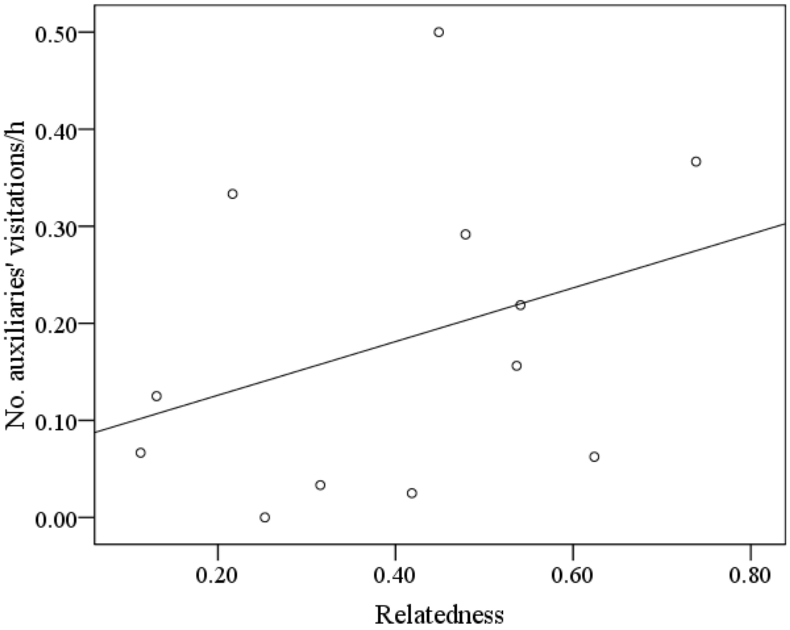

Figure 4.4 – Level of relatedness between auxiliaries and brood relative to auxiliary nest visitation rate during the nestling period in campo flickers (*Colaptes campestris campestris*) in central Brazil.

When we restricted our analyses to cooperative groups we observed that the number of hourly visits by parents during the nestling period was affected by the number of visits by auxiliaries (χ^2_1 = 10.82; P = 0.001; Fig. 4.3), but was not affected by the number of nestlings (χ^2_1 = 2.66; P = 0.102). However, neither the time spent at the nest by auxiliaries (χ^2_1 = 0.57; P = 0.449) nor the number of nestlings (χ^2_1 = 0.55; P = 0.457) affected the time the parents spent at the nest.

Concerning the investment made by auxiliaries, we observed that the number of their visits to the nest was directly and positively influenced by both their level of relatedness to the offspring (χ^2_1 = 4.79; P = 0.028; Fig. 4.4) as well as to the number of nestlings (χ^2_1 = 5.81; P = 0.016), but in this case the relationship between visitation rate and the number of nestlings was positive. However, the type of auxiliary (i.e. primary or secondary; χ^2_1 = 0.05; P = 0.822), their condition (χ^2_1 = 0.08; P = 0.775) or their sex (χ^2_1 = 0.03; P = 0.851) do not seem to be important regarding the investment made by auxiliaries. On the other hand, none of the response variables were considered important to affect the time the auxiliaries spent at the nest as the level of relatedness to the offspring (χ^2_1 = 0.68; P = 0.406), number of nestlings (χ^2_1 = 0.08; P = 0.767), type of auxiliary (χ^2_1 = 0.78; P = 0.375), condition (χ^2_1 = 0.84; P = 0.358) and sex (χ^2_1 = 1.75; P = 0.185).

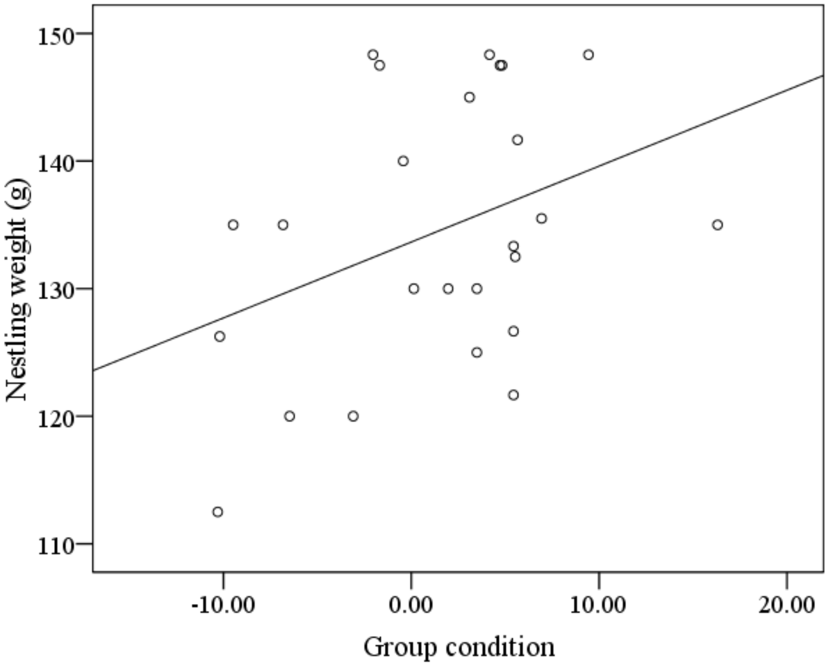

Figure 4.5 – Overall group condition relative to nestling weight at day 25th in campo flickers (*Colaptes campestris campestris*) in central Brazil.

We considered whether overall investment in a nest could influence the number of nestlings produced. Results show that average group condition (χ^2_1 = 0.58; P = 0.443), total frequency of nest visits (χ^2_1 =2.75; P = 0.09), and total time spent at the nest (χ^2_1 = 0.48; P = 0.486) did not affect the number of nestlings produced. Similarly, nest success was not affected by average group condition (χ^2_1 = 0.02; P = 0.879), total frequency of nest visits (χ^2_1 = 0.28; P = 0.590), or total time spent at the nest (χ^2_1 = 0.29; P = 0.590). Interestingly, we observed that fledgling weight was affected by the average condition of group members (χ^2_1 = 11.80; P < 0.001; Fig. 4.5), but not by the total frequency of nest visits (χ^2_1 = 1.27; P = 0.258) or the total time spent at the nest (χ^2_1 = 0.43; P = 0.512).

Finally, we observed that the total frequency of nest visits was not significantly affected by the presence of helpers at the nest ($\chi^2_1 = 0.27$; $P = 0.598$), but it was negatively affected by the number of nestlings ($\chi^2_1 = 13.35$; $P < 0.001$). The same was observed for the total time spent at the nest. Assisted groups did not spent more time as a whole at the nest than did unassisted groups ($\chi^2_1 = 0.87$; $P = 0.349$). However, we did observe again an effect of the number of fledglings ($\chi^2_1 = 5.14$; $P = 0.023$).

Discussion

Results revealed that none of the evaluated variables affected parental visitation rate during incubation. However, we observed that breeding females spent more time at the nest during daily incubation than did breeding males. Conversely, during the nestling period we observed a significant effect of parents' condition and the number of nestlings on the breeders' provisioning rate. Thus, campo flicker breeders in better condition presented elevated provisioning rates but a higher number of nestlings associated with decreased visiting rate. It has been found in some other species that the parents' condition often influences nestling survival, sex-ratio (Nager et al 2000) and nestling condition (Parker 2002). A similar result was observed when we evaluated the time spent at the nest by the breeders, which was marginally and positively influenced by the breeders' condition but negatively affected by the number of nestlings. The negative effect of number of nestlings upon both provisioning rate and time spent at the nest by the breeders must be related, among other reasons, to the fact that larger clutch sizes were predominantly found in cooperative groups (Chapter 1) for which we observed a considerable reduction in parental investment. Another possibility may be associated to variation in the amount of food transported by feeders in their crops. A study of the congener northern flicker (*Colaptes auratus*) revealed that larger broods induced an increase in the foraging time of feeders, but did not influence their visitation rate to the nest (Wiebe and Elchuk 2003).

The presence of auxiliaries does not seem to directly affect the breeders' parental investment, although non-assisted pairs invested considerable more than assisted pairs during the nestling period. The presence of auxiliaries may have an additive effect upon total provisioning of nestlings, and thus may not directly affect the provisioning effort of the breeders (Emlen and

Wrege 1991; Magrath and Yezerinac 1997). Alternatively, assisted breeders may reduce their own provisioning effort, but not the overall provisioning rate, because the investment made by the auxiliaries may compensate the reduction in care by the parents (compensatory) (Brown et al. 1978; Legge 2000b; Khan and Walters 2002). The latter is expected to occur especially in species where parental care is very costly (e.g. Reyer and Westerterp 1985). Studies have demonstrated that the occurrence of both effects in the same species it is also a possibility (Kingma et al. 2010; Meade et al. 2010). This seems to be the case for campo flickers. Although we did not observe a statistical effect of helping behavior on parents' provisioning levels we noticed that assisted breeders reduced their effort by 25-35% in comparison to non-assisted breeders similarly to Kingma et al. (2010). Additionally, the investment made by the auxiliaries was high, and comparable to the provisioning of breeders, as in Lloyd et al. (2009), especially when considering the females. Surprisingly, we observed that the overall number of visits to the nest and the total time spent at the nest were not statistically affected by the presence of auxiliaries, despite the fact that the overall contribution was apparently higher in cooperative groups.

Adjustments in the overall provisioning rate of offspring is known to directly affect several life-history aspects of avian breeders, such as increasing the number of breeding attempts per season (Russell and Rowley 1988) and adult survival until the next breeding season (Koenig and Mumme 1987), among others. A recent study of the purple-crowned fairy-wren (*Malurus coronatus*) revealed that auxiliaries contributed to the reduction of breeders' workload favoring higher breeder survival (Kingma et al. 2010). Campo flickers have an extended lifespan, and in the present study it was not possible to evaluate the effects of auxiliaries' effort on breeders' long-term survival.

Several studies have shown that the presence of auxiliaries can affect the investment level of breeders (Hatchwell 1999). However, only a few addressed the direct effects of the investment by auxiliaries' upon the investment made by breeders. When we evaluated how parents modulate their provisioning in relation to the provisioning made by auxiliaries, we found that the number of visits by parents during the nestling period was positively affected by the number of visits by auxiliaries. This result reinforces the conclusion that parents may attempt to increase overall investment and maybe productivity in favorable situations. Another important aspect that must be remembered in this situation is that campo flickers have high rates of nestling loss due to starvation (Chapter 1), a condition possibly associated with the occurrence of additive care (Hatchwell 1999).

Interestingly, the investment provided by auxiliaries measured as visitation rate was directly affected not only by their level of relatedness to the offspring, but also to the number of nestlings. However, the auxiliary condition or type (i.e. offspring sibling or subordinate female breeder) were not important to predict investment. Cooperative breeders generally evolve within kin-based circumstances, and auxiliaries are usually offspring from previous years (Brown 1987). This situation seems to favor kin-selected benefits, however, in some specific cases the strength of kin selection seems to be even higher, so that auxiliaries preferentially assist close kin or assist them at a higher rate (Mumme 1992; Komdeur 1994; Dickinson et al. 1996; Russell and Hatchwell 2001; Richardson et al. 2003; but see, Legge 2000b; Canestrari et al. 2005). Recent studies with the long-tailed tit (*Aegithalos caudatus*) and bell miner (*Manorina melanophrys*) indicated that the investment made by auxiliaries was affected by fine-scaled differences in relatedness, and was higher in broods to which they were related (Nam et al. 2010; Wright et al. 2010). Kin discrimination is likely to occur in species with a variable relatedness component within groups (Cornwallis et al. 2009). Molecular analyses point to such variability in relatedness for the campo flicker (Chapter 2), and we can thus expect some sort of kin discrimination mechanism to have developed in the species.

Based on results of previous studies (Doerr and Doerr 2007; Kingma et al. 2010) we expected to find that the overall investment per nest would be linked to the number of nestlings produced or nesting success. Our results show that campo flickers do not exhibit a similar pattern, since overall investment had no effect on nest productivity or nest fate. However, previous analyses controlling for individual and territory quality have shown that the presence of auxiliaries itself enhances fledgling production (Chapter 3). This result suggested that the observed enhancement in productivity may be associated to different factors related with the presence of auxiliaries, other than provisioning rate. Another possibility is that the non-significant difference in the higher overall investment of cooperative groups is indeed relevant because auxiliaries may be bringing more food in their crops, which could be enough to affect productivity without necessarily increase the levels of provisioning. Surprisingly, fledgling weight was affected by the average condition of group members, but was not affected to the overall nest provisioning (Hatchwell et al. 2004; Lloyd et al. 2009). This result suggests that the condition of the produced fledglings was directly related to the quality of the group that reared the fledglings.

In conclusion, this study demonstrated that the condition of group members may be even more important than the presence of auxiliaries to determine the provisioning rates of the breeders and condition of the fledglings. Moreover, similar to other species, campo flickers seem to present both additive and compensatory effects in their nesting effort, with breeders reducing their investment but presenting a higher overall investment per nest of cooperative groups when compared with unassisted pairs. Although the comparison between cooperative groups and unassisted pairs did not produce statistically significant results, the difference in provisioning seems biologically significant. The relevance of variation in parental investment and total provisioning levels to several life-history traits, such as breeders' survival and fledglings' ability to find a mate, for example, still needs to be investigated. In addition, this study demonstrated that auxiliaries adjusted their provision based on differences in the levels of relatedness, investing more in nests where they had higher relatedness to the nestlings, suggesting that kin selection in the campo flicker may be more important than originally suspected (Chapter 3).

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