Universidade de Brasília
Instituto de Biologia
Departamento de Ecologia

IDENTIFICAÇÃO MATERNAL DE OVOS E ASSIMETRIA REPRODUTIVA EM POSTURAS COMUNITÁRIAS DE *Guira guira* (AVES, CUCULIDAE)

Mariana Otero Cariello

Tese apresentada ao Departamento de Ecologia da Universidade de Brasília como requisito para a obtenção do grau de Doutor em Ecologia.

Brasília – 2002

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Tese apresentada como requisito à obtenção do grau de Doutor em Ecologia pelo Departamento de Ecologia, Instituto de Biologia, Universidade de Brasília – UnB, à comissão formada pelos professores:

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Brasília, 16 de agosto de 2002

O ÔVO DE GALINHA

João Cabral de Melo Neto

§ Ao ôlho mostra a integridade
de uma coisa num bloco, um ôvo
Numa só matéria, unitária,
maciçamente ôvo, num todo

Sem possuir um dentro e um fora,
tal como as pedras, sem miolo:
é só miolo: o dentro e o fora
integralmente no contôrno.

No entanto, se ao ôlho se mostra
unânime em si mesmo, um ôvo,
a mão que o sopesa descobre
que nele há algo suspeitoso:

que seu pêso não é o das pedras,
inanimado, frio, gôro;
que o seu é um peso môrno, túmido,
um pêso que é vivo e não morto.

§ O ôvo revela o acabamento
a toda mão que o acaricia,
daquelas coisas torneadas
num trabalho de toda a vida.

E que se encontra também noutras
que entretanto mão não fabrica:
nos corais, nos seixos rolados
e em tantas coisas esculpidas

cujas formas simples são obra
de mil inacabáveis lixas
usadas por mãos escultoras
escondidas na água, na brisa.

No entretanto, o ôvo, e apesar
da pura forma concluída,
não se situa no final:
está no ponto de partida.

§ A presença de qualquer ôvo,
até se a mão não lhe faz nada,
possui o dom de provocar
certa reserva em qualquer sala.

O que é difícil de entender
se se pensa na forma clara
que tem um ôvo, e na franqueza
de sua parede caiada.

A reserva que um ôvo inspira
é de espécie bastante rara:
é a que se sente ante um revólver
e não se sente ante uma bala.

É a que se sente ante essas coisas
que conservando outras guardadas
ameaçam mais com disparar
do que com a coisa que disparam.

§ Na manipulação de um ôvo
um ritual sempre se observa:
há um jeito recolhido e meio
religioso em quem o leva.

Se pode pretender que o jeito
de quem qualquer ôvo carrega
vem da atenção normal de quem
conduz uma coisa repleta.

O ôvo porém está fechado
na sua arquitetura hermética
e quem o carrega, sabendo-o
prossegue na atitude regra:

procede ainda da maneira
entre medrosa e circunspecta,
quase beata, de quem tem
nas mãos a chama de uma vela.

Dedico essa tese ao meu avô Cariello e
à minha avó Anileda,

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FORMATAÇÃO

Essa tese apresenta a formatação exigida pelo Programa de Pós-graduação em Ecologia da Universidade de Brasília para teses cujos capítulos já foram submetidos para publicação em periódicos de circulação internacional.

Desse modo, cada capítulo dessa tese está escrito em língua inglesa e formatado de acordo com as normas do periódico para o qual foi submetido. A saber:

(a) Capítulo I submetido ao periódico Molecular Ecology;

(b) Capítulo II submetido ao periódico Journal of Avian Biology; e

(c) Capítulo III submetido ao periódico Behavioral Ecology and Sociobiology.

Adicionalmente, anterior aos capítulos, essa tese apresenta, em língua portuguesa, uma introdução geral sobre o comportamento reprodutivo da espécie estudada, uma seção para cada capítulo (que resume sua introdução e sua conclusão) e uma bibliografia geral.

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Material and Methods

Methods

Mariana Otero Cariello

IDENTIFICAÇÃO MATERNAL DE OVOS E ASSIMETRIA REPRODUTIVA EM POSTURAS COMUNITÁRIAS DE *Guira guira* (CUCULIDAE, AVES)

Aspectos Gerais do Comportamento Reprodutivo do Anu Branco (*Guira guira*)

Desde a última década, um estudo de longo-prazo sobre as estratégias reprodutivas da espécie comunitária *Guira guira* (Cuculidae, Aves), popularmente conhecida como anu branco, tem sido desenvolvido no Brasil Central (Macedo 1992, 1994; Quinn *et al.* 1994; Macedo & Bianchi 1997 a,b; Melo & Macedo 1997; Macedo & Melo 1999; Macedo *et al.* 2001). Nessa região, a reprodução da espécie ocorre principalmente durante a estação chuvosa, que se estende de meados de agosto a meados de março, sendo que alguns grupos podem se reproduzir até cinco vezes durante uma única estação reprodutiva (Melo 1997). Seus padrões de postura e destruição de ovos assemelham-se àqueles observados em outras espécies da subfamília Crotophaginae, que incluem *Crotophaga ani, C. major* e *C. sulcirostris* (Davis 1942; Vehrencamp 1977; Sick 1997). Os grupos reprodutivos de anu branco são compostos por dois a 15 adultos (moda = 6), que podem permanecer no mesmo território por, no mínimo, quatro anos (RH Macedo, com. pess. 2002). Tipicamente, os adultos de cada grupo constróem um ninho comunitário, onde as fêmeas reprodutivas colocam seus ovos. Tais ninhos podem conter de um a 26 ovos, que pesam, em média, 25.3 g (aproximadamente, 16% do peso do adulto), com comprimento e largura médios de 42.5 mm e 31.8 mm, respectivamente (Macedo 1992). Os ovos são azul turquesa, com um padrão variável de manchas calcáreas sobrepostas à casca, que se assemelham a uma malha de rede. Tais manchas possuem considerável espessura e podem ser removidas através de raspagem. Durante a postura, até quatro ovos podem ser colocados no mesmo dia em um ninho comunitário.

Embora a reprodução comunitária em *G. guira* integre aspectos de cooperação, que incluem a construção do ninho, o ataque a predadores e a alimentação dos filhotes, é a competição entre os indivíduos adultos, caracterizada pela destruição de ovos e filhotes, que a torna particularmente interessante para o estudo do comportamento animal. Considerando-se todas as ninhadas comunitárias monitoradas até o momento, houve ejeção de ovos em 72% (*n* = 176) delas (com médias de, aproximadamente, oito ovos colocados/ninhada e quatro ovos ejetados por/ninhada). Em 80% (*n* = 75) dos ninhos em que houve eclosão de filhotes, ocorreu redução de ninhada. No mínimo, 50% de tal redução foi devido ao infanticídio (Macedo & Melo 1999).

Um estudo genético de uma amostra limitada de quatro grupos reprodutivos mostrou que o parentesco entre os membros do grupo é, geralmente, baixo e bastante variável (Quinn *et al.* 1994). Dentre os 36 adultos presentes nos grupos, 21 (58%) foram amostrados e, dentre os 46 possíveis pares de combinações dentro de tais grupos, apenas seis (13%) apresentaram um coeficiente de similaridade (*D*) considerado suficiente para a caracterização de um alto grau de parentesco entre os indivíduos. Adicionalmente, tal estudo revelou que as oportunidades reprodutivas nos grupos não são equivalentes para todos os membros, porque alguns indivíduos podem não se reproduzir em algumas nidificações. Outro estudo revelou que os grupos de *G. guira* não sofrem constrangimentos ecológicos, ou seja, há espaços vazios de alta qualidade disponíveis para reprodução, caso indivíduos abandonem o grupo (Macedo & Bianchi 1997a). Estudos futuros são necessários para uma melhor compreensão das vantagens da reprodução comunitária em *G. guira*, a despeito do baixo parentesco entre os membros do grupo, da ausência de saturação do ambiente e dos conflitos reprodutivos caracterizados pela destruição de ovos e filhotes.

Seção I

Identificação Maternal de Ovos em Posturas Comunitárias de Anu Branco (*Guira guira*) Através de Análises Eletroforéticas de Proteínas da Gema

Em ninhos de aves onde há duas ou mais fêmeas reprodutivas, é fundamental identificar a maternidade dos ovos colocados para que seja possível compreender as estratégias de tais fêmeas, bem como seus subseqüentes benefícios reprodutivos. Tais situações ocorrem em sistemas sociais complexos, como espécies com reprodução comunitária (Craig 1979; Koenig 1981; Brown 1987; Vehrencamp *et al.* 1986; Brown & Brown 1990; Macedo 1992) ou em casos de parasitismo de ninho intraespecífico (Andersson & Eriksson 1982; Brown 1984; Mϕller 1987). No sistema de reprodução comunitária, três ou mais indivíduos se reproduzem usando, às vezes, o mesmo ninho (veja Brown 1987 para terminologia alternativa) e a participação de ajudantes pode incluir tanto adultos aparentados quanto não aparentados aos ninhegos (filhotes de ninhadas passadas que permaneceram no território). Em situações de parasitismo intraespecífico, fêmeas colocam ovos em ninhos de coespecíficos, sem o cuidado parental de seus ovos e filhotes. As fêmeas hospedeiras podem ter dificuldade em discriminar seus ovos daqueles colocados por fêmeas parasitas e, desse modo, em alguns casos, tais ovos são aceitos, enquanto que, em outros, rejeitados (Yom-Tov 1980b; Lahti & Lahti *in press*).

Alguns estudos de parasitismo intraspecífico identificaram a maternidade de ovos postos em um único ninho através de análises eletroforéticas de tecidos embriônicos (Gowaty & Karlin 1984; Wrege & Emlen 1987; Brown & Brown 1988; McKitrick 1990). Entretanto, a amostragem de tais tecidos pode subestimar a contribuição de cada fêmea no ninho, quando: (1) ocorrer redução de ninhada (como predação ou ejeção de ovos) antes do início da incubação; e (2) a captura de adultos da espécie estudada for difícil ou insuficiente, já que tais análises requerem amostragem parental para comparação de resultados. Em tais situações, o uso de ovos recém-colocados (ao invés de tecidos do embrião) pode ser uma alternativa útil para determinar a maternidade de ninhadas individuais (Smyth *et al.* 1993; Andersson & Åhlund 2001). A amostragem de ovos nos estágios iniciais da postura reduz a perda de informação devido à redução de ninhada. Adicionalmente, não é necessária a captura das fêmeas, porque as proteínas dos ovos não incubados são exclusivamente de origem maternal, ou seja, qualquer polimorfismo reflete o genótipo da fêmea (Manwell & Baker 1975).

Análises de alozimas da clara dos ovos foram usadas em estudos de parasitismo intraespecífico para diferenciar entre ninhadas de fêmeas hospedeiras e parasitas (Fleischer *et al.* 1985, Kendra *et al.* 1988, Smyth *et al.* 1993). Em tais estudos, houve a separação de produtos enzimáticos de diferentes alelos em um único *locus* (alozimas) baseada em suas cargas elétricas, sendo que alguns alelos produziram polimorfismo detectável, que permitiu a identificação de mais de uma fêmea reprodutiva em um único ninho. Tal técnica também pode usar amostras de gemas (Fleischer 1985, Fleischer & Smith 1992). Entretanto, como as alozimas podem não mostrar suficiente polimorfismo entre as fêmeas, porque diferentes fêmeas podem possuir os mesmos alelos, tais análises têm sido utilizadas, preferencialmente, para a exclusão de maternidade e não para uma precisa identificação maternal.

A despeito de sua potencial importância para o estudo de sucesso reprodutivo e estratégia de fêmeas em aves, as análises de eletroforese de proteínas da clara ou da gema nunca foram usadas especificamente para a identificação de posturas individuais em ninhos de espécies comunitárias. Como as análises de alozimas podem apresentar as limitações acima descritas, novos métodos usando proteínas totais são mais indicados para estudos dessa natureza, porque eles testam diferenças entre todas as proteínas presentes na amostra. Recentemente, Andersson e Åhlund (2001) desenvolveram uma técnica muito acurada, denominada "protein fingerprinting" para determinar a maternidade de ovos de hospedeiros e parasitas em ninhos do pato *Bucephala clangula*. Através de tal técnica, proteínas totais da clara foram separadas de acordo com sua carga em um campo elétrico aplicado sobre um gradiente de pH estável no gel, sendo que as amostras produziram um rico padrão de bandas de proteínas da clara, bastante variável entre as fêmeas.

No estudo representado por essa seção, uma nova técnica de análise de eletroforese de proteínas totais da gema foi empregada para determinar a origem maternal de ovos em posturas comunitárias do anu branco (*Guira guira*). Nessa espécie, é impossível determinar o número de fêmeas reprodutivas em cada grupo a partir de observações de campo, porque os adultos são sexualmente monocromáticos e monomórficos (Sick 1997). Adicionalmente, considerando-se que nem todos os membros do grupo se reproduzem, o número de fêmeas presentes em um grupo pode não refletir o número real de fêmeas reprodutivas. Outras dificuldades incluem (Macedo 1992): (1) a dificuldade de captura dos adultos em armadilhas, porque estes aprendem a evitá-las; (2) a assimetria sexual na captura, que favorece machos; e (3) a predominância de ejeção de ovos no início da postura (ou seja, antes da incubação), que reduz a amostragem de tecidos dos embriões. Os objetivos específicos desse trabalho foram: (1) determinar o número de fêmeas reprodutivas em ninhos comunitários de anu branco; (2) verificar o padrão de postura dentro dos ninhos; e (3) verificar se fêmeas colocam ovos em sucessivas nidificações do grupo. Para aumentar a resolução das diferenças entre os indivíduos nas análises, os extratos de proteínas foram digeridos com protease, semelhantemente ao uso de enzimas de restrição em métodos de DNA para a resolução de diferenças genéticas.

A validação da técnica de eletroforese de proteínas para a identificação maternal de ovos de anu branco incluiu análises de proteínas da gema em ninhadas de fêmeas de periquito australiano (*Melopsittacus undulatus*) mantidas isoladas em cativeiro e em oócitos de fêmeas de anu branco, além de aspectos biológicos. Cada fêmea de periquito ou de anu branco previamente conhecida apresentou um padrão particular de bandas de proteínas da gema, distinto das demais fêmeas da sua espécie. Tais resultados mostraram que, além dessa técnica ser bastante acurada para ser utilizada na determinação maternal dessas espécies, também poderia ser testada em outras espécies. Outras corroborações envolveram a observação cuidadosa dos padrões de bandas dentro e entre ninhos dos grupos silvestres de anu branco, que mostraram que: (1) todos os múltiplos ovos colocados em um único ninho num mesmo dia apresentaram diferentes padrões de bandas de proteínas, o que praticamente elimina a possibilidade de uma ou mais fêmeas terem sido representadas pelo mesmo padrão de bandas; (2) os ovos que apresentaram o mesmo padrão de bandas de proteínas não foram colocados no mesmo dia, mas em dias distintos, o que indica que algumas fêmeas colocaram ovos em dias sucessivos na postura comunitária. Ambos os resultados condizem com as limitações fisiológicas das aves de colocarem apenas um ovo por dia (van Tienhoven 1983).

Nem todas as fêmeas reprodutivas de anu branco contribuem para todas as nidificações de seus grupos e algumas colocam mais ovos que outras nas diferentes posturas comunitárias. Isso difere do que foi encontrado para fêmeas de anu *Crotophaga sulcirostris*, outra espécie de Crotophaginae com reprodução comunitária, onde as fêmeas do grupo contribuem para todas as nidificações (Vehrencamp 1977).

Em conclusão, a técnica de eletroforese de proteínas da gema, proposta por este trabalho, é, atualmente, o melhor método para a identificação da maternidade de ovos em ninhadas comunitárias de anu branco. Tal técnica pode elucidar benefícios reprodutivos individuais e estratégias de fêmeas em outras espécies de reprodução comunitária ou poligínica, ou em casos de parasitismo intraespecifico, especialmente se o uso de análises genéticas for restrito.

Seção II

Avaliação do Uso de Características dos Ovos Para a Identificação Maternal em Posturas Comunitárias de Anu Branco (*Guira guira*)

Em alguns complexos sistemas de reprodução em aves, como os comunitários ou polígínicos (Vehrencamp 1977; Bertram 1979; Craig 1979; Koenig 1981; Brown 1987; Brown & Brown 1990; Macedo 1992), ou em casos de parasitismo intraespecífico de ninho (Yom-Tov 1980b; Andersson & Eriksson 1982; Brown 1984; Mϕller 1987), mais de uma fêmea da mesma espécie pode colocar ovos em um único ninho. Em tais situações, a identificação maternal de ovos é importante para a avaliação das diferentes táticas reprodutivas adotadas pelas fêmeas.

Um método empregado por vários estudos para tal identificação é o uso da aparência dos ovos (cor, formato, tamanho, padrão de manchas), relacionando-se ovos semelhantes a uma mesma fêmea e ovos distintos a diferentes fêmeas (Vehrencamp 1977; Yom-Tov 1980a,b; Loflin 1983; Gibbons 1986; Mϕller 1987; Lyon 1997; Dugger & Blums 2001).

As características dos ovos podem ser bons indicativos de maternidade, especialmente para as espécies que apresentam uma maior variação entre indivíduos diferentes do que em um mesmo indivíduo (Baerends & Hogan-Warburg 1982; Fetterolf & Blokpoel 1984; Jackson 1992; Petersen 1992). Entretanto, para algumas espécies, há uma grande variação entre as características dos ovos de uma mesma fêmea, dependente de sua ordem de postura (Coulson 1963; Mills & Shaw1980; Runde & Barret 1981; Quinn & Morris 1986; Meathrel & Ryder 1987; Henriksen 1995; Viñuela 1997), que pode limitar seu uso para a identificação de maternidade. Há, então, um risco em se assumir que um ovo distinto dos demais em um ninho foi colocado por uma outra fêmea, especialmente se a ordem de postura dos ovos não for conhecida (Brown & Sherman 1989; McRae 1997).

Por essa razão, em espécies de aves em que nenhuma avaliação prévia da variabilidade dos ovos dentro e entre ninhadas de fêmeas foi realizada, a aparência dos mesmos não deve ser usada para distinguir ninhadas de diferentes fêmeas. Tais características podem ser utilizadas como um método confiável para determinação de maternidade se validadas, por exemplo, pela identificação de fêmeas, previamente marcadas, durante a postura ou através de métodos bioquímicos e/ou moleculares, como análises de eletroforese de proteínas ou de DNA (Bischoff & Murphy 1993; McRae & Burke 1996). A primeira alternativa pode despender muito tempo e produzir pequena amostragem (mas veja Brown 1984). A segunda é relativamente mais fácil e permite uma maior amostragem. Contudo, análises de DNA ou de eletroforese de proteínas de tecidos embriônicos podem subestimar o número de fêmeas reprodutivas em um ninho quando há redução de ninhada antes da incubação dos ovos ou quando a captura das fêmeas é insuficiente, já que tais análises requerem amostragem tanto dos filhotes quanto da mãe. Nessas situações, eletroforese de proteínas de ovos recém-colocados pode ser o método mais útil para a determinação da maternidade de posturas individuais (Fleischer 1985; Kendra *et al.* 1988; Smyth *et al.* 1993), e o uso de proteínas totais nas análises ao invés de alozimas permite uma identificação mais acurada (Andersson & Åhlund 2001; Cariello *et al.* no prelo).

O estudo representado por essa seção pretendeu verificar a aplicabilidade do uso das características dos ovos na determinação da maternidade de ninhadas individuais de fêmeas de anu branco em posturas comunitárias. Seu principal objetivo foi verificar se ovos de diferentes origens maternais postos em um mesmo ninho poderiam ser distinguidos com base em suas características. Adicionalmente, tal estudo pretendeu verificar se todos os ovos em um mesmo ninho poderiam ser corretamente associados a suas origens maternais com base em suas características. A correta maternidade dos ovos foi determinada previamente através de análises de eletroforese de proteínas da gema.

A maternidade dos ovos em posturas comunitárias do anu branco, identificada através da eletroforese de proteínas da gema, não pode ser corretamente determinada através do uso de características morfométricas e padrões da manchas dos ovos. Em média, menos da metade dos ovos foi associada à fêmea correta. Adicionalmente, as poucas diferenças encontradas entre fêmeas reprodutivas de um mesmo grupo ocorreram, principalmente, devido a uma única fêmea que diferiu das demais em apenas uma das variáveis dos ovos analisadas. Tais diferenças somente foram significativas em grupos em que havia um maior número de fêmeas reprodutivas. Desse modo, pode-se concluir que as características dos ovos de anu branco não são um critério confiável para discriminar entre posturas individuais de fêmeas em ninhos comunitários e, portanto, não devem ser utilizadas com essa finalidade para essa espécie.

O fato de ovos de diferentes fêmeas de *G. guira* não serem distinguidos por sua aparência contradiz o que foi proposto na literatura para duas outras espécies de Crotophaginae. Vehrencamp *et al.* (1986) mediu o tamanho dos ovos em posturas comunitárias do anu *Crotophaga sulcirostris* e verificou que as fêmeas que iniciam a postura colocam ovos maiores que as fêmeas que colocam ovos ao final desse período. Entretanto, a identificação de fêmeas nesse estudo foi baseada em um estudo anterior (Vehremcamp 1976) em que os primeiros ovos, que eram mais visualmente semelhantes entre si e mais distintos dos últimos ovos foram relacionados às mesmas fêmeas, enquanto que os últimos ovos foram considerados como tendo sido colocados por outras fêmeas. Adicionalmente, o número de fêmeas reprodutivas em um grupo foi determinado pelo número total de ovos encontrado no ninho, ou seja, assumiu-se que cada fêmea põe um número típico de ovos no ninho, dos quais grupos de ovos foram separados baseados em sua aparência e associados a uma ou mais fêmeas. Loflin (1983), que estudou ninhadas comunitárias do anu *Crotophaga ani*, também identificou fêmeas individuais através das dimensões, cor, formato e massa dos ovos, além do número total de ovos no ninho. Infelizmente, em tais estudos, nenhuma avaliação quantitativa da variabilidade dos ovos da mesma ou das diferentes fêmeas foi previamente realizada.

Alguns pesquisadores, após verificarem que a variação nas medidas dos ovos entre fêmeas foi maior que dentro de fêmeas, puderam usar, seguramente, as características dos ovos para detectar parasitismo intraespecífico (Fetterolf & Blokpoel 1984; Jackson 1992; Petersen 1992). Contrariamente, para outras espécies, isso não seria possível, porque a variância dentro de ninhadas individuais de fêmeas não diferiu suficientemente daquela entre ninhadas de outras fêmeas (Coulson 1963; Brown & Sherman 1989).

Uma das razões mais importantes para a realização de avaliações prévias da variabilidade dos ovos é o fato de que, em muitas espécies, um ovo na ninhada de uma fêmea, geralmente o último, pode diferir dos demais (Mills & Shaw 1980; Meathrel & Ryder 1987; Viñuela 1997) e tais diferenças podem ser maiores que as diferenças entre ninhadas de diferentes fêmeas. Em estudos de parasitismo intraespecífico de ninho, Brown e Sherman (1989) e McRae (1997) advertiram sobre o risco de se considerar o último ovo em uma ninhada como sendo colocado por outra fêmea baseado apenas em suas características. Tais autores recomendaram que os dados sobre aparência dos ovos fossem combinados aos de seqüência de postura e checagem diária de ninho para uma avaliação mais precisa da maternidade dos ovos. Tal metodologia pode ser útil em alguns estudos de parasitismo intraespecífico, mas pode ser uma tarefa impossível para a identificação de posturas individuais de fêmeas em ninhos comunitários. Em ninhos de anu branco, a checagem diária de ninhos e as análises de eletroforese da gema permitiram a verificação de que as diferentes fêmeas: (1) colocam seus ovos a intervalos irregulares; (2) não colocam a mesma quantidade de ovos nos ninhos; e (3) não seguem padrões semelhantes de postura. Desse modo, em tal espécie, há, provavelmente, constrangimentos comportamentais, além de fisiológicos, que limitam o número de ovos postos pelas fêmeas, bem como a ordem de entrada das fêmeas na postura, o que impede o uso da seqüência de postura para a determinação do número exato de fêmeas reprodutivas em um ninho.

Em conclusão, baseado em dados quantitativos prévios e em dados deste estudo, recomenda-se o uso das características dos ovos para a identificação de posturas individuais somente quando avaliações prévias de tais características confirmarem que ovos de uma mesma fêmea são semelhantes entre si, mas distintos significativamente de ovos de fêmeas diferentes.

Seção III

Assimetria Reprodutiva em Posturas Comunitárias de Anu Branco (*Guira guira*)

A regra de Hamilton e o modelo de valor adaptativo inclusivo (Hamilton 1964) têm servido como princípios-guia para a compreensão da evolução da socialidade entre os animais. Desde a década de 70, um número crescente de publicações relativas à evolução da reprodução comunitária em aves, mamíferos e insetos tem sido produzido (Vehrencamp 1977; Woolfenden & Fitzpatrick 1984; Brown 1987; Koenig & Mumme 1987; Jamieson 1997). A relevância evolutiva de tal sistema estimulou a realização de vários estudos de campo e também o desenvolvimento de modelos teóricos, que tentaram explicar padrões de oportunidades reprodutivas para indivíduos que vivem em grupos, geralmente, em termos do grau de assimetria reprodutiva em favor dos indivíduos dominantes (Vehrencamp 1979, 1983a,b; Emlen 1982; Emlen & Vehrencamp 1983, Reeve & Ratnieks 1993; Keller & Reeve 1994; Reeve 1998). Esses modelos de concessões assumem que os membros dominantes são capazes de controlar as oportunidades reprodutivas dentro do grupo e somente toleram alguma reprodução dos subordinados para que estes permaneçam no grupo. Em tais modelos, espera-se que a assimetria reprodutiva em favor dos dominantes seja alta, quando: (1) os membros do grupo são proximamente aparentados; (2) os constrangimentos ecológicos reduzem as chances de dispersão e de reprodução independente dos subordinados; e (3) a vida em grupo produz consideráveis vantagens para todos os membros.

Recentemente, alguns trabalhos testaram modelos de assimetria reprodutiva em insetos sociais (Heinze 1995; Reeve & Keller 1995). Para vertebrados sociais, entretanto, os dados sobre tal assimetria ainda são bastante escassos para que se faça uma avaliação da generalidade desses modelos (Johnstone & Cant 1999a). Evidências de conflitos em grupos de algumas espécies de vertebrados sugerem que os indivíduos dominantes, freqüentemente, são incapazes de exercer controle sobre a reprodução dos subordinados (Clutton-Brock 1998), o que tem levado ao desenvolvimento de modelos alternativos, que consideram questões anteriormente negligenciadas, como a partilha da reprodução entre subordinados em grupos multi-membros, a assimetria reprodutiva através do infanticídio e o controle incompleto da reprodução pelos dominantes (Reeve *et al.* 1998; Johnstone & Cant 1999a,b; Johnstone *et al.* 1999).

As espécies da subfamília Crotophaginae (Aves, Cuculidae) têm comportamentos bastante similares, que incluem a reprodução comunitária, com ovos sendo ejetados ou enterrados no ninho pelos membros do grupo (Vehrencamp 1977; Loflin 1983; Macedo 1992). Em seu estudo pioneiro sobre a reprodução comunitária do anu *Crotophaga sulcirostris*, Vehrencamp (1977) sugeriu que essas aves são um excelente exemplo de sociedade razoavelmente igualitária, em que também ocorre reprodução dos membros subordinados. Entretanto, a despeito de algum sucesso reprodutivo dos subordinados, Vehrencamp (1977) propôs a existência de uma hierarquia definitiva para as fêmeas dentro dos grupos, levando a significantes diferenças no número de ovos que cada fêmea põe no ninho.

No trabalho representado por essa seção, o sistema reprodutivo do anu branco (*Guira guira*) foi avaliado de acordo com previsões dos modelos de concessões. Considerando-se as características da espécie de baixo parentesco entre os membros reprodutivos e de relativa ausência de saturação do *habitat*, os modelos de concessões prevêem um baixo nível de assimetria reprodutiva entre as fêmeas dessa espécie. O sistema reprodutivo do anu branco também foi comparado ao proposto por Vehrencamp (1977) para o anu *C. sulcirostris*. A identificação de ninhadas individuais em posturas comunitárias de *G. guira* foi feita através de análises eletroforéticas de proteínas da gema.

Os índices de assimetria reprodutiva encontrados para os grupos de *G. guira* nem sempre foram baixos como previstos pelo modelo de concessões. As primeiras nidificações das estações reprodutivas apresentaram maior assimetria comparada às seguintes. Naquelas, houve o monopólio da reprodução por uma ou poucas fêmeas e, geralmente, houve a perda total do investimento no ninho antes que a postura estivesse completa devido à ejeção dos ovos e/ou ao abandono do ninho. Com o avanço da estação chuvosa e a redução de novas oportunidades reprodutivas, é possível que uma distribuição mais igualitária das oportunidades reprodutivas, relativa à postura dos ovos, contribua para o sucesso do ninho.

Os padrões de partilha reprodutiva de *G. guira*, elucidados nesse estudo, não apresentam similaridade ao que foi proposto para *C. sulcirostris*. Vehrencamp (1977) propôs que as fêmeas que colocam ovos ao fim da postura (designadas como "fêmeas-alfa") retardam a postura de seus ovos, enquanto que as fêmeas que colocam ovos no início da postura têm as suas ninhadas individuais reduzidas através da ejeção de ovos por aquelas do grupo que ainda não iniciaram suas próprias posturas. Tal hipótese foi baseada na identificação de ovos de fêmeas através de avaliação visual da morfologia dos ovos. As "fêmeas-alfa" foram as mais bem-sucedidas devido à assimetria no número de ovos incubados e filhotes que deixaram o ninho. Tal conclusão baseou-se nas seguintes suposições (Vehrencamp 1977): (1) as fêmeas não reconhecem seus próprios ovos em uma ninhada comunitária; (2) as fêmeas que iniciam a postura, embora produzam maior número de ovos, possuem menor sucesso reprodutivo que as "fêmeas-alfa", porque seus ovos são ejetados; (3) após todas as fêmeas iniciarem a postura de seus ovos, não ocorre mais ejeção; (4) as "fêmeas-alfa" não perdem ovos através da ejeção e possuem maiores sucessos reprodutivos; (5) as fêmeas mantém a ordem de entrada na seqüência de postura em repetidas nidificações do grupo; e (6) todas as fêmeas participam de todas as posturas do grupo.

A maioria dos resultados encontrados para *G. guira* são incompatíveis com tais premissas. As fêmeas que iniciam a postura comunitária, como em *C. ani* e *C. sulcirostris*, colocaram mais ovos que as demais. Entretanto, a pressão de ejeção dos ovos permaneceu constante, afetando todas as fêmeas independentemente de sua posição de entrada na seqüência de postura, o que difere do que foi observado para *C. ani* e *C. sulcirostris*. De fato, as fêmeas de *G. guira* que colocaram ovos ao fim da postura perderam mais de 50% dos ovos. Conseqüentemente, a maior proporção de ovos incubados pertencia às fêmeas que iniciaram a postura. Adicionalmente, as fêmeas dessa espécie não mantiveram a mesma ordem de entrada na postura e nem todas participaram das repetidas nidificações de seus grupos.

Há duas explicações para tais diferenças. Primeiro, podem haver sutis diferenças entre os sistemas socias de *G. guira*, *C. ani* e *C. sulcirostris*, que levam a estratégias reprodutivas em *Crotophaga* divergentes das de *G. guira*. Os grupos reprodutivos de *Crotophaga* têm, aparentemente, até quatro ou cinco fêmeas (Vehrencamp 1977; Loflin 1983), enquanto que os de *G. guira* podem conter até 10 fêmeas. Grupos de *Crotophaga* podem ser mais proximamente aparentados ou, então, os constrangimentos ecológicos para as espécies desse gênero (por exemplo, saturação de ambiente; Koford *et al.* 1986) não são relevantes para (ou não se aplicam a) *G. guira*. Seria interessante investigar se níveis crescentes de complexidade em termos de número de indivíduos no grupo, número de fêmeas e nidificações por estação reprodutiva nas espécies da família Crotophaginae (*C. sulcirostris, C. ani, C. major* e *G. guira*) levam a partilhas reprodutivas progressivamente mais eqüitativas, considerando-se o grau de parentesco entre os membros reprodutivos e a presença/ausência de constrangimentos ecológicos.

Uma segunda explicação para essa divergência encontrada nos padrões reprodutivos das espécies de Crotophaginae é que a metodologia usada por Vehrencamp (1976, 1977) e Loflin (1983) para determinar a maternidade dos ovos produziu resultados inacurados. Técnicas bioquímicas e/ou moleculares não eram prontamente disponíveis quando da realização desses estudos. Desse modo, a morfometria dos ovos (comprimento, largura, cor, peso) foi usada para distinguir ovos de fêmeas individuais em posturas comunitárias e o número de ovos colocados e ejetados, para estimar o número de fêmeas reprodutivas.

As características dos ovos podem ser menos variáveis em ninhadas de fêmeas individuais do que entre ninhadas de diferentes indivíduos (Kendeigh *et al.* 1956; Baerends & Hogan-Warburg 1982; Ricklefs 1984; Croxall *et al.* 1992; Reed & Oring 1997). Entretanto, é fundamental que se faça uma avaliação prévia da variabilidade individual das características dos ovos para usá-las com confiabilidade na identificação da maternidade dos ovos, principalmente em espécies em que ovos de várias fêmeas são encontrados no mesmo ninho.

A aparente flexível hierarquia das fêmeas em grupos de *G. guira* pode explicar por que os indivíduos se associam e permanecem nos grupos a despeito do alto grau de conflito, que envolve perdas de ovos e filhotes. Em uma hierarquia rígida, poderia ser mais vantajoso para uma fêmea subordinada que consistentemente perdesse seus ovos através da ejeção abandonar o grupo e se reproduzir independentemente, especialmente na existência de ambientes disponíveis. Posturas comunitárias são a opção reprodutiva mais comum para os Crotophaginae, o que parece indicar que há significativas vantagens reprodutivas para todos os membros do grupo.

Desse modo, é difícil saber se há ou não fêmeas dominantes em termos de partilha reprodutiva em grupos de *G. guira*. As fêmeas que iniciam a postura, aparentemente, têm uma vantagem em relação às demais, já que estas produzem mais ovos, que não sofrem maior pressão de ejeção que aqueles colocados por fêmeas ao fim da postura. Entretanto, como as fêmeas não mantêm suas posições na seqüência de postura em repetidas nidificações do grupo e nem contribuem para todas as tentativas reprodutivas, as posições de entrada na seqüência de postura parecem mudar entre as fêmeas. Interessantemente, para fêmeas de *G. guira*, a melhor estratégia reprodutiva parece ser a freqüência com que cada fêmea contribui para repetidas nidificações do grupo. As fêmeas com maior proporção de ovos incubados em uma estação reprodutiva foram aquelas que contribuíram para duas nidificações.

O sistema comunitário dos crotofagíneos apresenta um desafio no teste dos diferentes modelos de assimetria reprodutiva atualmente disponíveis. Para *G. guira*, as hierarquias de dominância entre os membros reprodutivos parecem ser fracas, devido a um baixo grau de parentesco entre os membros e à falta de constrangimentos ecológicos, concomitantes ao alto número de fêmeas potencialmente reprodutivas e a uma estação reprodutiva bastante longa, que permite repetidas oportunidades para nidificações.

Em conclusão, a despeito da rápida expansão da teoria e modelos de assimetria reprodutiva, a maioria dos modelos tem sido aplicada na elucidação do comportamento de insetos sociais. Nossos resultados não concordam completamente com tais modelos, e podem refletir o fato de que sociedades de vertebrados exibem uma maior variabilidade e flexibilidade comportamental.

Bibliografia Geral

Andersson M, Åhlund M (2001) Protein fingerprinting: a new technique reveals extensive conspecific brood parasitism. *Ecology*, **82**, 1433-1442.

Andersson M, Eriksson MOG (1982) Nest parasitism in goldeneyes *Bucephala clangula*: some evolutionary aspects. *American Naturalist*, **120**, 1-16.

Baerends GP, Hogan-Warburg AJ (1982) The external morphology of the egg and its variability. *Behaviour*, **82,** 1-32.

Bertram BCR (1979) Ostriches recognise their own eggs and discard others. *Nature*, **279**, 233-234.

Bischoff CM, Murphy MT (1993) The detection of and responses to experimental brood parasitism in eastern kingbirds. *Animal Behaviour*, **45**, 631-638.

Brown CR (1984) Laying eggs in a neighbor's nest: benefit and cost of colonial nesting in swallows. *Science*, **224**, 518-519.

Brown CR, Brown MB (1988) Genetic evidence of multiple parentage in broods of cliff swallows. *Behavioral Ecology and Sociobiology*, **23**, 379-387.

Brown CR, Sherman LC (1989) Variation in the appearance of swallow eggs and the detection of intraspecific brood parasitism. *Condor*, **91**, 620-627.

Brown JL (1987) *Helping and Communal Breeding in Birds*. Princeton University Press, Princeton, NJ.

Brown JL, Brown EL (1990) Mexican jays: uncooperative breeding. In: *Cooperative Breeding in Birds: Long-term Studies of Ecology and Behavior* (eds Stacey PB, Koenig WD), pp. 269-288. Cambridge University Press, Cambridge.

Cariello M, Schwabl H., Lee R, Macedo RH (no prelo). Individual female clutch identification through yolk protein electrophoresis in the communally-breeding guira cuckoo (*Guira guira*). *Molecular Ecology*.

Clutton-Brock TH (1998) Reproductive skew, concessions and limited control. *Trends in Ecology and Evolution*, **13,** 288-292.

Coulson JC (1963) Egg size and shape in the kittiwake (*Rissa tridactyla*) and their use in estimating age composition of populations. *Proceedings of the Zoological Society of London*, **140**, 211-227.

Craig JL (1979) Habitat variation in the social organization of a communal gallinule, the Pukeko (*Porphyrio porphyrio*). *Behavioral Ecology and Sociobiology*, **5**, 331-358.

Croxall JP, Rothery P, Crisp A (1992) The effect of maternal age and experience on egg-size and hatching success in wandering albatrosses *Diomedea exulans*. *Ibis*, **134**, 219-228.

Davis DE (1942) The phylogeny of social nesting habits in the Crotophaginae. *Quarterly Review of Biology*, **17**, 115-134.

Dugger BD, Blums P (2001) Effect of conspecific brood parasitism on host fitness for tufted duck and common pochard. *Auk*, **118**, 717-726.

Emlen ST (1982) The evolution of helping I. An ecological constraints model. *American Naturalist*, **119**, 29-39.

Emlen ST, Vehrencamp SL (1983) Cooperative breeding strategies among birds. In: *Perspectives in Ornithology* (eds Brush AH, Clark JGA), pp 93-133. Cambridge University Press, Cambridge.

Fetterolf PM, Blokpoel H (1984) An assessment of possible intraspecific brood parasitism in ring-billed gulls. *Canadian Journal of Zoology*, **62**, 1680-1684.

Fleischer RC (1985) A new technique to identify and assess the dispersion of eggs of individual brood parasites. *Behavioral Ecology and Sociobiology*, **17**, 91-99.

Fleischer RC, Murphy MT, Hunt LE (1985) Clutch size increase and intraspecific brood parasitism in the yellow-billed cuckoo. *Wilson Bulletin*, **97**, 125-127.

Fleischer RC, Smith NG (1992) Giant cowbird eggs in the nests of two icterid hosts: the use of morphology and electrophoretic variants to identify individuals and species. *Condor*, **94**, 572-578.

Gibbons DW (1986) Brood parasitism and cooperative nesting in the moorhen, *Gallinula chloropus*. *Behavioral Ecology and Sociobiology*, **19**, 221-232.

Gowaty PA, Karlin AA (1984) Multiple maternity and paternity in single broods of apparently monogamous eastern bluebirds (*Sialia sialis*). *Behavioral Ecology and Sociobiology*, **15**, 91-95.

Hamilton WD (1964) The genetical evolution of social behavior. I and II. *Journal of Theoretical Biology*, **7**, 1-52.

Heinze J (1995) Reproductive skew and genetic relatedness in *Leptothorax* ants. *Proceedings of the Royal Society of London B*, **261**, 375-379.

Henriksen K (1995) Intraclutch variation in egg volume of great crested grebes. *Condor*, **97**, 826-828.

Jackson WM (1992) Estimating conspecific nest parasitism in the northern masked weaver based on within-female variability in egg appearance. *Auk*, **109**, 435-443.

Jamieson IG (1997) Testing reproductive skew models in a communally breeding bird, the pukeko, *Porphyrio porphyrio*. *Proceedings of the Royal Society of London B*, **264**, 335-340.

Johnstone RA, Cant MA (1999a) Reproductive skew and indiscriminate infanticide. *Animal Behaviour*, **57**, 243-249.

Johnstone RA, Cant MA (1999b) Reproductive skew and the threat of eviction: a new perspective. *Proceedings of the Royal Society of London B*, **266**, 275-279.

Johnstone RA, Woodroffe R, Cant MA, Wright J (1999) Reproductive skew in multimember groups. *American Naturalist*, **153**, 315-331.

Keller L, Reeve HK (1994) Partitioning of reproduction in animal societies. *Trends in Ecology and Evolution*, **9**, 98-102.

Keller L, Vargo EL (1993) Reproductive structure and reproductive roles in colonies of eusocial insects. In: *Queen Number and Sociality in Insects* (ed Keller L), pp. 16-44. Oxford University Press, Oxford.

Kendeigh SC, Kramer TC, Hamerstrom F (1956) Variations in egg characteristics of the house wren. *Auk*, **73**, 42-65.

Kendra PE, Roth RR, Tallamy DW (1988) Conspecific brood parasitism in the house sparrow. *Wilson Bulletin*, **100**, 80-90.

Koenig WD (1981) Reproductive success, group size, and the evolution of cooperative breeding in the acorn woodpecker. *American Naturalist*, **117**, 421-443.

Koenig WD, Mumme RL (1987) *Population Ecology of the Cooperatively Breeding Acorn Woodpecker*. Princeton University Press, Princeton, NJ.

Koford RR, Bowen BS, Vehrencamp SL (1986) Habitat saturation in groove-billed anis (*Crotophaga sulcirostris*). *American Naturalist*, **127**, 317-337.

Lahti DC, Lahti AR (*in press*) How precise is egg discrimination in weaverbirds? *Animal Behaviour*.

Loflin RK (1983) *Communal Behaviors of the Smooth-billed Ani.* Ph.D. dissertation, University of Miami, Coral Gables, FL.

Lyon BE (1997) Spatial patterns of shiny cowbird brood parasitism on chestnut-capped blackbirds. *Animal Behaviour*, **54**, 927-939.

Macedo RH (1992) Reproductive patterns and social organization of the communal guira cuckoo *(Guira guira)* in central Brazil. *Auk*, **109**, 786-799.

Macedo RH (1994) Inequities in parental effort and costs of communal breeding in the guira cuckoo. *Neotropical Ornithology*, **5**, 79-90.

Macedo RH, Bianchi CA (1997a) Communal breeding in tropical guira cuckoos *Guira guira*: sociality in the absence of a saturated habitat. *Journal of Avian Biology*, **28**, 207-215.

Macedo RHF, Bianchi, CA (1997b) When birds go bad: circumstantial evidence for infanticide in the communal South-American guira cuckoo. *Ethology, Ecology and Evolution*, **9**, 45-54.

Macedo RHF, Cariello M, Muniz L (2001) Context and frequency of infanticide in communally breeding guira cuckoos. *Condor*, **103**, 170-175.

Macedo RH, Melo C (1999) Confirmation of infanticide in the communally breeding Guira cuckoo. *Auk*, **116**, 847-851.

Manwell C, Baker CMA (1975) Molecular genetics of avian proteins XIII. Protein polymorphism in three species of Australian passerines. *Australian Journal of Biological Sciences*, **28**, 545-557.

McKitrick MC (1990) Genetic evidence for multiple parentage in eastern kingbirds (*Tyrannus tyrannus*). *Behavioral Ecology and Sociobiology*, **26**,149-155.

McRae SB (1997) Identifying eggs of conspecific brood parasites in the field: a cautionary note. *Ibis*, **139**, 701-704.

McRae SB, Burke T (1996) Intraspecific brood parasitism in the moorhen: parentage and parasite-host relationship determined by DNA fingerprint *Behavioral Ecology and Sociobiology*, **38**, 115-129.

Meathrel CE, Ryder JP (1987) Intraclutch variation in the size, mass and composition of ring-billed gull eggs. *Condor*, **89**, 364-368.

Melo C (1997) *Reprodução Comunitária em Guira guira Gmelin 1788 (Cuculidae, Aves): Aspectos Eto-ecológicos*. Masters thesis, Universidade de Brasília, Brazil.

Melo C, Macedo RHF (1997) Mortalidade em ninhadas de *Guira guira* (Cuculidae): competição por recursos? *Ararajuba*, **5**, 49-56.

Mills JA, Shaw PW (1980) The influence of age on laying date, clutch size, and egg size of the white-fronted tern, *Sterna striata*. *New Zealand Journal of Zoology*, **7**, 147-153.

Mϕller AP (1987) Intraspecific nest parasitism and anti-parasite behaviour in swallows, *Hirundo rustica*. *Animal Behaviour*, **35**, 247-254.

Petersen MR (1992) Intraspecific variation in egg shape among individual emperor geese. *Journal of Field Ornithology*, **63**, 344-354.

Quinn JS, Macedo R, White BN (1994) Genetic relatedness of communally-breeding guira cuckoos. *Animal Behaviour*, **47**, 515-529.

Quinn JS, Morris RD (1986) Intraclutch egg-weight apportionment and chick survival in caspian terns. *Canadian Journal of Zoology*, **64**, 2116-2122.

Reed JM, Oring LW (1997) Intra- and inter-clutch patterns in egg mass in the spotted sandpiper. *Journal of Field Ornithology*, **68**, 296-301.

Reeve HK (1998) Game theory, reproductive skew, and nepotism. In: *Game Theory and Animal Behavior* (eds Dugatkin L, Reeve HK), pp 118-145. Oxford University Press, Oxford.

Reeve HK, Emlen ST, Keller LF(1998) Reproductive sharing in animal societies: reproductive incentives or incomplete control by dominant breeders? *Behavioral Ecology*, **9**, 267-278.

Reeve HK, Keller L (1995) Partitioning of reproduction in mother-daughter versus sibling associations: a test of optimal skew theory. *American Naturalist*, **145**, 119-132.

Reeve HK, Ratnieks FLW (1993) Queen-queen conflict in polygynous societies: mutual tolerance and reproductive skew. In: *Queen Number and Sociality in Insects* (ed Keller L), pp 45-85. Oxford University Press, Oxford.

Ricklefs RE (1984) Variation in the size and composition of eggs of the European starling. *Condor*, **86**, 1-6.

Runde OJ, Barrett RT (1981) Variations in egg size and incubation period of the kittiwake *Rissa tridactyla* in Norway. *Ornis Scandinavica*, **12**, 80-86.

Sick H (1997) *Ornitologia Brasileira*. Nova Fronteira, Rio de Janeiro.

Smyth AP, Orr BK, Fleischer RC (1993) Electrophoretic variants of egg white transferrin indicate a low rate of intraspecific brood parasitism in colonial cliff swallows in the Sierra Nevada, California. *Behavioral Ecology and Sociobiology*, **32**, 79-84.

van Tienhoven A (1983) *Reproductive Physiology of Vertebrates*, 2nd edn. Cornell University Press, Ithaca & London.

Vehrencamp SL (1976) *The Evolution of Communal Nesting in Groove-billed Anis*. Ph.D. dissertation, Cornell University, Cornell.

Vehrencamp SL (1977) Relative fecundity and parental effort in communally nesting anis, *Crotophaga sulcirostris*. *Science*, **197**, 403-405.

Vehrencamp SL (1979) The roles of individual, kin and group selection in the evolution of sociality. In: *Social Behavior and Communication* (eds Marler P, Vandenbergh J), pp 351-394. Plenum Press, New York.

Vehrencamp SL (1983a) A model for the evolution of despotic versus egalitarian societies. *Animal Behaviour*, **31**, 667-682.

Vehrencamp SL (1983b) Optimal degree of skew in cooperative societies. *American Zoologist*, **23**, 327-335.

Vehrencamp SL, Bowen BS, Koford RR (1986) Breeding roles and pairing patterns within communal groups of groove-billed anis. *Animal Behaviour*, **34**, 347-366.

Viñuela J (1997) Adaptation vs. constraint: intraclutch egg-mass variation in birds. *Journal of Animal Ecology*, **66**,781-792.

Woolfenden GE, Fitzpatrick JW (1984) *The Florida Scrub Jay: Demography of a Cooperative-breeding Bird*. Princeton University Press, Princeton.

Wrege PH, Emlen ST (1987) Biochemical determination of parental uncertainty in white-fronted bee-eaters. *Behavioral Ecology and Sociobiology*, **20**, 153-160.

Yom-Tov Y (1980a) Intraspecific nest parasitism among dead sea sparrows *Passer moabiticus*. *Ibis*, **122**, 234-237.

Yom-Tov Y (1980b) Intraspecific nest parasitism in birds. *Biological Review*, **55**, 93-108.

CAPÍTULO I

Individual female clutch identification through yolk protein electrophoresis in the communally-breeding guira cuckoo (*Guira guira*)

Keywords: Guira guira, guira cuckoo, communal breeding, egg maternity, yolk protein electrophoresis, egg ejection.

Running title: YOLK PROTEIN ELECTROPHORESIS IN GUIRA CUCKOOS

Abstract

Avian communal breeding systems generate alternative behavioral strategies for females, resulting in differences in reproductive success. Identifying eggs of different females in such systems is problematic, however, due to egg destruction before incubation, difficulty of capturing adults, and/or inaccuracy of egg identification based on egg morphometry. Here, we describe a technique that uses electrophoresis of yolk proteins to determine egg ownership, which we applied to communally breeding guira cuckoos (*Guira guira*). Validation of the method included identical yolk protein banding patterns in all eggs of the same female, but different patterns in eggs of different females in budgerigars (*Melopsittacus undulatus*), and identical patterns in yolk follicles of the same females in guira cuckoos. We applied the protocol to 195 guira cuckoo eggs from 34 joint nests in two years. All multiple guira cuckoo eggs laid on the same day in single nests had distinct banding patterns of yolk proteins, practically eliminating the possibility of more than one female being represented by the same pattern. Some identical banding patterns were repeated in different days within a nesting bout, indicating that some females laid several eggs in shared nests. Identical patterns occasionally occurred in re-nestings of groups, indicating that some females lay eggs in consecutive nestings. Yolk protein electrophoresis is a useful tool to identify egg maternity in other circumstances, such as polygynous mating systems with joint nests and intraspecific parasitism. Additionally, it is an alternative method for species where electrophoresis of egg white proteins does not show sufficient polymorphism.

Introduction

In avian nests where two or more females deposit eggs, it is crucial to identify the ownership of individual eggs to understand the strategies of the laying females and their subsequent reproductive benefits. These situations occur in species with complex social systems, such as communal breeders (Craig 1979; Koenig 1981; Brown 1987; Vehrencamp *et al.* 1986; Brown & Brown 1990; Macedo 1992), or in cases of intraspecific nest parasitism (Andersson & Eriksson 1982; Brown 1984; Mϕller 1987). In communal reproduction three or more individuals in a group breed simultaneously, sometimes using a joint nest (see Brown 1987 for alternative terminology). In this system, helpers at the nest may include adults genetically related to the nestlings (former offspring that remained in the group), as well as adults unrelated to the brood. In intraspecific nest parasitism females lay eggs in nests of conspecifics without caring for their offspring. The host females are faced with the difficulty of discriminating between their own eggs and parasitic ones, which may be very similar in appearance to their own, leading to acceptance of parasitic eggs in some cases and rejection in others (Yom-Tov 1980; Lahti & Lahti 2002).

In some intraspecific nest parasitism studies, egg maternity has been determined through electrophoresis using embryonic tissue (Gowaty & Karlin 1984; Wrege & Emlen 1987; Brown & Brown 1988; McKitrick 1990). However, embryonic sampling may underestimate the contribution of each female to the nest under two conditions. First, when clutch reduction occurs before incubation starts (i.e., before embryo development). For instance, a significant sampling of embryos may not be possible in cases where egg loss due to predation or egg ejection is high at the onset of laying, or in the case of infertile eggs. Second, when the capturing of adults in the study species is unsuccessful or insufficient, since embryonic tissue analysis requires parental sampling for comparison of the results.

In such situations, protein electrophoresis of recently laid eggs can be useful as an alternative method for determining the maternity of individual clutches (Smyth *et al.* 1993; Andersson & Åhlund 2001). Egg sampling in the early stages of laying avoids the loss of information due to clutch reduction. In addition, female capturing is not necessary because proteins of undeveloped (non-incubated) eggs are exclusively maternally derived, and any polymorphism reflects maternal genotype. Therefore, if different protein phenotypes and therefore genotypes occur among eggs within a single nest it is very likely that more than a single female laid eggs in that clutch (Manwell & Baker 1975).

Allozyme analysis of egg white has been used in studies of avian intraspecific nest parasitism to differentiate between clutches of hosts and parasites in the yellow-billed cuckoo *Coccyzus americanus* (Fleischer *et al.* 1985), the house sparrow *Passer domesticus* (Kendra *et al.* 1988) and the cliff swallow *Hirundo pyrrhonota* (Smyth *et al.* 1993). These studies used the separation of enzyme products of different alleles at a single locus (allozymes) based on electrical charges. Some alleles yielded detectable polymorphism that allowed the identification of more than one laying female in a single nest. Although egg white proteins have been more widely used for identifying individual clutches in nest parasitism studies, yolk proteins may also be used for this purpose. Fleischer (1985) and Fleischer and Smith (1992) used yolk proteins in allozyme analysis in studies of interspecific parasitism to verify if two or more cowbird females parasitized the same host nests. Sufficient polymorphism of egg white proteins may not exist in all species and, in these cases, it has been suggested that polymorphic electromorphs of yolk proteins could be good markers (Fleischer 1985). However, since allozyme analysis may not show sufficient differences among females because different females may possess the same alleles, they have been used mostly for exclusion of maternity, and not for a precise maternal identification of eggs.

Despite its potential importance for studying individual reproductive success and female strategies in birds, electrophoresis of egg white or yolk proteins has never been used specifically for identifying individual female clutches in joint nests of communal breeders. Since allozyme analysis may present the limitation mentioned above, new methods using total proteins rather than allozymes would be better indicated for such studies. More recently, Andersson and Åhlund (2001) developed a very accurate "protein fingerprinting" technique, based on egg white sampling and isoelectric focusing electrophoresis (IEF) to determine egg maternity of hosts and parasites in nests of the common goldeneye duck *Bucephala clangula*. Through this technique, total proteins of egg white are separated according to their charge in an electric field applied over a stable pH gradient in the gel, and the samples analyzed produced a rich banding pattern of egg white proteins that was very variable among females. Thus, methods using total proteins may allow a better resolution of differences among females (compared to allozyme methods) because they test for differences among all proteins present in the sample.

For the past decade, an ongoing study concerning the reproductive strategies of the South American communallly-breeding guira cuckoo (*Guira guira*) has been carried out in central Brazil (Macedo 1992, 1994; Quinn *et al.* 1994; Macedo & Bianchi 1997 a,b; Melo & Macedo 1997; Macedo *et al.* 2001). These birds occur in reproductive units varying from 2 to 15 adults (mode = 6) and their egg laying and egg ejection patterns resemble those of other species in the Crotophaginae (Davis 1942; Vehrencamp 1977; Sick 1997). Typically, adults in each group build a single nest, in which reproductive females of the group lay their eggs. The communal clutch may range from one to as many as 26 eggs during a single nesting bout. During the breeding season, up to five successive nesting bouts may occur for groups in the same territory (Melo 1997). The social behavior of group members includes cooperation (e.g. nest construction, predator defense, territorial defense, nestling feeding) as well as competition (e.g. egg ejection, infanticide). The groups are composed of related and unrelated individuals, and reproductive opportunities are not equivalent for all group members because some individuals may not reproduce in some of the nesting bouts (Quinn *et al.* 1994).

Past studies have focused upon clarifying aspects related to individual reproductive success and interactions leading to egg ejection and infanticide within guira cuckoo breeding groups. However, it is impossible to determine the number of females within each group from field observations because the species is sexually monochromatic and monomorphic (Sick 1997). Additionally, the number of females in a group may not be equivalent to the sub-group that is actually reproducing in any given nesting bout. Added difficulties in this species are (Macedo 1992): (1) adults are not easily caught in nets and also learn to avoid traps; (2) there exists a strong bias in capture, favoring males; and (3) egg ejection is prevalent at the onset of laying, before incubation starts, thus excluding the possibility of using embryonic tissue.

In this study, a new method was employed to ascribe maternal origin in guira cuckoo communal clutches from electrophoretic banding patterns of yolk proteins. To increase resolution of differences among individuals, protein extracts were digested with a protease analogously to the use of restriction enzymes in DNA based methods of resolving genetic differences. Our specific objectives were to determine the number of females laying eggs in guira cuckoo communal nests, the pattern of laying within nests, and whether females lay eggs in successive nesting bouts of the group. Such method should be widely applicable to other avian species, and highly suitable in the resolution of problems in evolutionary studies and related areas.

Materials and methods

Study area and species

We conducted the field study in the central Brazilian Plateau in a semi-urban area of 30 km^2 near Brasilia (15°47'S, 47°56'W; altitude 1.158 m) that includes fragments of the native Brazilian savanna (known as "cerrado"), cultivated fields, housing developments and residential gardens. Preferred nesting sites for guira cuckoos in this area include the introduced monkey puzzle trees, *Araucaria angustifolia*. Reproductive activity is mostly restricted to the rainy season, from mid-August to mid-March, with the peak reproductive activity of the guira cuckoos in our study area occurring in September and October.

Egg and female collection

In July 1998 and 1999 we searched for old nests, commonly re-used by breeding groups, and placed nylon fishing nets (mesh=15mm) camouflaged with *Araucaria* branches underneath nests in good conditions to collect ejected eggs. From August to December 1998 and 1999, we visited the nests every other day, and once reproductive activity was detected, we visited them daily. We recorded the maximum number of birds seen around the nest in successive visits. This may be considered an estimate of group size, but may be an underestime since birds were not banded. We collected ejected eggs from the net and non-ejected fresh eggs from the nest, which were substituted by dummy eggs. The birds readily accepted these dummy eggs, which were hand-painted chicken and pigeon eggs, or guira cuckoo eggs collected in another area. The dummy eggs were numbered sequentially as they were substituted to record any subsequent ejection. The fresh eggs were taken to the laboratory, measured in length and width, weighed, photographed, and then broken apart to separate the yolk and egg white (which were weighed separately and frozen at –20°C). The eggshell was also weighed and stored at room temperature. For validation of the yolk protein electophoresis method for the guira cuckoo we collected three guira cuckoo females in another area towards the end of the breeding season. Their ovaries were immediately frozen at –20°C. The yolk of individual follicles of these ovaries was used to validate that the yolk protein banding pattern of individual females is identical among their follicles and hence their eggs, and that different females have different patterns.

Polyacrylamide gel electrophoresis (PAGE)

To develop the protocol, we used 36 recently-laid eggs from nine individually-housed captive budgerigars, *Melopsittacus undulatus*. These analyses included eggs laid in the same and in different clutches of known females. We ran entire clutches on single gels to compare yolk protein polymorphism of eggs. We used this protocol then for the guira cuckoo.

We thawed the frozen yolks and punctured their membranes to extrude contents. Yolks were diluted 1:1 with distilled water and homogenized. Samples of the homogenate were then either: (1) mixed with 67 mM potassium phosphate buffer (pH 7.6) in a 1(homogenate):9 (buffer) proportion; or (2) mixed with 1 mM HCl plus trypsin (for protein digestion; SIGMA: bovine pancreas) and 67 mM potassium phosphate buffer in a 1(homogenate):2(enzyme):18 (buffer) proportion. The samples containing trypsin were incubated for 2 h at room temperature, and the digestions were stopped after incubation by heating to 94°C for 30 seconds. All samples (with or without the enzyme) were frozen until further analyses.

In PAGE analyses, all samples were diluted 1:4 with loading buffer (0.0625 M Tris-HCl (pH 6.8), 10% glycerol, 2% SDS, 5% 2-mercaptoethanol. 0.05% bromophenol blue), heated at 94°C for 90 seconds, and then cooled on ice. Gels were run under denaturing conditions using standard procedures based on Laemmli's (1970) protocol, i.e., proteins were separated according to their size. Separating gels consisted of 7.5% or 12.0% acrylamide as detailed by Laemmli (1970). Gels ran for 2 h at 20 mA and 80V at room temperature in Mini-PROTEAN Electrophoresis Cell (BIO-RAD). To maximize sensitivity and resolution proteins were visualized by silver staining (Morrissey 1981). To test for instability of the samples and reproducibility of the protein digestion procedure, replicate digestions and analyses were conducted on freeze/thawed yolk samples.

For determining maternity in guira cuckoo eggs we did not compare gels that belonged to different groups, since we were interested only in identifying eggs belonging to females within groups. However, for calculating probability that two random females shared the same banding patterns, we compared bands across gels to determine presence or absence in different individuals. Only well defined bands that could be reliably scored were used for maternal identification.

The validation of the protocol for guira cuckoos was made through PAGE analyses of yolk proteins in ovarian follicles from known females. Yellow, yolk-containing follicles, measuring 1.5-2.0 mm in diameter, were collected and processed according to Tyler (1993), with the following modifications: (1) Four individual follicles from each of the three females were manually homogenized separately in distilled water (w: v = 1:100); (2) the homogenate was then centrifuged at 1400 *g* for one min. Aliquots of 25 µl of supernatant were transferred to two new tubes and buffer only or enzyme and buffer were added to the tubes in the same proportion as for the egg yolk procedure. The subsequent protocol procedures for the follicle yolks were the same as for egg yolks (see above).

Results

The protocol that best resolved the bands for both budgerigar and guira cuckoo egg and follicle yolks was trypsin digestion followed by resolution on a 12.0% separating gel. Although we could see slight differences among samples not treated with enzyme, they were not as visible as those we obtained when trypsin was added. Therefore we applied trypsin digestion of yolk proteins before PAGE.

Results for the budgerigar analyses showed identical banding patterns of yolk proteins for all of the eggs from the same females, including eggs in the same and in different clutches, and different patterns for all of the eggs of distinct females (Fig. 1). For guira cuckoos, banding patterns for yolk proteins of follicles from the same female were identical, but different from follicles of distinct females.

During egg production, meiotic division starts just before ovulation, when the egg has already been yolked (Burley & Vadhera 1989). So the yolk proteins are gene products of a diploid situation, and maternally derived only. Thus, the identical banding patterns in eggs or follicles in the same female and the different pattern in eggs and follicles of different females can be used to identify eggs of unknown individual females. After this validation we applied the method to eggs of free-living guira cuckoo groups for further biological validation.

We monitored 27 nesting bouts from 14 active groups and 21 nesting bouts from 10 groups of guira cuckoos in 1998 and 1999, respectively. The number of adults seen at the nests ranged from one to eight individuals, with varying numbers of females (as determined by the electrophoresis of yolk proteins), and communal clutches in joint nests ranged from one to 19 eggs (Table 1). These were reduced through egg ejection, which averaged about 3.3 eggs/clutch for the two years. Of the total 171 eggs collected in 1998, 40 eggs were not suitable or available for electrophoresis because we found them during incubation, and 18 eggs were lost due to ejection (we found their eggshells broken on the ground). In 1999 we collected 126 eggs, of which 19 were not available for PAGE analyses (same reasons) and 25 were lost due to ejection. The majority of eggs for which we obtained no data (67% of 43 ejected eggs in both years) were first-laid eggs that were promptly ejected before the net was installed. Although we observed guira cukoos building new nests, we could not disturb them by placing nets until the birds started laying, to avoid desertion. Finally, for the PAGE analyses we used 113 eggs from 17 nesting bouts in 12 active groups in 1998 and 82 eggs from 17 nesting bouts in 10 groups in 1999.

There were no cases of identical yolk protein banding patterns of eggs laid on the same day within any nest. This includes 22 cases where two eggs were laid in the same nest on the same day, and 12 cases where three eggs were laid in a single nest on the same day. For example, in Figure 2 we show that all of the eggs laid within days 3, 4 and 5 in a joint nest have distinct banding patterns, indicating that in day 3 there were two females laying, and in days 4 or 5, three females contributed one egg each to the communal clutch. This further substantiates our method, because it is consistent with laying physiology which does not allow the laying of more than one egg per day by female birds (van Tienhoven 1983).

Identical banding patterns were always from eggs laid on different days of a nesting bout, which indicates that some females laid more than one egg in the laying sequence of the communal nest. This is also clearly seen in Figure 2 wherein of the 13 total eggs analyzed from that nesting bout, one female (B) laid four eggs, two females

(A and C) each laid three eggs, and one female (D) laid two eggs in different days. Only one female (E) laid a single egg in the total analyzed.

Additionally, in guira cuckoo groups in which we monitored renestings we frequently observed that some banding patterns of yolk proteins were repeated in successive nesting bouts, while others were not. At least 13 females laid eggs in two nesting bouts of the same group and at least one female laid eggs in three renestings of the same group. For example, in group D5, female C laid eggs in all three nesting bouts of her group, two of which (1 and 3) are shown in Figure 3. First laid eggs lost due to tossing or eggs being incubated in complete clutches were not analyzed, thus some laying females were possibly missed in our study.

Banding patterns of yolk proteins per communal clutch indicated that various females contribute eggs to nests, in repeated nesting bouts of each group. In rare instances, as many as seven females may lay simultaneously in a joint nest. In this exceptional case, seven females laid a total of 16 eggs: one female laid a single egg, three females each laid two eggs and three females each laid three eggs. The communal nature of reproduction in guira cuckoos is shown by the positive correlation between the number of laying females in joint nests with communal clutch size ($r^2 = 0.90$, $p < 0.001$, $n = 16$, Fig. 4), indicating a fairly equal partitioning of reproductive opportunities.

Discussion

The validation of our protocol included known female budgerigar clutches, guira cuckoo egg follicles, and biological aspects. Results suggest that yolk protein electrophoresis is an excellent tool for identifying maternity of eggs. The budgerigar and guira cuckoo analyses showed not only that our protocol was accurate in determining egg maternity for these birds due to sufficient female polymorphism for yolk proteins (Manwell & Baker 1975), but that with high likelihood the protocol can be used with other species.

Other supporting evidence for the protocol involves the scrutiny of banding patterns within and among nests of free-living guira cuckoo groups. First, all multiple eggs laid in a single nest on the same day had different patterns of proteins, practically nullifying the possibility that two or more females are represented by the same banding pattern. Second, eggs with the same pattern of bands were not laid on the same day but were repeated on different days, indicating that some females lay multiple eggs in successive days in a communal nest. Both results are also in agreement with physiological restraints that allow female birds to produce a single egg per day (van Tienhoven 1983).

Thirty-two (1998) and sixteen (1999) females were involved in laying of multiple eggs in a single nest on the same day. For these cases, within each year, no identical banding patterns of yolk proteins occurred in nests of different groups. However, even if none of the eggs laid on the same day within a nest had identical banding patterns, there is a probability that two different females share the same banding pattern. We estimated this probability using Andersson and Åhlund's (2001) approach for albumen bands in common goldeneye duck eggs. This estimate uses expressions applied in individual identification in DNA fingerprinting studies (Jeffreys et al. 1985 a,b; Burke & Bruford 1987; Georges et al. 1988). For this, we used eggs laid in nests at least 1 km apart, considerably reducing the chances that the same female laid eggs in more than one of these nests. We sampled a unique banding pattern from nests where there were no cases of multiple eggs laid within a 24h period (three eggs laid in three nests total) plus two or more banding patterns from nests where multiple eggs were laid on the same day (24 eggs from nine groups). Since a female cannot lay more than one egg per day, multiple eggs found in joint nests within a period of 24 h clearly belonged to different females. We estimated x, the mean probability (across all bands) that a band in one individual also occurs in another random individual; f, the mean number of bands per individual (we eliminated bands not clearly defined for this estimate); and q, the allele (band) frequency, estimated from $x = 2q - q^2$ (Jeffreys et al. 1985b). The results were: $x = 0.135$, $f = 4.81$, and $q = 0.070$. The mean probability that two random individuals will share identical banding patterns is thus $x^f = 0.135^{4.81} \approx 6.56 \times 10^{-5}$.

We also estimated this probability for the case of closely related females, although Quinn et al. (1994) showed that kinship among adults in guira cuckoo nests is relatively low. The probability of band sharing between siblings (p_{sib}) is $(1 + q - q^2) / (2 - q)$ or $(4 + 5q - 6q^2 + q^3) / 4\ (2 - q)$ depending on whether the band variation is non-allelic or allelic in Hardy-Weinberg equilibrium (Jeffreys et al. 1985 a). Using the first expression, two sisters will have a mean probability of $Ps = (p_{\text{sib}})^f = 0.552^{4.81} \approx 5.74 \times 10^{-2}$ of sharing an identical banding pattern. And using the second expression, this probability is $0.560^{4.81} \approx 6.15 \times 10^{-2}$. Additionally, we estimated the probability of a mother and a daughter sharing the same banding pattern (as seen in DNA fingerprinting (Georges et al. 1988; Rabenold et al. 1991), and in protein fingerprinting (Andersson &

Åhlund 2001)). Considering that the expected number of bands a female has inherited from her mother is $n_m = f(1 + q - q^2) / (2 - q)$, the expected number of bands she has inherited exclusively from her father is thus $n_p = f(1-(1 + q - q^2) / (2 - q))$. For the daughter and mother share identical banding pattern, these n_p bands inherited from the father must also be present in the mother. For each band, this probability is x. Thus, the probability that mother and daughter will have the same pattern of bands is $P_{m\text{-}d} = x^{n_p} = 0.135^{2.155} \approx 1.34 \times 10^{-2}$.

In our study there was one clutch to which seven females contributed eggs. Genetic analyses to determine maternity would require parental sampling for comparison of results, which would have been quite difficult to obtain given the high number of females and the difficulty in capturing them. And using only field observations, the occurrence of three new eggs per day in this nest would have indicated only that a minimum of three females were laying, when there were, in fact, seven females.

Some previous studies used allozymes analyses to discriminate between eggs of more than a single parasitic female of the same species laying in one nest (Fleischer 1985, Fleischer *et al.* 1985, Kendra *et al.* 1988, Fleischer & Smith 1992, Smyth *et al.* 1993). However, most of these studies could not reveal the exact number of females contributing eggs to nests, but only the minimum number. This occurred because detection of genetic differences was limited, and different females might present the same alleles and be mistakenly identified as the same female. This does not seem to occur in our analysis because every known budgerigar or guira cuckoo female had a particular banding pattern of yolk proteins. Andersson and Åhlund (2001) also found unique patterns of bands for egg white proteins of eggs from known common goldeneye duck females.

In this study we collected whole eggs (ejected as well as retained in the nests) because egg yolk, egg white and eggshell masses were required for other objectives. However, if only the identification of egg maternity is desirable, small yolk samples (mg) may be obtained by puncturing the eggshell with a needle to sample the yolk without embryonic damage, and eggs can be returned to the nest to continue development (see Schwabl 1993 for details).

Although guira cuckoo groups contain a number of females, they do not always contribute to each nesting bout of the group, and some females contribute more eggs to different clutches than do other females. This is somewhat different from what has been proposed in the literature for the groove-billed ani (*Crotophaga sulcirostris*), another crotophagine communal breeder, where females in the group are reported to contribute eggs to all nesting bouts (Vehrencamp 1977).

Thus, the biochemical technique proposed is the best current method to identify individual clutches in guira cuckoos and may be broadly applicable in other ornithological studies, especially when the use of genetic analyses is impossible. As in "protein fingerprinting" of egg white, this technique has several advantages over DNA methods, such as the ease in application and low cost involved (Andersson & Åhlund 2001). It can elucidate individual reproductive benefits and strategies of females in communal and cooperative breeding species, polygynous mating systems with joint nests, and in cases of intraspecific nest parasitism.

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References

Andersson M, Åhlund M (2001) Protein fingerprinting: a new technique reveals extensive conspecific brood parasitism. *Ecology*, **82**, 1433-1442.

Andersson M, Eriksson MOG (1982) Nest parasitism in goldeneyes *Bucephala clangula*: some evolutionary aspects. *American Naturalist*, **120**, 1-16.

Brown CR (1984) Laying eggs in a neighbor's nest: benefit and cost of colonial nesting in swallows. *Science*, **224**, 518-519.

Brown CR, Brown MB (1988) Genetic evidence of multiple parentage in broods of cliff swallows. *Behavioral Ecology and Sociobiology*, **23**, 379-387.

Brown JL (1987) *Helping and Communal Breeding in Birds*. Princeton University Press, Princeton, NJ.

Brown JL, Brown EL (1990) Mexican jays: uncooperative breeding. In: *Cooperative Breeding in Birds: Long-term Studies of Ecology and Behavior* (eds Stacey PB, Koenig WD), pp. 269-288. Cambridge University Press, Cambridge.

Burke T, Bruford MW (1987) DNA fingerprinting in birds. *Nature*, **327**, 149-152.

Burley RW, Vadhera DV (1989) *The Avian Egg: Chemistry and Biology*. John Wiley, New York.

Craig JL (1979) Habitat variation in the social organization of a communal gallinule, the Pukeko (*Porphyrio porphyrio*). *Behavioral Ecology and Sociobiology*, **5**, 331-358.

Davis DE (1942) The phylogeny of social nesting habits in the Crotophaginae. *Quarterly Review of Biology*, **17**, 115-134.

Fleischer RC (1985) A new technique to identify and assess the dispersion of eggs of individual brood parasites. *Behavioral Ecology and Sociobiology*, **17**, 91-99.

Fleischer RC, Murphy MT, Hunt LE (1985) Clutch size increase and intraspecific brood parasitism in the yellow-billed cuckoo. *Wilson Bulletin*, **97**, 125-127.

Fleischer RC, Smith NG (1992) Giant cowbird eggs in the nests of two icterid hosts: the use of morphology and electrophoretic variants to identify individuals and species. *Condor*, **94**, 572-578.

Georges M, Lequarré A-S, Castelli M, Hanset R, Vassart G (1988) DNA fingerprinting in domestic animals using four different minisatellite probes. *Cytogenetics and Cell Genetics*, **47**, 127-131.

Gowaty PA, Karlin AA (1984) Multiple maternity and paternity in single broods of apparently monogamous eastern bluebirds (*Sialia sialis*). *Behavioral Ecology and Sociobiology*, **15**, 91-95.

Jeffreys AJ, Brookfield JFY, Semenoff R (1985a) Positive identification of an immigration test-case using DNA fingerprints. *Nature*, **317**, 818-819.

Jeffreys AJ, Wilson V, Thein SL (1985b) Individual-specific 'fingerprints' of human DNA. *Nature*, **316**, 76-79.

Kendra PE, Roth RR, Tallamy DW (1988) Conspecific brood parasitism in the house sparrow. *Wilson Bulletin*, **100**, 80-90.

Koenig WD (1981) Reproductive success, group size, and the evolution of cooperative breeding in the acorn woodpecker. *American Naturalist*, **117**, 421-443.

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, **227**, 680-685.

Lahti DC, Lahti AR (2002) How precise is egg discrimination in weaverbirds? *Animal Behaviour*, **63**, 1135-1142.

Macedo RH (1992) Reproductive patterns and social organization of the communal guira cuckoo *(Guira guira)* in central Brazil. *Auk*, **109**, 786-799.

Macedo RH (1994) Inequities in parental effort and costs of communal breeding in the guira cuckoo. *Neotropical Ornithology*, **5**, 79-90.

Macedo RH, Bianchi CA (1997a) Communal breeding in tropical guira cuckoos *Guira guira*: sociality in the absence of a saturated habitat. *Journal of Avian Biology*, **28**, 207-215.

Macedo RHF, Bianchi CA (1997b) When birds go bad: circumstantial evidence for infanticide in the communal South American guira cuckoo. *Ethology, Ecology & Evolution*, **9**, 45-54.

Macedo RHF, Cariello M, Muniz L (2001) Context and frequency of infanticide in communally breeding guira cuckoos. *Condor*, **103**, 170-175.

Manwell C, Baker CMA (1975) Molecular genetics of avian proteins XIII. Protein polymorphism in three species of Australian passerines. *Australian Journal of Biological Sciences*, **28**, 545-557.

McKitrick MC (1990) Genetic evidence for multiple parentage in eastern kingbirds (*Tyrannus tyrannus*). *Behavioral Ecology and Sociobiology*, **26**,149-155.

Melo C (1997) *Reprodução comunitária em Guira guira Gmelin 1788 (Cuculidae, Aves): aspectos eto-ecológicos*. Masters thesis, Universidade de Brasília, Brazil.

Melo C, Macedo RHF (1997) Mortalidade em ninhadas de *Guira guira* (Cuculidae): competição por recursos? *Ararajuba*, **5**, 49-56.

Mϕller AP (1987) Intraspecific nest parasitism and anti-parasite behaviour in swallows, *Hirundo rustica*. *Animal Behaviour*, **35**, 247-254.

Morrissey JH (1981) Silver stain for proteins in polyacrylamide gels: a modified procedure with enhanced uniform sensitivity. *Analytical Biochemistry*, **117**, 307-310.

Quinn JS, Macedo R, White BN (1994) Genetic relatedness of communally-breeding guira cuckoos. *Animal Behaviour*, **47**, 515-529.

Rabenold P, Rabenold KN, Piper WH, Decker MD, Haydock J (1991) Using DNA fingerprinting to assess kinship and genetic structure in avian populations. In: *The Unity of Evolutionary Biology* (ed Dudley EC), pp.611-620. Dioscorides, Portland.

Schwabl H (1993) Yolk is a source of maternal testosterone for developing birds. *Proceedings of the National Academy of Sciences of the USA*, **90**, 11446-11450.

Sick H (1997) *Ornitologia Brasileira*. Nova Fronteira, Rio de Janeiro.

Smyth AP, Orr BK, Fleischer RC (1993) Electrophoretic variants of egg white transferrin indicate a low rate of intraspecific brood parasitism in colonial cliff swallows in the Sierra Nevada, California. *Behavioral Ecology and Sociobiology*, **32**, 79-84.

Tyler C (1993) Electrophoretic patterns of yolk proteins throughout ovarian development and their relationship to vitellogenin in the rainbow trout, *Oncorhynchus mykiss*. *Comparative Biochemistry and Physiology Part B*, **106**, 321-329.

van Tienhoven A (1983) *Reproductive Physiology of Vertebrates*, 2nd edn. Cornell University Press, Ithaca & London.

Vehrencamp SL (1977) Relative fecundity and parental effort in communally nestings anis, *Crotophaga sulcirostris*. *Science*, **197**, 403-405.

Vehrencamp SL, Bowen BS, Koford RR (1986) Breeding roles and pairing patterns within communal groups of groove-billed anis. *Animal Behaviour*, **34**, 347-366.

Wrege PH, Emlen ST (1987) Biochemical determination of parental uncertainty in white-fronted bee-eaters. *Behavioral Ecology and Sociobiology*, **20**, 153-160.

Yom-Tov Y (1980) Intraspecific nest parasitism in birds. *Biological Review*, **55**, 93-108.

Author information box

Mariana Cariello, a doctoral student at Universidade de Brasilia, Brazil, focused on maternal reproductive investment in guira cuckoos, a study within an ongoing project on the behavioural ecology of guira cuckoos in Brazil. She conducted the electrophoresis within a collaborative project between H Schwabl and RH Macedo. The field project was coordinated by RH Macedo while H Schwabl coordinated the development of the yolk protein electrophoresis method. R Lee, a professor in environmental physiology at Washington State University, has interests in the application of biochemical/molecular approaches to ecological investigations and provided expertise with the fine-tuning of the analyses.

Fig. 1. SDS-PAGE of yolk proteins of eggs from four individually-housed captive budgerigars. Note that each female had a unique pattern of bands, which was repeatable within and between clutches. Egg yolk proteins were digested by trypsin and fragments visualized by silver staining. The migration positions of the molecular weight standards are indicated on the left. On the right we indicate which bands were used in the diagnosis of maternity.

Fig. 2. SDS-PAGE of yolk proteins of eggs from the second nesting bout of guira cuckoo group B8, in which 13 of 15 eggs laid were analyzed. All eggs laid in each of days 3, 4 and 5 of the laying sequence had different banding patterns. Some identical banding patterns were repeated on different days. Egg yolk proteins were digested by trypsin and fragments visualized by silver staining. The migration positions of the molecular weight standards are indicated on the left. On the right we indicate which bands were used in the diagnosis of maternity.

Fig. 3. SDS-PAGE of yolk proteins of eggs from the first and third nesting bouts of the same guira cuckoo group (D5) indicating that the female represented by banding pattern C laid eggs in both bouts. Egg yolk proteins were digested by trypsin and fragments visualized by silver staining. The migration positions of the molecular weight standards are indicated on the left. On the right we indicate which bands were used in the diagnosis of maternity.

Fig 4. The relationship between the number of different females and communal clutch size for nests in which maternity of all eggs could be determined ($r^2 = 0.90$, $n = 16$, $P < 0.001$).

Table 1. Characteristics of reproductive groups of guira cuckoos from central Brazil in 1998 and 1999.

Variable	1998		1999	
	Mean ± SD (n)	Range	Mean ± SD (n)	Range
Number of adults / nest*	4.4 ± 1.9 (21)	1-7	4.7 ± 2.1 (16)	1-8
Number of females / nest**	3.2 ± 2.0 (17)	1-7	2.5 ± 1.4 (17)	1-5
Clutch size	6.6 ± 5.5 (26)	1-19	7.3 ± 3.4 (19)	1-17
Eggs tossed	2.2 ± 2.5 (25)	0-8	4.2 ± 4.0 (17)	0-12

* Maximum number of (unbanded) adults seen simultaneously in each nest visit.

** Minimum number of laying females in the nesting bout, considering that maternity of some eggs could not be determined.

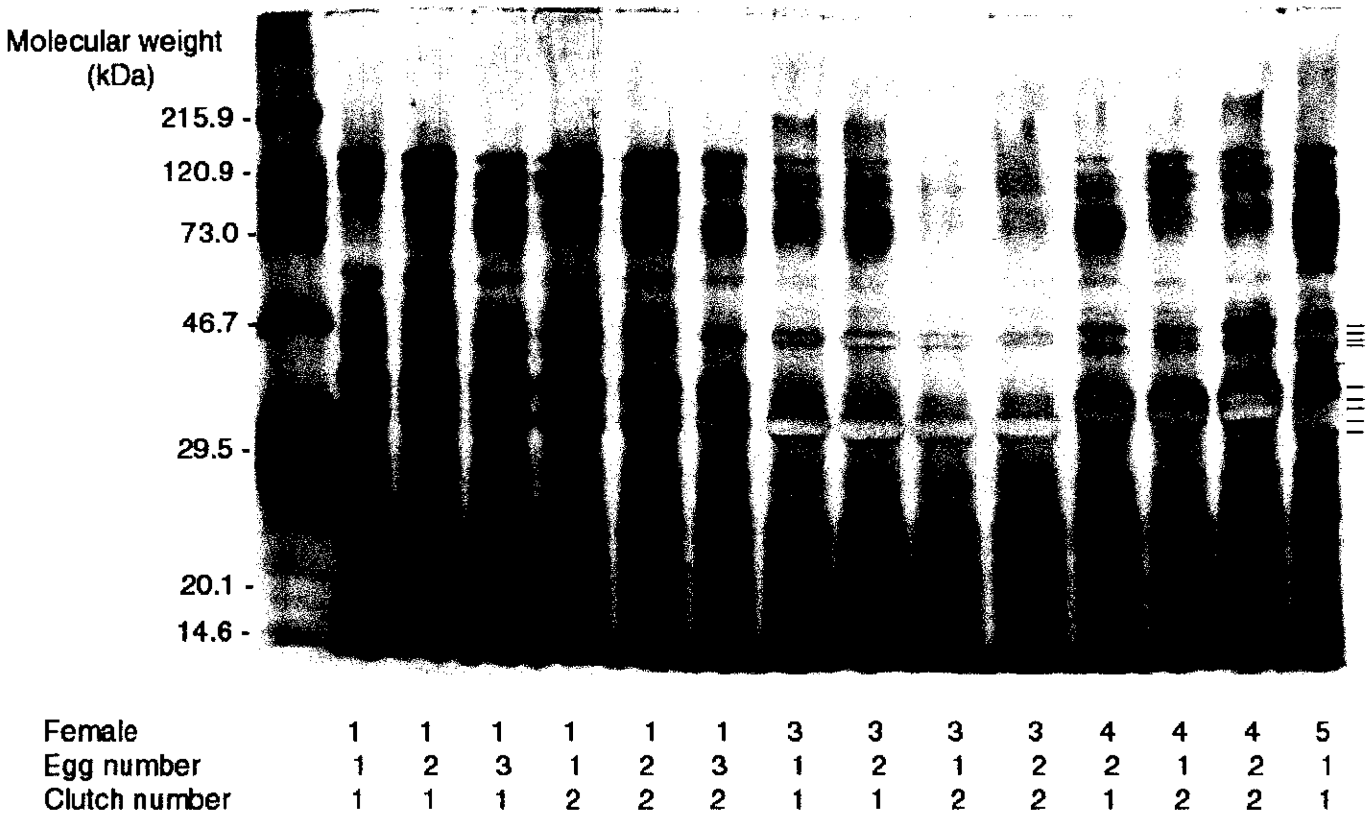

Figure 1

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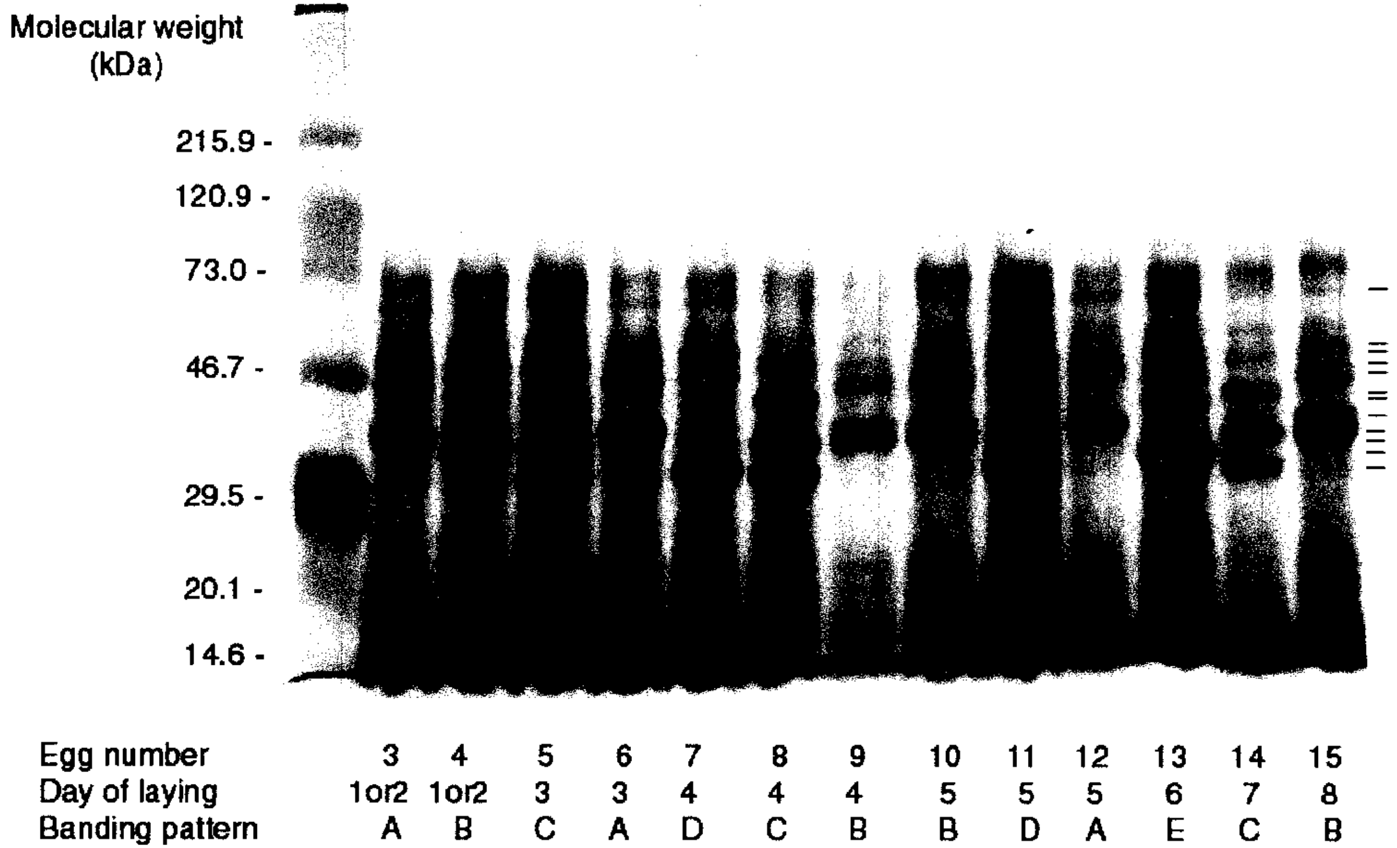

Figure 2

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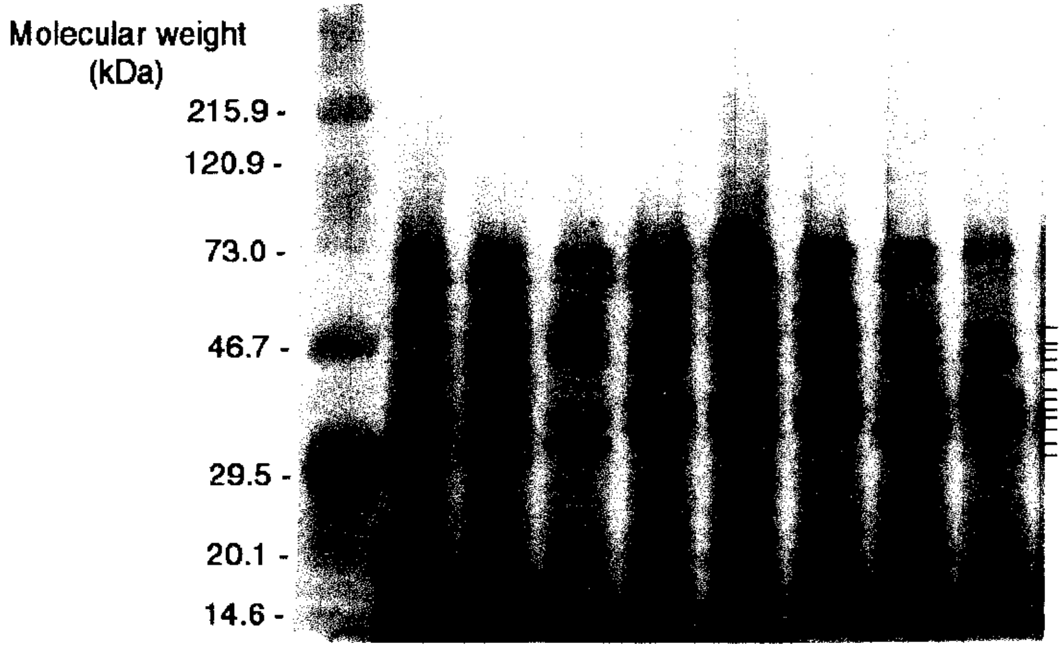

Nesting bout of group	1	1	1	1	1	1	3	3
Egg number	2	3	4	5	6	7	1	2
Day of laying	1	3	5	5	7	9	1	4
Banding pattern	A	A	B	C	C	C	D	C

Figure 3

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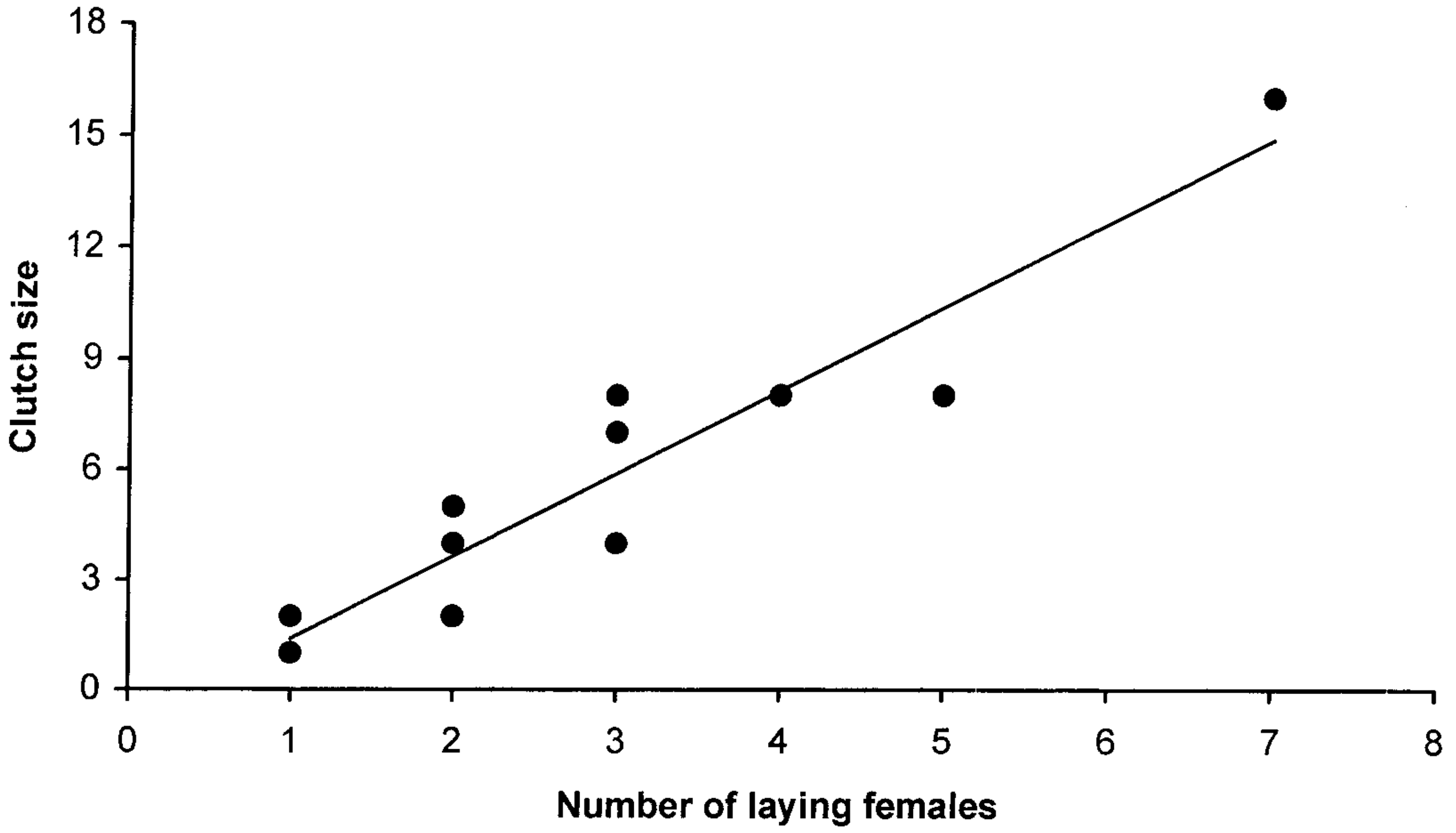

Figure 4

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CAPÍTULO II

Evaluating the use of egg characteristics to determine maternity in communal clutches of guira cuckoos (*Guira guira*)

Keywords: Guira guira, guira cuckoo, communal breeding, egg maternity, yolk protein electrophoresis, egg characteristics

Running title: EGG CHARACTERISTICS AND MATERNITY IN GUIRA CUCKOOS

Abstract

For determining egg maternity for birds in situations where two or more females lay eggs in a single nest (such as communal breeding systems or in cases of intraspecific parasitism), a few studies have used egg characteristics (e.g. shape, volume, eggshell speckling pattern), and have reported similar eggs as belonging to the same female and different eggs as laid by distinct females. Here, we assessed the applicability of egg morphometry and eggshell appearance in ascribing egg ownership in communal clutches of guira cuckoos (*Guira guira*), a species where up to seven females may lay eggs in a joint nest. We used both combined variables, which included egg mass, length, width, shape and two eggshell variables (number of color changes along a transect on eggshell and percentage of white color speckling), and shape alone to test whether eggs laid by each female were similar but different from eggs laid by other females. Additionally, we conducted discriminant function analyses to verify if eggs could be correctly classified to their mothers based on their characteristics. These analyses considered the correct maternity of freshly-laid eggs as determined by electrophoresis of yolk proteins. Individual female chutches were separated through egg characteristics or shape alone in 29.4% and 41.2% of the groups tested, respectively. In both cases where this occurred, differences were mostly due to a single female that differed from her nest-mates in a unique egg variable. On average, 55.5% of the eggs analyzed were not assigned to the correct mother using egg dimensions and eggshell speckling pattern. In conclusion, egg characteristics do not reliably indicate maternity in guira cuckoo commnunal clutches.

Introduction

In communal and polygynous breeders with complex reproductive tactics (Vehrencamp 1976, Bertram 1979, Craig 1979; Koenig 1981, Brown 1987, Brown and Brown 1990, Macedo 1992), or in cases of intraspecific nest parasitism (Yom-Tov 1980b, Andersson and Eriksson 1982, Brown 1984, MΦller 1987), multiple females of the same species may lay eggs in a joint nest. In these situations, egg maternity identification is important to evaluate reproductive tactics used by different females.

One criterion to identify individual female clutches in such situations is the use of egg appearance (e.g. color, shape, size, speckling pattern), with several studies attributing similar eggs to the same female, and different eggs to distinct females (Vehrencamp 1977, Yom-Tov 1980a, Yom-Tov 1980b, Loflin 1983, Gibbons 1986, MΦller 1987, Lyon 1997, Dugger and Blums 2001). Egg characteristics may be a good predictor of maternity, especially in species where inter-individual variation is high and intra-individual variation is low. This has been reported, for instance, for the herring gull *Larus argentatus* (Baerends and Hogan-Warburg 1982), the ring-billed gull *Larus delawarensis* (Fetterolf and Blokpoel 1984), the northern masked weaver *Ploceus taeniopterus* (Jackson 1992), and the emperor goose *Chen canagicus* (Petersen 1992).

For some bird species, however, there may be high variability in egg characteristics within clutches laid by the same female, limiting the use of these variables in correctly ascribing maternity. In particular, a female's first egg in the clutch (Henriksen 1995) or most commonly last egg (Coulson 1963, Mills and Shaw1980, Runde and Barret 1981, Quinn and Morris 1986, Meathrel and Ryder 1987, Viñuela 1997) are frequently different from her other eggs. Thus, a common pitfall is to assign the odd egg in a clutch to a different female, especially if the laying sequence is unknown (Brown and Sherman 1989, McRae 1997).

For this reason, in avian species where no previous evaluation of egg variability within and among female clutches has been performed, egg appearance should not be used to discriminate among eggs laid by potentially different females. Egg characteristics should only be used as a reliable method for ascribing maternity if validated, for instance, by identification of marked females during egg-laying or by biochemical methods such as protein electrophoresis or DNA fingerprinting (Bischoff and Murphy 1993, McRae and Burke 1996). The former may be time consuming and usually produces small sample sizes (but see Brown 1984). The latter is relatively easier and allows a larger sampling. However, DNA fingerprinting and electrophoresis of embryonic tissue may underestimate female contributions to the nest when clutch losses occur before embryo development or when female capturing is difficult, since these techniques require tissue sampling of both nestling and mother. In these situations, protein electrophoresis of recently-laid eggs may be the most useful tool for determining maternity of individual clutches (Fleischer 1985, Kendra *et al.* 1988, Smyth *et al.* 1993), and the use of total proteins rather than allozymes allows a more accurate maternal identification (Andersson and Åhlund 2001, Cariello et al. in press). Proteins of freshly-laid eggs are exclusively maternally derived, and any polymorphism reflects maternal genotype (Manwell and Baker 1975). Additionally, early egg sampling avoids losses due to clutch reduction.

Populations of the South American communally-breeding guira cuckoo (*Guira guira*) of central Brazil reproduce during the rainy season, from mid-August to mid-March, and up to five renests by breeding group have been recorded in one season

(Macedo 1992, Melo 1997). Reproductive guira cuckoo groups that may contain from two to 15 related and unrelated individuals use a joint nest, and may both cooperate (e. g. nest construction and defense, nestling feeding) and compete (e.g. egg ejection and infanticide) during breeding opportunities (Macedo 1992, 1994, Melo and Macedo 1997, Macedo et al. 2001). Approximately half of all eggs laid are lost due to ejection, which is prevalent at the onset of laying and is responsible for most of the communal clutch reduction. Joint-nests may contain up to 26 eggs, averaging 25.3 g (approximately 16% of adult weight), with average egg length and width of 42.5 mm and 31.8 mm, respectively (Macedo 1992). The eggs are turquoise, overlaid with a lace-like calcareous pattern of white splotches and streaks of considerable thickness (speckling), widely variable among eggs. During egg laying it is common to find two, three or even four eggs laid in a joint nest on the same day. The identification of individual clutches in communal nests of guira cuckoos is important to understand female reproductive behavior and strategies.

A new methodology for determining maternity of guira cuckoo eggs through egg yolk protein electrophoresis was developed recently, showing that up to seven females may contribute eggs to a communal clutch (Cariello et al. in press). This technique used yolk proteins in recently-laid (non-incubated) eggs, and allowed the sampling of eggs that were subsequently lost due to ejection. Additionally, female capturing was not necessary (egg proteins were of maternal origin only), which is particularly useful in the case of guira cuckoos, because capturing adults is very difficult and favors males, thus reducing the possibility of sampling female tissue for DNA analyses.

Our objective in this study was to evaluate the applicability of egg characteristics for ascribing egg ownership in communal clutches of guira cuckoos. We asked whether individual female clutches within joint nests could be separated on the basis of egg characteristics. We also evaluated whether all eggs laid in the joint nests could be correctly assigned to their mothers based on these characteristics. We considered the correct maternity of eggs as determined previously by electrophoresis of yolk proteins.

Materials and methods

Study area and field methods

The 30 km^2 study area is located near Brasilia, Distrito Federal, Brazil (15°47'S, 47°56'W; altitude 1,158 m), and includes fragments of the native savanna vegetation (known as "cerrado"), urbanized plots and cultivated fields. In this area, guira cuckoos nest predominantly in the introduced monkey puzzle trees, *Araucaria angustifolia*, and their peak reproductive activity occurs in September and October. In both field season years, 1998 and 1999, we started our activities in July by placing nylon fishing nets below nests in good conditions. Since guira cuckoos commonly re-use nests in different years and in consecutive nesting bouts within years, we used this procedure to collect eggs ejected from these nests. From August to December we visited old nests every two days until reproduction was initiated, then monitored them daily. Fresh non-ejected eggs were collected from nests and substituted by dummy eggs. Fresh ejected eggs were collected from the fishing nets without substitution. Dummy eggs were guira cuckoo eggs collected outside our study area, or chicken and pigeon eggs that resembled real guira cuckoo eggs in size and shape, and were painted to match guira cuckoo eggs in background color and speckling pattern. The introduction of dummy eggs did not disrupt patterns of egg laying and ejection (details in Macedo et al. submitted).

Egg characteristics

Fresh eggs collected in the field were taken to the laboratory where they were measured in maximum length (*L*) and width (*W*) with digital caliper (precision 0.1 mm), and weighed with a digital scale (precision 0.1 g). A shape index was calculated using the ratio of maximum diameter to length multiplied by 100 (Romanoff and Romanoff 1949), which was logarithmically transformed in all analyses (Zar 1999).

We photographed eggs using a 35 mm Canon camera with 50 mm lens with +1 and +2 magnification rings. All photographs were taken against a neutral background and at minimum focal distance. We developed the photographs in paper, scanned them, and analyzed images with the software UTHSCSA Image Tool for Windows (version 2.00 alpha 3). The scanned photographs of eggs had their maximum length and width calibrated appropriately by the software, and two speckling pattern measures were taken of each egg image by one person only. First, the average percentage of white speckling (n=5 measures) in a square was measured in the midsection of the egg. The square root of each average percentage was arcsine transformed for all analyses (Zar 1999). Second, the number of times a color change (blue/white) occurred along a transect of known length at 1/2 of total length on eggshell. Since most guira cuckoo eggs are equally elliptical at both ends, any measure of speckling pattern originating at the upper or lower quarters of the egg, or of the blunt or pointed ends (e.g., diameter at 1/4 or 3/4 length from a specific end) would be ambiguous in our study. Thus, although it would have been desirable to have a higher number of speckling variables measured, the guira cuckoo eggs with symmetrical ends prevent using them as points of reference.

After photographs were taken, we broke the eggs, weighed the yolk and albumen separately and froze them at –20°C. Eggshells were also weighed and stored at room temperature. This procedure was required for objectives other than those of this study. Yolk samples were further used in electrophoresis analysis of proteins for egg maternity identification (see below). However, for maternal identification only, minute yolk samples may be obtained by puncturing the eggshell with a needle, allowing the eggs to continue development with no embryonic damage (see Schwabl 1993 for details).

Female identification

The yolk protein electrophoresis analyses for maternal identification of guira cuckoo eggs are detailed elsewhere (Cariello et al. in press). Briefly, samples of homogenized yolk from recently-laid guira cuckoo eggs were digested by trypsin, incubated, and heated to 94°C for 30 seconds. Samples were diluted 1:4 with loading buffer (0.0625 M Tris-HCl (pH 6.8), 10% glycerol, 2% SDS, 5% 2-mercaptoethanol. 0.05% bromophenol blue), heated at 94°C for 90 seconds, and cooled on ice. Then, samples were run on 12% denaturing polyacrilamide gels according to Laemmli's (1970) protocol for 2 h at 20 mA and 80V at room temperature. Proteins were visualized by silver staining (Morrissey 1981). Yolk proteins in freshly-laid eggs are maternally derived, and thus identical patterns of bands indicated eggs laid by the same female, while different patterns indicated eggs laid by distinct females. This was validated for guira cuckoos through electrophoretic analyses of yolk proteins from oocytes from wild-living guira cuckoo females and from eggs of known female budgerigar *Melopsittacus undulatus* kept in captivity.

Statistical analyses

In statistical analyses we used all six egg characteristics measured: mass, length, width, shape, white speckling (%), and number of color changes. Discriminant function analysis was used to determine whether egg characteristics can distinguish eggs laid by each female within a group, and how well these variables do discriminate individual clutches. This procedure yields Jackknifed classification matrices to determine the percentage of correct assignment to mothers in each group. Means of all egg characteristics were compared among females of the same breeding group by multivariate analysis of variance (MANOVA). Additionally, we conducted a univariate analysis of variance (ANOVA) for testing whether shape alone differed for eggs laid by distinct females. We treated the variable shape separately because it is often the only characteristic found by researchers to discriminate among eggs of different females. All statistical analyses were conducted with SYSTAT software (version 9.0).

Results

We collected 297 eggs from 48 nests of 24 guira cuckoo groups during 1998 and 1999. Overall mean values (±SD; n) for each variable based on all eggs from all clutches were: mass 24.3g (±2.9; n=221); length 42.7 mm (±2.1; n=242); width 31.8 mm (±1.3; n=241); shape index 74.7 (± 3.6; n=241); percentage of white speckling in the mid-section of the eggshell 0.54 (±0.11; n=185); and number of times color change occurs along a transect at 1/2 of length 1.067 times/mm (±0.267; n=185). Maternal identification was available for 195 fresh-laid eggs. The remaining unidentified eggs included 59 developing eggs found during incubation in complete clutches and 43 eggs lost due to ejection; most of these were first-laid eggs in recently-built nests, for which we had to await clutch initiation to place nets and avoid desertion.

For the ANOVA, MANOVA and discriminant functions, we did not include nests where only a single female laid eggs or nests where all females each laid a single egg because, in the first case, we had no other female for comparative purposes, and in the second case, there was no variance to conduct analyses. Thus, for analyses we used 177 eggs belonging to 28 communal clutches from 17 guira cuckoo groups. We considered all the repeated nesting bouts of each reproductive group within each year together in the analyses because some females may contribute eggs to more than one bout of the same group (Cariello et al. in press, Macedo et al. submitted). The number of laying females in the groups studied was variable, averaging 4.4 and ranging from two to 10. Breeding groups laid on average 13.3 eggs in the season, ranging from three eggs laid by Group 10 over two nesting bouts to 28 eggs laid by Group 4 distributed over three bouts (Table 1).

In general, discriminant function analyses yielded low percentages of correct classifications of eggs to their mothers (22 to 75%), averaging 44.5% (Table 2). In only 29.4% of the groups were individual female clutches within communal nests separated through egg characteristics by the MANOVA analysis (Table 2). In these cases, however, the most useful variable for discriminating among females differed among the groups (mass, length, width or color changes per mm) and, in most cases (83,3%), the significant result was due to a single female that differed from others in her group. For instance, in Group 6, where at least 10 guira cuckoo females contributed 25 eggs to the commnunal clutches in a season (from which 24 were analyzed), only female E laid eggs that differed from those laid by females A, C, G and H. Eggs laid by female E had smaller length, and no other females differed in this group for this or other egg variables. In Group 9, female B laid broader eggs compared to eggs laid by females A, D and F, and this was the only difference among eggs laid by the six females in this group (Table 2). For those groups where MANOVA separated at least one laying female from the others based on egg appearance, there was, on average, 6.4 females, a significantly higher number compared to the average 3.6 laying females in groups where no difference between females was detected in any egg variable (*T*-test, t=-2.770, P=0.034; n=17). This indicates that with more females in a group, there is a greater chance that eggs from one or two differ from those of nest-mates with respect to at least one egg variable.

The egg shape variable was distinct among females in 41.2% of the analyzed groups. The only sources of differences detected included cases where two females differed from each other (42.9% of the cases) or a single female differed from the others in her group (57.1% of the cases; Table 3). In Group 12, only female A laid eggs with different shape compared to those laid by females B, C, D and E. Her eggs were broader and shorter compared to the eggs laid by other females in this group.

Thus, eggs laid by the same guira cuckoo female may be highly different with regard to some traits (Fig. 1), while eggs laid by different females within a group may be quite similar (Fig. 2). This makes it impossible to use egg characteristics to reliably determine individual clutches in most communal nests of this species, either through simple visual inspection of the eggs or by quantitative statistical analyses.

Discussion

Some previous studies have found that the same females identified by allozyme (Manwell and Baker 1975, Fleischer 1985, Fleischer and Smith 1992) or DNA analyses (McRae and Burke 1996) lay more similar eggs, morphometrically, in comparison to those laid by other females. For guira cuckoos, however, we found that egg maternity, determined through yolk protein electrophoresis, could not be correctly and/or consistently assigned using egg characteristics. On average, less than half (44.5%) of the eggs within breeding groups were assigned correctly to the mother. Additionally, the few differences detected among laying females within groups were due mostly to a single female differing from one or more nest-mates with regard to one egg variable, and such differences only occurred in groups where a higher number of females contributed eggs to the communal clutch. Thus, our results indicate that egg characteristics are not a reliable criterion to discriminate individual female clutches in guira cuckoo communal nests and should not be used for this purpose in this species.

Guira cuckoo females laying eggs that cannot be distinguished by their appearance in joint nests contradicts what has been proposed and used for two other crotophagine species. Vehrencamp et al. (1986) measured egg characteristics in communal nests of groove-billed anis *Crotophaga sulcirostris* and found that first layers laid eggs significantly larger than those laid by last layers. However, female identification was based on a previous study (Vehrencamp 1976) where first eggs, which were more similar to each other and different from later eggs, were assigned to the same females (designated first layers), while last eggs were considered as laid by other females (last layers). Additionally, the number of laying females in a nest was determined by the number of eggs found in the nest, i.e., it was assumed that each female contributes a typical number of eggs to the nest, from which groups of eggs were separated based on their appearance and assigned to one or more females. Loflin (1983), in a study conducted with the communally-breeding smooth-billed ani *Crotophaga ani*, also identified individual females based on egg dimensions, color, shape and weight, in addition to total communal clutch size. Unfortunately, in both groove-billed and smooth-billed ani studies, no quantitative assessment of within and between female variability of egg characteristics in marked individuals was conducted.

For guira cuckoos, as proposed previously for the two other crotophagine species, communal clutch size and number of laying females are positively correlated (Cariello et al. in press). However, the high variability in features of eggs laid by the same female indicates that this is an inaccurate method for ascribing maternity in this species.

Baerends and Hogan-Warburg (1982) showed for herring gulls *Larus argentatus* that although the last-laid egg within a clutch tended to deviate from the other two in size, shape, color and speckling pattern, this variance was significantly smaller than that among eggs from 100 clutches studied. Thus, after assurance that variation in egg measurements among females is higher than variation within females, researchers can safely use egg characteristics for detecting intraspecific nest parasitism in some cases (Fetterolf and Blokpoel 1984, Jackson 1992, Petersen 1992). On the contrary, in studies with some species (e.g. Coulson 1963: kittiwake *Rissa tridactyla*) no evidence was found that eggs within a single female clutch were sufficiently similar in appearance to accurately infer maternity, and most females could not be identified through egg characteristics in subsequent years. Brown and Sherman (1989) found that although the degree of variability in egg appearance within clutches was smaller than between clutches of cliff swallows *Hirundo pyrrhonota* and barn swallows *Hirundo rustica* when sample size was 26-30 nests, such differences tended not to occur when smaller numbers of nests were analyzed. Additionally, since intra-clutch variance in egg traits was the same for nests known to be parasitized and nonparasitized, the authors concluded that differences in egg appearance probably are not reliable indicators of brood parasitism in these species.

One of the most important reasons for conducting previous evaluation of variability in eggs is that in many species one egg in a female's clutch (usually the last one) may differ from the others (Mills and Shaw 1980, Meathrel and Ryder 1987, Viñuela 1997), and such difference may be greater than that between different females' eggs. In studies of intraspecific nest parasitism, Brown and Sherman (1989) and McRae (1997) admonish caution in assigning the last-laid egg of a clutch to another female based solely on its characteristics, and recommend that egg appearance variables be used combined with laying sequence data and daily checking. This methodology may be useful in some intraspecific nest parasitism studies, but may be an impossible task for identifying individual female clutches in joint nests. In guira cuckoo communal nests, daily checking and yolk electrophoretic analyses allowed us to verify that females: (1) lay eggs at irregular intervals; (2) do not contribute the same numbers of eggs to the joint nest; and (3) do not follow similar patterns of laying. Thus, in this communal joint nester, there are probably physiological as well as behavioral contraints limiting the number of eggs females lay and their order of entering the laying sequence. This makes it impossible to use laying sequence data to predict the exact number of laying females at a nest.

Egg morphometry and other egg characteristics have proven useful in defining maternity in a number of avian species. However, based on quantitative data from previous studies and on our own results, we strongly recommend that egg characteristics be used for determining egg ownership in shared nests only when prior assesment in egg dimensions and/or speckling patterns confirm that eggs laid by different females can be distinguished by their features.

In the context of female reproductive strategies, it is worthwhile to speculate why guira cuckoo females lay eggs with such high variability. In some species it is thought that the high variation in egg traits among individuals may have evolved as a counteradaptation to nest parasitism. This would function in species with both intra- as well as interspecific parasitism because females may be able to reject eggs according to their degree of difference in appearance from their own eggs (Lahti and Lahti 2002). Egg characteristics of guira cuckoos may be under similar selection pressures. It may be profitable, from the individual perspective, to maintain high egg variability within the clutch and decrease the possibility of egg recognition and possible ejection by nest-mates. Thus, the maintenance of communal breeding in this species may partly derive from individual variability in egg traits. If females cannot recognize their own eggs, then there may be a cost associated with the ejection of any egg from the nest. In other words, in guira cuckoo nests, where up to seven females may lay eggs, if each female has a unique egg pattern and is able to recognize it, possibly they would be able to eject all other females' eggs and communal clutches would fail. We suggest that this hypothesis is compatible with several predictions concerning female reproductive strategies that are not mutually exclusive: (1) females do not recognize their own eggs and eject only before they start laying their own clutches; (2) females (and males) that are being excluded from a nesting bout engage in egg ejection and do not need to recognize eggs for ejection purposes; (3) some females, which do produce unique eggs and can distinguish them from other females', engage heavily in ejection of others' eggs; (4) females that do not recognize their own eggs eject in proportion to the number of eggs they have laid; in other words, if they have laid few eggs they exhibit a high ejection rate which will decrease as they lay more of their own eggs in the joint nest. Future studies that include observation of egg ejection by marked individuals are necessary to substantiate any of the above predictions.

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References

Andersson M, Åhlund M (2001) Protein fingerprinting: a new technique reveals extensive conspecific brood parasitism. *Ecology*, **82**, 1433-1442.

Andersson M, Eriksson MOG (1982) Nest parasitism in goldeneyes *Bucephala clangula*: some evolutionary aspects. *American Naturalist*, **120**, 1-16.

Baerends GP, Hogan-Warburg AJ (1982) The external morphology of the egg and its variability. *Behaviour*, **82**, 1-32.

Bertram, BCR (1979) Ostriches recognise their own eggs and discard others. *Nature*, **279**, 233-234.

Bischoff CM, Murphy MT (1993) The detection of and responses to experimental brood parasitism in eastern kingbirds. *Animal Behaviour*, **45**, 631-638.

Brown CR (1984) Laying eggs in a neighbor's nest: benefit and cost of colonial nesting in swallows. *Science*, **224**, 518-519.

Brown CR, Sherman LC (1989) Variation in the appearance of swallow eggs and the detection of intraspecific brood parasitism. *Condor*, **91**, 620-627.

Brown JL (1987) *Helping and Communal Breeding in Birds*. Princeton University Press, Princeton, NJ.

Brown JL, Brown EL (1990) Mexican jays: uncooperative breeding. In: *Cooperative Breeding in Birds: Long-term Studies of Ecology and Behavior* (eds Stacey PB, Koenig WD), pp. 269-288. Cambridge University Press, Cambridge.

Cariello M, Schwabl H., Lee R, Macedo RH (in press). Individual female clutch identification through yolk protein electrophoresis in the communally-breeding guira cuckoo (*Guira guira*). *Molecular Ecology*.

Coulson JC (1963) Egg size and shape in the kittiwake (*Rissa tridactyla*) and their use in estimating age composition of populations. *Proceedings of the Zoological Society of London*, **140**, 211-227.

Craig JL (1979) Habitat variation in the social organization of a communal gallinule, the Pukeko (*Porphyrio porphyrio*). *Behavioral Ecology and Sociobiology*, **5**, 331-358.

Dugger BD, Blums P (2001) Effect of conspecific brood parasitism on host fitness for tufted duck and common pochard. *Auk*, **118**, 717-726.

Fetterolf PM, Blokpoel H (1984) An assessment of possible intraspecific brood parasitism in ring-billed gulls. *Canadian Journal of Zoology*, **62**, 1680-1684.

Fleischer RC (1985) A new technique to identify and assess the dispersion of eggs of individual brood parasites. *Behavioral Ecology and Sociobiology*, **17**, 91-99.

Fleischer RC, Smith NG (1992) Giant cowbird eggs in the nests of two icterid hosts: the use of morphology and electrophoretic variants to identify individuals and species. *Condor*, **94**, 572-578.

Gibbons DW (1986) Brood parasitism and cooperative nesting in the moorhen, *Gallinula chloropus*. *Behavioral Ecology and Sociobiology*, **19**, 221-232.

Henriksen K (1995) Intraclutch variation in egg volume of great crested grebes. *Condor*, **97**, 826-828.

Jackson WM (1992) Estimating conspecific nest parasitism in the northern masked weaver based on within-female variability in egg appearance. *Auk*, **109**, 435-443.

Kendra PE, Roth RR, Tallamy DW (1988) Conspecific brood parasitism in the house sparrow. *Wilson Bulletin*, **100**, 80-90.

Koenig WD (1981) Reproductive success, group size, and the evolution of cooperative breeding in the acorn woodpecker. *American Naturalist*, **117**, 421-443.

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, **227**, 680-685.

Lahti and Lahti (2002). How precise is egg discrimination in weaverbirds? *Animal Behaviour*, **63**, 1135-1142.

Loflin RK (1983) Communal behaviors of the smooth-billed ani. Ph.D. dissertation, University of Miami, Coral Gables, FL.

Lyon BE (1997) Spatial patterns of shiny cowbird brood parasitism on chestnut-capped blackbirds. *Animal Behaviour*, **54**, 927-939.

Macedo R H (1992) Reproductive patterns and social organization of the communal guira cuckoo *(Guira guira)* in central Brazil. *Auk*, **109**, 786-799.

Macedo RH (1994) Inequities in parental effort and costs of communal breeding in the guira cuckoo. *Neotropical Ornithology*, **5**, 79-90.

Macedo RHF, Cariello M, Muniz L (2001) Context and frequency of infanticide in communally breeding guira cuckoos. *Condor*, **103**, 170-175.

Macedo RHF, Cariello MO, Schwabl H (submitted) Reproductive partitioning in communally breeding guira cuckoos, *Guira guira*. *Behavioral Ecology and Sociobiology*.

Manwell C, Baker CMA (1975) Molecular genetics of avian proteins XIII. Protein polymorphism in three species of Australian passerines. *Australian Journal of Biological Sciences*, **28**, 545-557.

McRae SB (1997) Identifying eggs of conspecific brood parasites in the field: a cautionary note. *Ibis*, **139**, 701-704.

McRae SB, Burke T (1996) Intraspecific brood parasitism in the moorhen: parentage and parasite-host relationship determined by DNA fingerprint. *Behavioral Ecology and Sociobiolgy*, **38**, 115-129.

Meathrel CE, Ryder JP (1987) Intraclutch variation in the size, mass and composition of ring-billed gull eggs. *Condor*, **89**, 364-368.

Melo C (1997) *Reprodução comunitária em Guira guira Gmelin 1788 (Cuculidae, Aves): aspectos eto-ecológicos*. Masters thesis, Universidade de Brasília, Brazil.

Melo C, Macedo RHF (1997) Mortalidade em ninhadas de *Guira guira* (Cuculidae): competição por recursos? *Ararajuba*, **5**, 49-56.

Mills JA, Shaw PW (1980) The influence of age on laying date, clutch size, and egg size of the white-fronted tern, *Sterna striata*. *New Zealand Journal of Zoology*, **7**, 147-153.

Møller AP (1987) Intraspecific nest parasitism and anti-parasite behaviour in swallows, *Hirundo rustica*. *Animal Behaviour*, **35**, 247-254.

Morrissey JH (1981) Silver stain for proteins in polyacrylamide gels: a modified procedure with enhanced uniform sensitivity. *Analytical Biochemistry*, **117**, 307-310.

Petersen MR (1992) Intraspecific variation in egg shape among individual emperor geese. *Journal of Field Ornithology*, **63**, 344-354.

Quinn JS, Macedo R, White BN (1994) Genetic relatedness of comunally-breeding guira cuckoos. *Animal Behaviour*, **47**, 515-529.

Quinn JS, Morris RD (1986) Intraclutch egg-weight apportionment and chick survival in caspian terns. *Canadian Journal of Zoology*, **64**, 2116-2122.

Romanoff AL, Romanoff AJ (1949) *The avian egg*. Jonh Wiley & Sons, Inc., New York.

Runde OJ, Barrett RT (1981) Variations in egg size and incubation period of the kittiwake *Rissa tridactyla* in Norway. *Ornis Scandinavica*, **12**, 80-86.

Schwabl H (1993) Yolk is a source of maternal testosterone for developing birds. *Proceedings of the National Academy of Sciences of the USA*, **90**, 11446-11450.

Smyth AP, Orr BK, Fleischer RC (1993) Eletrophoretic variants of egg white transferrin indicate a low rate of intraspecific brood parasitism in colonial cliff swallows in the Sierra Nevada, California. *Behavioral Ecology and Sociobiology*, **32**, 79-84.

Systat 9 statistics (1999) SPSS Inc.

UTHSCSA image tool for windows version 2.00 alpha 3 (1998) The University of Texas Health Science Center in San Antonio.

Vehrencamp SL (1976) The evolution of communal nesting in groove-billed anis. Ph.D. dissertation, Cornell University, Cornell.

Vehrencamp SL (1977) Relative fecundity and parental effort in communally nesting anis, *Crotophaga sulcirostris*. *Science*, **197**, 403-405.

Vehrencamp SL, Bowen BS, Koford RR (1986) Breeding roles and pairing patterns within communal groups of groove-billed anis. *Animal Behaviour*, **34**, 347-366.

Viñuela J (1997) Adaptation vs. constraint: intraclutch egg-mass variation in birds. *Journal of Animal Ecology*, **66**,781-792.

Yom-Tov Y (1980a) Intraspecific nest parasitism among dead sea sparrows *Passer moabiticus*. *Ibis*, **122**, 234-237.

Yom-Tov Y (1980b) Intraspecific nest parasitism in birds. *Biological Review*, **55**, 93-108.

Zar, JH (1999) Biostatistical analysis. 4th ed. Prentice Hall, Upper Saddle River, N.J.

Fig.1. Two eggs laid by female C in the same nesting bout of Group 5. Egg characteristics for egg on left were: mass 21.8g; length 40.5mm; width 30.8 mm; shape 76.0; white speckling percentage 0.54; color changes/mm 0.748. Egg characteristics for egg on right were: mass 22.9g; length 42.5mm; width 31.4 mm; shape 73.9; white speckling percentage 0.95; color changes/mm 0.389.

Fig. 2. Two eggs laid by different females in consecutive nesting bouts of the Group 13. Egg on left was laid by female A and its measures were: mass 21.8g; length 39.0mm; width 31.8 mm; shape 81.5; white speckling percentage 0.60; color changes/mm 1.521. Egg on right was laid by female C and its measures were: mass 22.8g; length 40.1mm; width 31.9 mm; shape 79.6; white speckling percentage 0.69; color changes/mm 1.299. In this group, eggs laid by females A and C were not different with regard to their characteristics.

Table 1. Reproductive parameters of the 17 guira cuckoo groups monitored in 1998 and 1999 in central Brazil used in the uni- and multivariate analyses of variance and in the discriminant functions for determining maternity of eggs based on their characteristics.

Year	Group	Number of nesting bouts	Total eggs in communal clutches	Number of laying females identified
1998	1	1	9	4
	2	1	4	2
	3	1	5	2
	4	3	28	6
	5	1	14	5
	6	3	25	10
	7	1	11	5
	8	1	15	5
	9	1	15	6
	10	2	3	2
1999	11	3	22	6
	12	2	24	5
	13	2	9	3
	14	1	4	3
	15	2	15	4
	16	2	17	5
	17	1	6	2

Table 2. Percentage of correct maternal classification of eggs, and difference among individual female clutches based on egg characteristics in 17 communally-breeding guira cuckoo groups monitored in 1998 and 1999 in central Brazil.

Year	Group	Correct classification of all eggs (%)[1]	Difference among females within groups[2]	Most important variable discriminating females	Different females[a]
1998	1	22	no	-	-
	2	75	no	-	-
	3	40	no	-	-
	4	52	yes	mass / color changes per mm	B≠D; C≠D / A≠B; B≠C; B ≠ D
	5	62	yes	mass	A≠B; A≠E; B≠C; B≠D; C≠E; D≠E
	6	38	yes	length	A≠E; C≠E; E≠G; E≠H
	7	33	no	-	-
	8	23	no	-	-
	9	64	yes	width	A≠B; B≠D; B≠F
	10	67	no	-	-
1999	11	50	no	-	-
	12	53	yes	length	A≠B; A≠C; A≠D
	13	44	no	-	-
	14	25	no	-	-
	15	33	no	-	-
	16	36	no	-	-
	17	40	no	-	-

[1] By Jackknifed classification matrix.

[2] MANOVA using Wilks' λ test; significant level at $P < 0.05$.

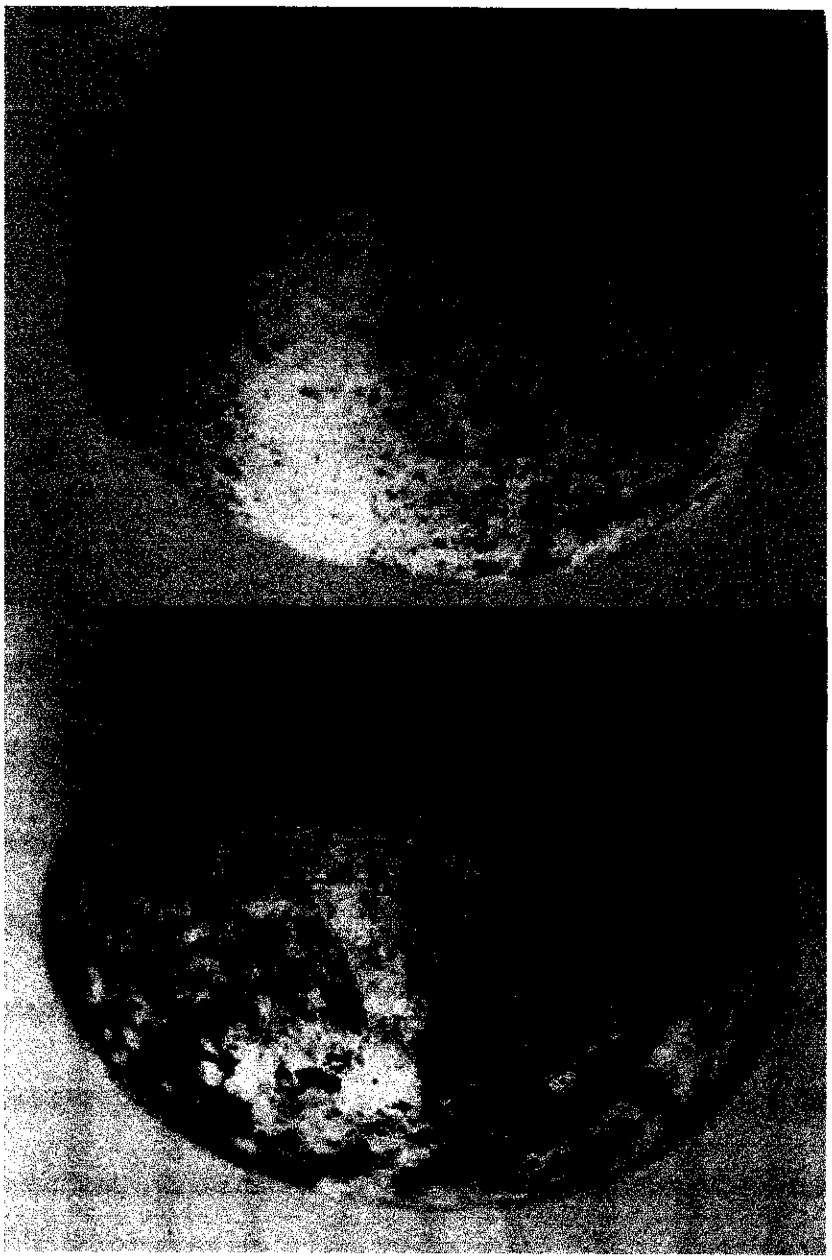
[a] Each letter designates a single female within her group.

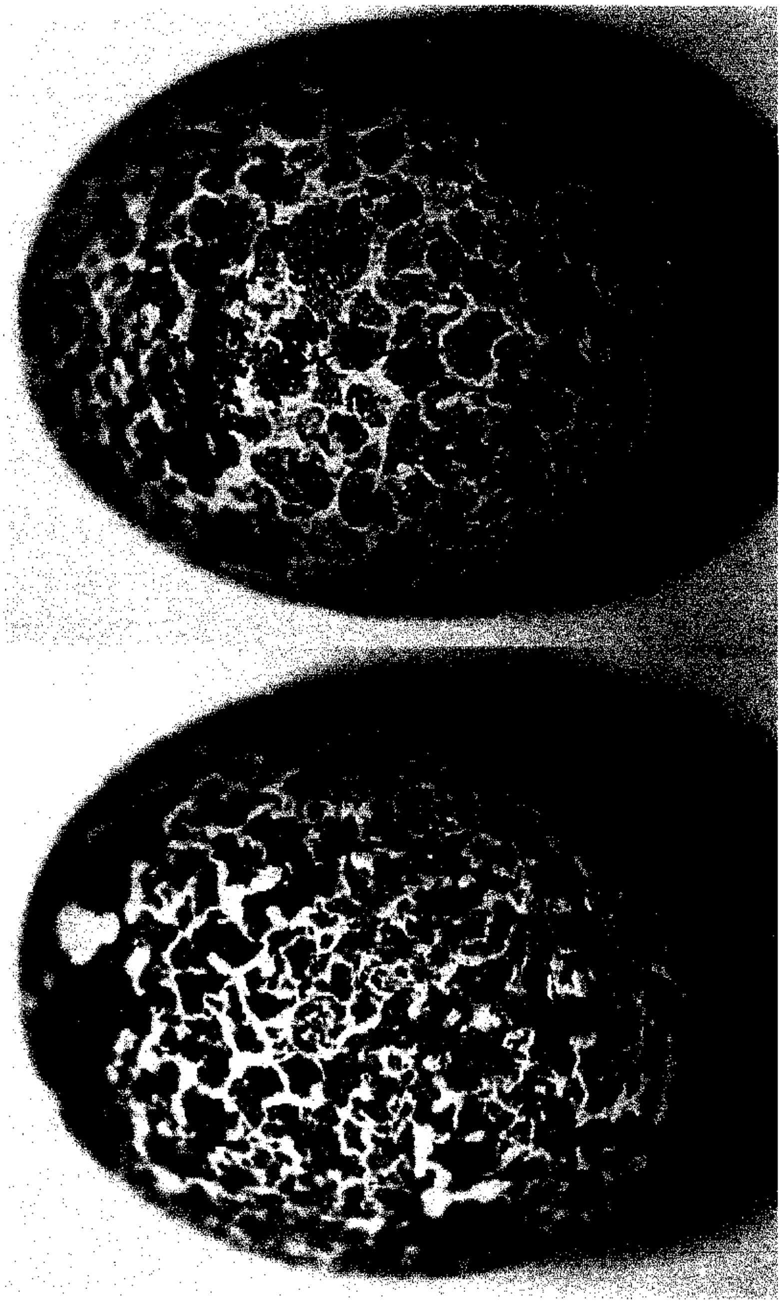
Table 3. Comparison of shape variables among eggs laid by different females in communal clutches of 17 guira cuckoo groups monitored in 1998 and 1999 in central Brazil.

Year	Group	ANOVA test		Different females[a]
		F-ratio	*P*	
1998	1	2.738	0.176	-
	2	4.685	0.163	-
	3	2.967	0.183	-
	4	1.486	0.252	-
	5	2.044	0.181	-
	6	4.516	0.006*	A≠F; A≠G; A≠I
	7	1.185	0.437	-
	8	3.962	0.046*	trend for A≠C
	9	5.561	0.041*	A≠B
	10	3.022	0.332	-
1999	11	4.143	0.045*	B≠E
	12	10.999	<0.001*	A≠B; A≠C; A≠D; A≠E
	13	10.690	0.011*	A≠B; B≠C
	14	0.878	0.602	-
	15	4.189	0.079	-
	16	6.374	0.024*	A≠C; C≠E
	17	0.681	0.470	-

[a] Each letter designates a single female within her group.

* *P* significance at 0.05.





CAPÍTULO III

Reproductive partitioning in communally breeding guira cuckoos, *Guira guira*

RRH: REPRODUCTIVE PARTITIONING IN GUIRA CUCKOOS

Abstract

Guira cuckoos, *Guira guira*, exhibit a rare polygynandrous reproductive system with groups containing several male and female breeders, which is suitable for testing reproductive skew models. Female reproductive strategies involve leaving the group, varying clutch size, egg ejection and infanticide, among others. Here we examined the predictions of reproductive skew models relative to reproductive partitioning among females in groups. We used yolk protein electrophoresis to identify individual females' eggs in joint nests. We found that reproductive partitioning favors early-laying females, which lay and incubate more eggs compared to females that enter laying later. Because females do not retain the order of entering the laying sequence in repeated nesting bouts of groups, nor always contribute eggs to each nesting bout, females tend to obtain fairly equitable reproductive success over time. The pattern of reproductive partitioning differs from that described for anis, another crotophagine joint-nester. We calculated reproductive skew indices for groups in two years, for both laying and incubation, as well as an overall population value. Varying degrees of skew were found for different groups, and also across sequential nesting bouts of the same groups. Nests that reached incubation tended to have lower skew values during the laying phase. Groups had higher reproductive skew indices in their first nesting bout of the season, and these nests frequently were unsuccessful. These results illustrate the importance of social organization in determining not only individual, but group success, in reproduction, and highlight the flexibility of vertebrate social behavior.

Keywords: communal breeding, guira cuckoo, *Guira guira*, reproductive skew, yolk protein electrophoresis.

Introduction

Hamilton's rule and inclusive fitness model (Hamilton 1964) have served as guiding principles to understand the evolution of sociality. Since the 1970s there has been an explosion in the number of published studies on the evolution of communal breeding, in which adults share parentage of offspring, for birds, mammals and insects (Woolfenden and Fitzpatrick 1984; Brown 1987; Koenig and Mumme 1987; Jamieson 1997). The relevance of evolutionary theory to these systems instigated numerous field studies and the development of theoretical models. Several of these models, produced over a decade ago (Vehrencamp 1979, 1983a,b; Emlen 1982; Emlen and Vehrencamp 1983) and more recently (Reeve and Ratnieks 1993; Keller and Reeve 1994; Reeve 1998), have attempted to explain patterns of reproductive opportunities for individuals living in groups, usually in terms of the degree to which reproduction is biased, or skewed, in favor of dominant individuals. These transactional or 'concessions' models assume that dominants can control breeding opportunities within the group and only provide sufficient reproduction to persuade subordinates to stay. Such models predict high skew when group members are closely related, when ecological constraints reduce the chances of dispersal and independent breeding by subordinates, and when group living yields considerable advantages.

Recent work testing reproductive skew models has been done primarily on social insects (Bourke and Heize 1994; Heinze 1995; Reeve and Keller 1995). Data on the reproductive skew of communally breeding vertebrates are still too scarce to evaluate the merits or generality of skew models (Johnstone and Cant 1999b). Social vertebrates are especially important to test these models because of their social plasticity (Jamieson 1997). Evidence of obvious conflict within groups in some species suggests that dominants frequently are unable to exert control over subordinate reproduction (Clutton-Brock 1998), and has led to the development of alternative models. In these, some important and previously neglected issues were addressed, including the partitioning of reproduction among subordinates in multimember groups, skew through infanticide, and incomplete reproductive control by dominants (Reeve et al. 1998; Johnstone and Cant 1999a,b; Johnstone et al. 1999).

This study attempts to assess reproductive skew patterns in a polygynandrously breeding bird with a complex and variable social structure. Guira cuckoos (*Guira guira*) are joint nesting, communally breeding birds that occupy savanna regions of South America. They live and reproduce in groups that may have upwards of 15 members, and only rarely nest in pairs. Membership in groups is retained over at least four years (RH Macedo, pers. observ. 2002), and probably beyond. A long-term study of a population in central Brazil revealed that these birds are polygynandrous joint nesters, with communal clutches of as many as 26 eggs (Macedo 1992; Quinn et al. 1994). Reproductive conflict in this species is evident: 72% of communal clutches (n = 176) monitored to date exhibited egg ejection (mean communal clutch = 8.01 eggs and mean ejected eggs = 4.30). Once chicks hatch, broods are usually further reduced (80% of 75 broods monitored to date), with at least 50% of this reduction probably due to infanticide (Macedo and Melo 1999).

In a genetic study of a limited sample of four breeding groups, it was found that kinship among co-breeders is generally low and quite variable among groups (Quinn et al. 1994). Of 36 adults in the groups, 21 (58%) were sampled, and of the possible 46 pairwise combinations of adults within the groups, only six pairs (13%) had high enough similarity coefficient scores (D) to be considered closely related. Moreover, a study on ecological constraints in this population suggested that the habitat is not only unsaturated, but that empty sites are of high quality and would be available for nesting, if individuals were to leave their groups (Macedo and Bianchi 1997).

Guira cuckoos and anis (subfamily Crotophaginae, Cuculidae) are taxonomically closely related and also exhibit striking similarity in their reproductive behaviors, which include joint nesting and egg ejection or burying behavior (Vehrencamp 1977; Loflin 1983; Macedo 1992). In her pioneer work on communal breeding in anis *Crotophaga sulcirostris*, Vehrencamp (1977) suggested that these birds provide an excellent example of a fairly egalitarian society, where subordinates are allowed some reproduction. However, despite some subordinate reproductive success, Vehrencamp (1977) proposed the existence of a definitive female hierarchy within the group, leading to significant differences in the number of eggs each female retains in the nest.

Here we assess the guira cuckoo system in light of the predictions generated by the concessions models. Given the above characteristics of the species—low relatedness among co-breeders and relative lack of ecological constraints—the concessions models predict a low level of reproductive skew among females. We also compare the guira cuckoo reproductive system with the more specific reproductive model proposed by Vehrencamp (1977) for anis. We used a newly developed biochemical method to identify the eggs of individual guira cuckoo females in communal clutches.

Methods

Study Site and Egg Collection

We monitored reproduction of guira cuckoo groups from a population in a semi-urban area of 30 km^2 (15°47'S, 47°56'W) near Brasilia, Brazil from August to December of

1998 and 1999. After nesting activity was detected, we visited nests daily to check for freshly laid eggs. We collected 297 eggs from nests (most within five hours of laying, and some after incubation started), substituting them with dummy eggs. These dummy eggs were numbered sequentially during substitution and monitored to record ejection activity, which was possible by the placement of nylon nets beneath nests to catch both real and dummy eggs that were ejected. Visits to the nests ceased when the birds had started incubating the clutch of dummy eggs, or until ejection of all eggs, which is common in unmanipulated nests, eventually led to desertion.

Dummy eggs were either: (a) guira cuckoo eggs collected outside the study area; or (b) guinea hen or pigeon eggs that approximated guira cuckoo eggs in size and shape. The non-guira cuckoo dummy eggs were hand-painted with non-toxic acrylic paint to match the blue-green background color of guira cuckoo eggs, while the eggshell markings were replicated using white-out paint.

To detect whether dummy eggs disrupted normal ejection patterns, we compared the percentage of ejected eggs per nest from these nests with those studied during the same period in other years (1987, 1988, 1994), where no dummy eggs were used. We performed a normalizing arcsine transformation (Zar 1999) on this variable. There was no difference in the percentage of tossed eggs among years when no dummy eggs were used (ANOVA, $F_{1,59} = 0.029$, $P=0.867$; $n=61$ nests) and between years when dummy eggs were used (T-test, $t=-0.494$, $P=0.624$; $n=42$ nests). Thus, we pooled the data for all years where no dummy eggs were used (1987, 1988 and 1994) for comparison with the pooled data from years where dummy eggs were used (1998 and 1999). There was no difference between the proportion of ejected eggs between years with or without dummy eggs (*T*-test, *t*=-1.118, *P*=0.267), which indicates that the introduction of dummy eggs did not interfere with the normal process of egg laying and ejection.

Egg maternity determination

We did not identify which individuals laid which eggs, an impossible task given the height of the nests, difficulty in capturing and banding females, and impossibility of continuous observation of a large sample of nests. Instead, we applied an indirect method to determine female egg ownership. The collected eggs, both ejected in the net and from nests, were opened in the laboratory and yolk samples were taken of non-incubated eggs only, which were frozen and later used in a newly-developed protein electrophoresis method that allowed us to determine the maternity of eggs (Cariello et al. in press). Briefly, yolk proteins in freshly laid eggs are maternally derived, and identical banding patterns are expected in eggs laid by the same female, while different females should present different patterns of yolk proteins.

We ran whole clutches on single SDS-PAGE gels (modified from Laemmli (1970)) for comparison of yolk protein polymorphism, and used silver staining for visualizing the proteins. We validated our results by conducting preliminary analyses using known female budgerigar (*Melopsittacus undulatus*) eggs and ovarian egg follicles obtained from known guira cuckoo females. The technique was subsequently applied in the maternal identification of eggs in communal clutches of free living guira cuckoos in the study population. An example of a gel from a guira cuckoo nest is shown in Figure 1.

The female order of entering the laying sequence was determined by identifying the maternity of each egg as it was laid. Thus, the first egg laid in the communal clutch was labeled as laid by female A, the second egg that differed from that egg's pattern as female B, and so on. New eggs with identical protein patterns to previously laid ones were assigned to the same female. In subsequent nesting bouts of the group, eggs were compared to those laid in the first bout and maternal identification was labeled accordingly. When two or more females initiated laying on the same day, they shared the designation of position of entering the laying sequence, and the values for numbers of eggs laid, tossed and incubated were averaged for use in statistical analyses. In the groups monitored, the total number of females contributing eggs to any single nesting bout varied from a solitary female to seven females. We did not consider single-female nesting bouts in analyses comparing reproductive parameters according to laying position. We also did not use the single nesting bout containing seven females, because the sample was too small to allow comparisons with bouts that had from two to six females.

We were thus able to ascertain the total or minimum number of laying females per nest, the order in which they entered the laying process, the total or minimum number of eggs each female laid and lost through ejection, and the identity of the females represented in the final communal clutch incubated and in successive nesting bouts of groups. The non-incubated eggs for which we were unable to establish maternal identity were usually first-laid eggs in the communal clutch, ejected before the net was in place. But because females seldom lay only a single egg, we are confident that, despite the under-estimation of individual clutch size in some instances, this resulted in only a negligible error when determining the representation of females within nests.

In this study we collected the entire egg to answer questions concerning individual investment in eggs, in addition to identifying their maternal origin. However, the extraction of only a small sample of yolk (Schwabl 1993) or albumen (Andersson and Åhlund 2000) through a hole in the eggshell allows the same methodology (protein electrophoresis) and has the added advantage of allowing eggs to hatch.

Determination of Reproductive Skew Indices

Reeve and Ratnieks (1993) and Keller and Vargo (1993) developed an index that quantifies reproductive skew, which varies from 0 (low skew: equitable reproductive partitioning among group members) to 1 (high skew: monopoly of reproduction by a single individual). Another formula for reproductive skew (S) was derived by Pamilo and Crozier (1996): $S = (N_T - Q_E) / (N_T - 1)$, where N_T is the total number of potential breeders and Q_E is the 'effective number' of breeders defined as $Q_E = 1 / \Sigma p_i^2$, where p_i is the reproductive contribution of the ith female. We used this formula instead of the previous ones because Pamilo and Crozier (1996) argue that former ones had certain statistical characteristics that led to discontinuities in an otherwise linear relationship. Using this formula, we calculated the reproductive skew for females only, during the egg laying and incubation phases of each group-year, and averaged these values to derive estimates when comparing repeated nesting bouts or nests that failed to reach incubation with those that reached incubation.

Potential female breeders included females identified in consecutive nesting bouts with the yolk protein electrophoresis method. To estimate the total number of potentially breeding females for calculating reproductive skew, we used either: (a) groups that attempted breeding at least twice, giving ample opportunity for all females to breed; or (b) singular breeding groups where at least three females participated in the breeding attempt. Thus, we did not include in the analyses those singular breeding groups that had less than three females represented. This was done to avoid under-estimating the number of potentially breeding females in a group.

Results

In the two study years, we monitored 24 groups that produced 48 nests of which 43 were analyzed in this study due to the availability of information on different aspects of reproduction. If a nest was found after the first egg was laid, it was not included in analyses that refer to female order of entering laying. We usually knew approximately how many eggs had been ejected previous to finding a nest, as ejected eggs were found either in the nets or broken on the ground. This allowed us to determine to what extent the inclusion of such nests in some analyses could compromise the quality of results, and to discard some nests where too much information was lost.

We used 195 non-incubated eggs for the PAGE analyses. For 16 of 34 nests (47%), all laid eggs were identified as to maternal origin, for eight nests (23.5%), at least 80% of eggs were identified, and for an additional eight nests (23.5%), at least 50% of egg identity was known. For the remaining two nests (6%), less than 50% of egg identity was known.

We first analyzed the variability of reproductive parameters (eggs laid, ejected and incubated) for females that had the same position in the order of entering the laying sequence in different groups. For this, we used an ANCOVA that had the number of co-breeding females as the covariate, thus eliminating the variability that could obscure real differences in the dependent variables analyzed (reproductive parameters). We found that only for number of eggs laid was there a statistical variation ($F_{5,1}$=3.094, P=0.025). None was found for number of eggs ejected ($F_{5,1}$=0.344, P=0.881) or eggs incubated ($F_{4,1}$=0.544, P=0.706). Namely, the fact that nesting bouts differed in the number of co-breeding females did not greatly affect the final reproductive output for females in the same position in the laying order.

It was evident, when considering all females together and notwithstanding the number of co-breeding females in the group, that those in the first laying positions laid more eggs relative to those in later positions (Pearson r=-0.46, P<0.01), lost equivalent numbers of eggs due to ejection (Pearson r=-0.03, P=0.86), and ended up with a greater number of eggs in the incubated communal clutch (Pearson r=-0.40, P=0.04; Fig. 2). In fact, a direct comparison of the early laying positions (females entering first and second; N=20) with later positions (fourth through sixth; N=8) shows that the former laid more eggs than the latter (Mann-Whitney U=126, P=0.01). The individual clutch size difference between early and late-laying females was of one to two eggs. However, egg ejection equally affected females in early or late positions (Mann-Whitney U=61, P=0.73).

Ten groups, which had from two to 10 females, were involved in two to three repeated nesting bouts within seasons, of which we monitored 32 bouts with variable amounts of information on female identity. Despite the loss of information due to occasional eggs being ejected outside the nets, or the fact that some nests were found after laying had started, some interesting patterns emerged. In none of the groups did all females enter all nesting bouts, although some females contributed eggs to all nesting bouts in a season. In repeated nesting bouts, different proportions (17 to 100%; Table 1) of the groups' females contributed to the communal clutch. The first nesting bout of the season had an average of 38.8% participation of the groups' females (n=7 groups). Female participation peaked in the second nesting bout (80.7%, n=9 groups), and then declined in the third nesting bout (70%, *n*=6 groups). The percentage of group females that contributed to the communal clutch was associated with the percentage of eggs that survived (r=0.47, P=0.045, n=19 bouts).

We were able to show that the female order of entering laying changed within groups over the course of the season (Table 1: restricted to data from nests where the identification of most eggs allowed us to determine the order of entering laying for a majority of females). There appears to be no consistent pattern overall, though first-laying females may have occasionally initiated laying in a second or third bout. The first bout was often characterized by the laying of only one female, whose single or few eggs were habitually ejected, and which then usually participated in subsequent nesting bouts. Two or even three females sometimes laid eggs synchronously on the same day. In none of the nests monitored did the last laying female(s) in one bout continue in that position in a subsequent bout.

Two of these groups had three nesting bouts with fairly complete information (76% of all eggs) concerning both egg fate (incubated versus ejected) and maternal identity. We assume that the loss of information concerning the non-identified eggs affected all females equally. Thus, these data allowed us to examine the partitioning of reproductive opportunities over a whole season for females with different laying tactics. Sixteen females participated in the six nesting bouts of the two groups (Table 2). Individual clutch size for these females differed greatly, and ranged from one to six eggs. This difference decreased after egg ejection; in the first group the incubated eggs for females ranged from one to five eggs, while in the second group the range was of zero to three eggs. Only one female laid in all three nesting bouts of her group. Most females (n=11) laid in one bout only. Females that contributed to only one nesting bout in the season laid significantly less eggs, and retained fewer of them for incubation, than did females that partook in two or three nesting bouts. The most successful females, however, were those that laid in two nesting bouts, both in terms of absolute number as well as in the proportional ownership over all eggs laid by the group in a season (calculated from the raw data, not means). However, egg ejection altered the proportions of eggs females owned initially in fresh clutches relative to what they eventually retained in the incubated communal clutches. For example, females that laid only once increased their representation from an average of 8.55% of all eggs laid by the group to 9.25% of all eggs incubated. Similarly, females that laid twice went from 20.28% of eggs laid to 21.20% for eggs incubated. However, the single female that laid in three nesting bouts decreased her representation, going from 25% of all eggs laid to 13.30% of the incubated eggs in the season. Thus, in proportional terms, laying three times in a season involved a cost in terms of energetic investment made and then lost for the single female that adopted this tactic.

We calculated the reproductive skew index for both laying and incubation phases for groups with single and multiple nesting bouts in both years (Table 3). First nesting bouts of groups (*n*=9) comprised a single female in more than half the cases (*n*=5), and were characterized by a total reproductive monopoly in laying (*RS* = 1.000), but resulted in nest failure (ejection of eggs and discontinuity of nesting bout) in three cases. In the other two cases, either a single egg or two eggs were incubated.

An analysis of reproductive skew values (Table 3) reveals that although nests that failed to reach incubation were not statistically different from those that reached incubation (Mann-Whitney *U*=115, *P*=0.11), approximately 60% of them had skew values greater than 0.500, with an average *RS* of 0.637 (±0.11). In contrast, only 38% of nests that reached incubation had a reproductive skew in laying above 0.500, and averaged *RS* = 0.387 (±0.09).

A comparison of reproductive skew levels between first versus later nesting bouts showed that first bouts were characterized by higher skew values for both the laying and incubation phases, as compared to later bouts (Fig. 3), though this difference was not statistically significant for the incubation phase (Laying: Mann-Whitney *U*-test, U=116.5, P=0.03; Incubation: Mann-Whitney *U*-test, U=25.5, P=0.43).

For an overall population index of reproductive skew we discarded nesting bouts where there was only a single female laying eggs, since these clutches were almost always doomed to failure, and usually comprised an incomplete clutch of 1-2 eggs. Thus, the average skew for the laying and incubation phases for both years were 0.353 (±0.063) and 0.467 (±0.088), respectively.

Discussion

Classical skew models predict that dominant group members should increase reproductive skew in their favor when ecological constraints prevent subordinate independent breeding and when relatedness is high among co-breeders. Given that previous studies of guira cuckoos showed that these conditions were not met (Quinn et al. 1994; Macedo and Bianchi 1997), reproductive skew was expected to be quite low.

Available genetic data are too limited yet to estimate the variability in kinship levels among groups, and to establish whether low relatedness is a general phenomenon. This aspect needs further research, especially to determine whether variability in kinship in different groups leads to variable levels of skew enforced by some members.

The observed skew indices for groups were not always low, as predicted by concessions model. The study revealed varying degrees of skew among groups and within groups across repeated nesting bouts. While average reproductive skew for all groups in the laying and incubation phases is moderate, it varies quite widely in a case by case analysis. First nesting bouts in the season had higher reproductive skew than later attempts. It is possible that there are physiological as well as ecological restrictions for the participation of females in different nesting attempts of the same group. Since guira cuckoos also forage in groups, if some females are dominant with regard to food access, possibly these females will have better body condition than others, and will be more prepared to initiate laying. There may be considerable variability among females in their ability to reproduce depending also on their age and/or breeding experience. Interestingly, these first bouts, where one or a few females monopolized reproduction, generally resulted in total nest failure before a complete clutch was laid, due to ejection of eggs and nest desertion. Thus, it appears that in this species, birds' behavioral mechanisms operate to avoid complete monopoly of reproductive opportunities within groups. Females, whatever their status, apparently are able to eject each other's eggs and undermine any attempt of control. This is a similar situation to that of the acorn woodpecker, a polygynandrous breeder that also nests jointly (Haydock and Koenig 2002). As the rainy season advances and chances for new reproductive opportunities decrease, it is possible that a more equitable distribution of reproductive opportunities among group members during the egg laying phase contributes toward nesting success.

Reproductive partitioning patterns of guira cuckoos elucidated in this study bear no similarity to what has been proposed for the joint nesting anis. Vehrencamp (1977) indicated that last-laying 'alpha' females of groove-billed anis postpone egg-laying while earlier-laying females have their individual clutches reduced through egg ejection by those in the group that have not initiated egg laying. This hypothesis was based upon the identification of individual female eggs through visual assessment of egg morphology. The 'alpha female', as designated in that model, was the most successful due to a skew in incubated eggs and fledged young. This conclusion was based on the following assumptions (Vehrencamp 1977): (1) females do not recognize their own eggs within a mixed communal clutch; (2) early-laying females, though producing more eggs, have a lower reproductive success than females laying later, due to egg ejection; (3) once all females in a group are laying, egg ejection ceases; (4) last-laying females do not lose any eggs and have a higher reproductive success; (5) females retain their order of entrance in the laying sequence in repeated nesting bouts of the group; and (6) all females participate in all laying bouts of the group.

Most of our results are incompatible with the above premises. We found that egg ownership favors early-laying females, and not last-laying ones, as reported for anis by Vehrencamp (1977) and Loflin (1983). Early-laying guira cuckoo females, like the anis, laid more eggs than late-laying females. However, the egg ejection pressure remained constant, affecting all females independently of their position in entering the laying sequence, differently from what was reported for anis. In fact, last-laying females in our study lost over 50% of the eggs they laid. This resulted in more incubated eggs for early-laying females. Additionally, our study showed that females neither retain the order of entering laying, nor all contribute eggs in repeated nesting bouts of their groups.

There are two arguments to explain these differences. First, there may be subtle yet important differences between the ani and guira cuckoo social systems, leading to reproductive strategies and patterns in anis that diverge from those of the guira cuckoo. Ani groups apparently have only up to four or five females (Vehrencamp 1977; Loflin 1983), whereas guira cuckoo groups may contain up to 10 reproductive females. Anis may have a higher coefficient of relatedness within groups, or the ecological constraints for anis (e.g., habitat saturation; Koford et al. 1986) are not relevant (do not apply to) for guira cuckoos. It would be of interest to investigate whether increasing levels of complexity in terms of group size, number of females, and nestings per season in the crotophagine species (*C. sulcirostris, C. ani, C. major* and *G. guira*) lead to progressively more equitable reproductive partitioning, taking into consideration degree of relatedness among co-breeders and the presence/absence of ecological constraints.

A second argument that could explain the divergence found in the reproductive patterns of these species is that the methodology used by Vehrencamp (1976, 1977) and Loflin (1983) to determine female egg ownership produced inaccurate results. At the time of their studies, molecular biochemical techniques were not readily available, and egg morphometry (length, width, weight) was applied to distinguish individual female eggs within the communal clutch, and also the numbers of eggs laid and ejected to estimate the number of laying females. This was a suitable methodology at the time, given that for many species, egg characteristics such as size, shape, color and eggshell patterns may be less variable within the clutch of individual females than among clutches of different individuals (Kendeigh et al. 1956; Baerends and Hogan-Warburg 1982; Ricklefs 1984; Croxall et al. 1992; Reed and Oring 1997).

However, it may be crucial to make a previous assessment of individual variability in egg morphometry in order to use egg characteristics to approach some degree of reliability in identification of maternal egg ownership. This would be especially important in species such as anis and guira cuckoos, where eggs of several females are found in the same nest. Distinguishing individual female eggs in joint-nesting species is crucial to understand broader issues involving reproductive skew, individual strategies, group membership and, ultimately, aspects pertaining to the evolution of sociality.

The large number of females in guira cuckoo groups may lead to a more egalitarian reproductive partitioning during egg laying than has been previously suggested for crotophagines, for it may be impossible for a female to maintain a dominant position through an entire reproductive season. Thus, as in acorn woodpeckers (Haydock and Koenig 2002), behaviorally dominant guira cuckoo females may not be able to either control reproductive partitioning within the group nor grant reproductive concessions to subordinates.

In our study we found that the last-laying female laid fewer eggs, which is comparable to what has been found for anis. These eggs are often laid toward the very end of the laying period, sometimes even after incubation has started. But contrary to anis, these eggs are frequently ejected. Possibly, egg ejection is carried out not only by females participating in the current nesting bout, but also by group females not involved in that nesting attempt.

The apparent flexible female reproductive hierarchy in guira cuckoo groups may explain why individuals join and remain in groups despite a high degree of conflict, involving both egg and chick losses. In a rigid hierarchy, a subordinate female with continuous and consistent ejection of her eggs might find it more profitable to leave the group and breed independently, especially in the case of an unsaturated habitat. That joint nests are the most common breeding option in guira cuckoos and anis seems to indicate that there are significant reproductive advantages for all group members. Egg ejection during the laying period decreases the amount of skew in the final incubated communal clutch.

Based on our results, it would be difficult to presume whether or not there is a dominant female in terms of reproductive partitioning. Early-laying females apparently have an advantage, considering the number of eggs that eventually survive to incubation, since they invest heavily in egg production but do not suffer a greater relative loss than later-laying females. However, since females apparently may not retain their position in the laying sequence in repeated nesting bouts of the group, nor contribute to all nesting bouts (possibly as an effect of behavioral, physiological and/or ecological mechanisms), these early and late positions appear to rotate among females. Additionally, although varying degrees of reproductive skew occur in sequential nesting attempts of a group, these values do not always apply to the same group of individuals.

The position in the laying sequence may be important, but only within a broader context, because the best overall strategy seems to be the frequency with which a female contributes to repeated nesting bouts of the group. Females with the highest proportion of incubated eggs over an entire season were those that contributed to two nesting bouts. We are cautious about making generalizations concerning energetic losses for females that contributed eggs to more than two nesting bouts in a season, given that only one female was found to do so. However, the scarcity of females that adopted this tactic may be an indication that it is not an advantageous one. Obviously, the tactic of choice may ultimately depend upon the groups' ability to renest frequently and upon female body condition, age and/or breeding experience (that may reflect their social status within groups). Additionally, factors not considered in this study (e.g. infanticide and differences in parental care) may contribute to overall reproductive skew patterns.

The communal breeding system of the crotophagines presents a challenge in testing the different models of reproductive skew currently available. For guira cuckoos, dominance hierarchies among breeders seem to be feebly enforced, resulting from a combination of factors. There appears to be a low degree of relatedness among breeders and a lack of ecological constraints, which act in concurrence with a high number of potentially reproductive females and a very long breeding season, allowing repeated opportunities for nesting. There is mixed maternity within nests, lack of reproductive dominance, and females apparently can subvert monopoly by egg ejection.

In conclusion, despite the rapid expansion of skew theory and modeling approaches, most of the models have been applied toward the elucidation of social behavior among the hymenopteran insects. Our results do not consistently agree with the concessions model, and this may reflect the fact that vertebrate societies exhibit greater variability and behavioral flexibility. The increasing number of sophisticated biochemical and molecular tools could help to resolve previously unknown kinship levels among individuals, perhaps the most important variable in the study of reproductive skew.

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References

Andersson M, Åhlund M (2000) Host-parasite relatedness shown by protein fingerprinting in a brood parasitic bird. Proc Natl Acad Sci USA 97:13188-13193.

Baerends GP, Hogan-Warburg AJ (1982) The external morphology of the egg and its variability. Behaviour 82:1-32.

Bourke AFG, Heinze J (1994) The ecology of communal breeding: the case of multiple-queen leptothoracine ants. J Phil Trans R Soc Lond B 345:359-372.

Brown JL (1987) Helping and communal breeding in birds. Princeton Univ Press, Princeton, New Jersey.

Cariello M, Schwabl H., Lee R, Macedo RH (in press). Individual female clutch identification through yolk protein electrophoresis in the communally-breeding guira cuckoo (*Guira guira*). Mol Ecol

Clutton-Brock TH (1998) Reproductive skew, concessions and limited control. Trends Ecol Evol 13:288-292.

Croxall JP, Rothery P, Crisp A (1992) The effect of maternal age and experience on egg-size and hatching success in wandering albatrosses *Diomedea exulans*. Ibis 134:219-228.

Emlen ST (1982) The evolution of helping I. An ecological constraints model. Am Nat 119:29-39.

Emlen ST, Vehrencamp SL (1983) Cooperative breeding strategies among birds. In: Brush AH, Clark JGA (eds) Perspectives in ornithology. Cambridge Univ Press, Cambridge, UK, pp 93-133.

Hamilton WD (1964) The genetical evolution of social behavior. I and II. J Theor Biol 7:1-52.

Haydock J, Koenig WD (2002) Reproductive skew in the polygynandrous acorn woodpecker. Proc Natl Acad Sci 99:7178-7183.

Heinze J (1995) Reproductive skew and genetic relatedness in *Leptothorax* ants. Proc R Soc Lond B 261:375-379.

Jamieson IG (1997) Testing reproductive skew models in a communally breeding bird, the pukeko, *Porphyrio porphyrio*. Proc R Soc Lond B 264:335-340.

Johnstone RA, Cant MA (1999a) Reproductive skew and indiscriminate infanticide. Anim Behav 57:243-249.

Johnstone RA, Cant MA (1999b) Reproductive skew and the threat of eviction: a new perspective. Proc R Soc Lond B 266:275-279.

Johnstone RA, Woodroffe R, Cant MA, Wright J (1999) Reproductive skew in multimember groups. Am Nat 153:315-331.

Keller L, Reeve HK (1994) Partitioning of reproduction in animal societies. Trends Ecol Evol 9:98-102.

Keller L, Vargo EL (1993) Reproductive structure and reproductive roles in colonies of eusocial insects. In: Keller L (ed) Queen number and sociality in insects. Oxford Univ Press, UK, pp 16-44..

Kendeigh SC, Kramer TC, Hamerstrom F (1956) Variations in egg characteristics of the house wren. Auk 73:42-65.

Koenig WD, Mumme RL (1987) Population ecology of the cooperatively breeding acorn woodpecker. Princeton Univ Press, Princeton.

Koford RR, Bowen BS, Vehrencamp SL (1986) Habitat saturation in groove-billed anis (*Crotophaga sulcirostris*). Am Nat 127:317-337.

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of Bacteriophage T4. Nature 227: 680-685.

Loflin RK (1983) Communal behaviors of the smooth-billed ani. Ph.D. dissertation, Univ Miami, Coral Gables, FL.

Macedo RH (1992) Reproductive patterns and social organization of the communal guira cuckoo (*Guira guira*) in central Brazil. Auk 109:786-799.

Macedo RH, Bianchi C (1997) Communal breeding in tropical guira cuckoos *Guira guira*: sociality in the absence of a saturated habitat. J Avian Biol 28:207-215.

Macedo RH, Melo C (1999) Confirmation of infanticide in the communally breeding guira cuckoo. Auk 116:847-851.

Pamilo P, Crozier RH (1996) Reproductive skew simplified. Oikos 75:533-535.

Quinn JS, Macedo RH, White BN (1994) Genetic relatedness of communally breeding guira cuckoos. Anim Behav 47:515-529.

Reed JM, Oring LW (1997) Intra- and inter-clutch patterns in egg mass in the spotted sandpiper. J Field Ornithol 68:296-301.

Reeve HK (1998) Game theory, reproductive skew, and nepotism. In: Dugatkin L, Reeve HK (eds) Game theory and animal behavior. Oxford Univ Press, Oxford, UK, pp 118-145.

Reeve HK, Emlen ST, Keller LF(1998) Reproductive sharing in animal societies: reproductive incentives or incomplete control by dominant breeders? Behav Ecol 9:267-278.

Reeve HK, Keller L (1995) Partitioning of reproduction in mother-daughter versus sibling associations: a test of optimal skew theory. Am Nat 145:119-132.

Reeve HK, Ratnieks FLW (1993) Queen-queen conflict in polygynous societies: mutual tolerance and reproductive skew. In: Keller L (ed). Queen number and sociality in insects. Oxford Univ Press, Oxford, UK, pp 45-85.

Ricklefs RE (1984) Variation in the size and composition of eggs of the European starling. Condor 86:1-6.

Schwabl H (1993) Yolk is a source of maternal testosterone for developing birds. Proc Natl Acad Sci USA 90:11446-11450.

Vehrencamp SL (1976) The evolution of communal nesting in groove-billed anis. Ph.D. dissertation, Cornell Univ, Cornell.

Vehrencamp SL (1977) Relative fecundity and parental effort in communally nesting anis, *Crotophaga sulcirostris*. Science 197:403-405.

Vehrencamp SL (1979) The roles of individual, kin and group selection in the evolution of sociality. In: Marler P, Vandenbergh J (eds) Social behavior and communication. Plenum Press, New York, pp 351-394

Vehrencamp SL (1983a) A model for the evolution of despotic versus egalitarian societies. Anim Behav 31:667-682.

Vehrencamp SL (1983b) Optimal degree of skew in cooperative societies. Am Zool 23:327-335.

Woolfenden GE, Fitzpatrick JW (1984) The Florida scrub jay: demography of a cooperative-breeding bird. Princeton Univ Press, Princeton.

Zar, JH (1999) Biostatistical analysis. 4th ed. Prentice Hall, Upper Saddle River, N.J.

Table 1. Female identity (designated by capital letters) in the order of entering the laying sequence in repeated nesting bouts of the same group, followed by the proportion of the groups' females contributing to each nesting bout. Slashes cluster new females that entered laying within each day. Parentheses cluster females entering laying in an unknown sequence, as a result of nest being found after the onset of laying. Question marks designate unidentified females due to eggs being ejected outside nets.

		Female sequence within nestings (proportion of female participation)		
Group / Year	Females in group	First nest	Second nest	Third nest
C13 / 98	6	A (17%)	(?,B,C) / A / D / E / F (100%)	B / D, E (50%)
D5 / 98	10	? / A / B,C (30%)	(C,D,E,F,G,H) / I (70%)	J / C (20%)
A9 / 98	2	A (50%)	B (50%)	
C13 / 99	3	A (33%)	(A,B) / C (100%)	
D5 / 99	6	(?,A) (17%)	A / B / C / D (67%)	(?,C,E) / F (50%)
A6 / 99	5	?	? / A,B (40%)	(?,B,C,D,E) / A (100%)
B11 / 99	5	?	A / ?,B / C (60%)	(A,B) / C,D / E (100%)
C11 / 99	4	A / ? (25%)	(?,A,B,C,) / D (100%)	

Table 2. Average number of eggs laid and incubated over an entire season for females in two groups, which contributed to one, two or three nesting bouts of their groups. In parenthesis is the average percent of eggs owned by females over all eggs laid and incubated in the season by her group.

Female participation	No. females	Seasonal average for individual females' eggs (proportion in communal clutch)	
		Total laid	Total incubated
One nesting bout	11	2.00 (8.55)	1.41 (9.25)
Two nesting bouts	4	4.25 (20.28)	3.50 (21.20)
Three nesting bouts	1	6.00 (25.00)*	2.00 (13.30)**

*Number of eggs laid by females in only one nesting bout versus those laying two or three times (pooled), Mann-Whitney $U = 2.00$, $P < 0.01$.

** Number of eggs incubated by females in only one nesting bout versus those laying two or three times (pooled), Mann-Whitney $U = 6.50$, $P = 0.01$.

Table 3. Reproductive skew indices for groups of guira cuckoos in the study population in the years 1998-1999. Only nests that followed criteria detailed in text were included in the calculations of reproductive skew, the formula of which is given in the text.

		Females in		Communal	Reproductive skew		
Year / Group	Bout	Group	Bout	clutch	Laying	Incubation	Nest fate
1998 / Alema	2	5	5	13	0.169	0.501	Success
1998 / Bigode	1	6	1	1	1.000	-	Fail
	2		6	12	0.172	0.486	Success
	3		3	8	0.618	0.618	Success
1998 / Cafezal	1	2	1	1	1.000	1.000	Success
	2		2	5	0.077	0.077	Success
1998 / Evani	2	4	4	12	0.132	-	Fail
1998 / PedraV.	1	6	6	11	0.150	-	Fail
1998 / Pirauba	1	2	1	2	1.000	-	Fail
	2		1	1	1.000	-	Fail
1998 / Quaresm	1	5	5	9	0.182	0.583	Success
1998 / Simonato	3	4	4	9	0.157	-	Fail
1998 / Tche	1	10	3	6	0.825	-	Fail
	2		7	16	0.403	0.494	Success
	3		2	2	0.889	-	Fail
1999 / Bigode	1	3	1	2	1.000	1.000	Success
	2		3	7	0.062	0.003	Success

Table 3. Cont.

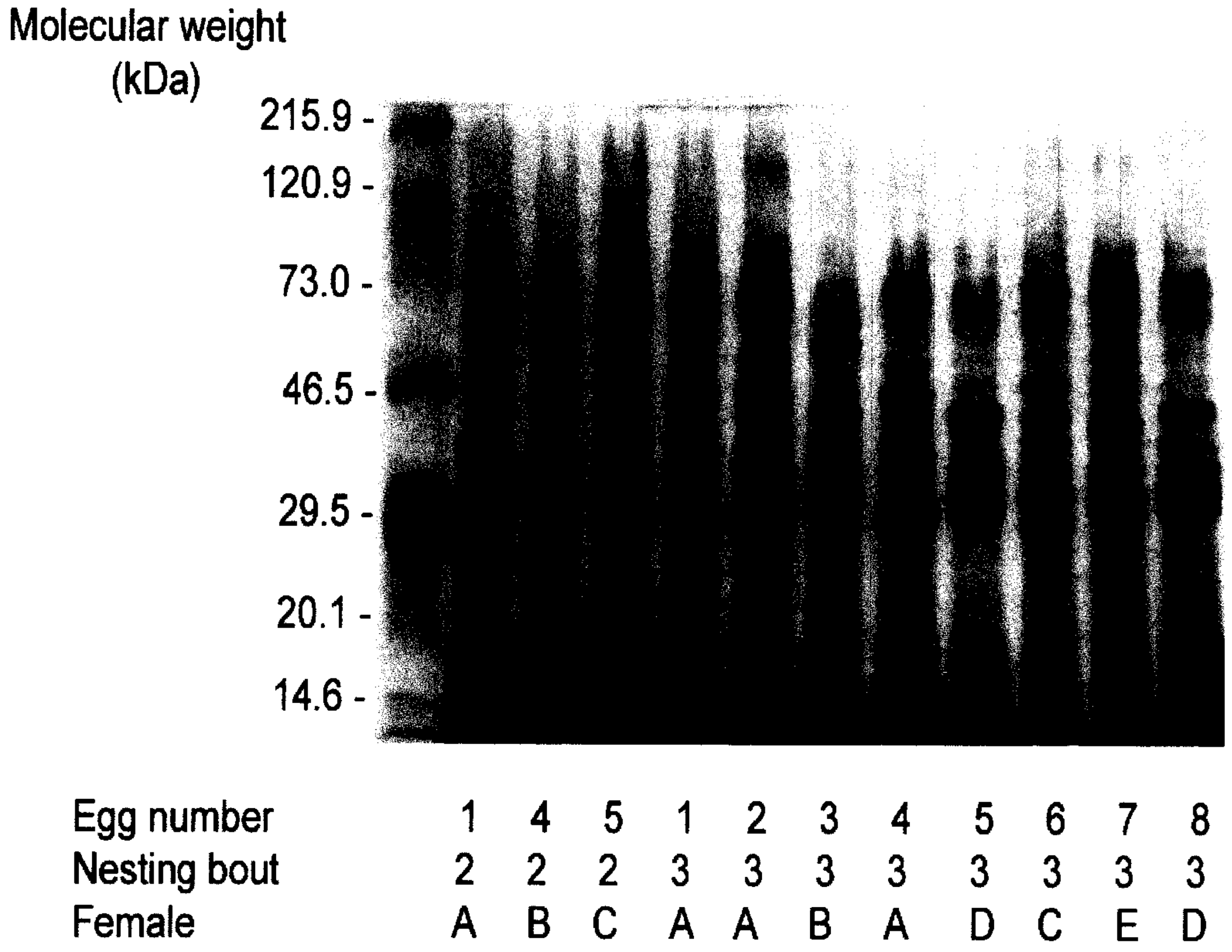
Year / Group	Bout	Females in		Communal clutch	Reproductive skew		Nest fate
		Group	Bout		Laying	Incubation	
1999 / Evani	2	5	2	10	0.750	1.000	Success
	3		5	10	0.114	0.750	Success
1999 / Simonato	1	3	3	4	0.167	0.003	Success
1999 / Tche	1	6	1	1	1.000	-	Fail
	2		4	8	0.560	0.644	Success
	3		3	5	0.745	-	Fail
1999 / Vargem	2	5	3	3	0.500	-	Fail
	3		5	8	0.250	-	Fail
1999 / Village	2	4	4	5	0.143	0.444	Success

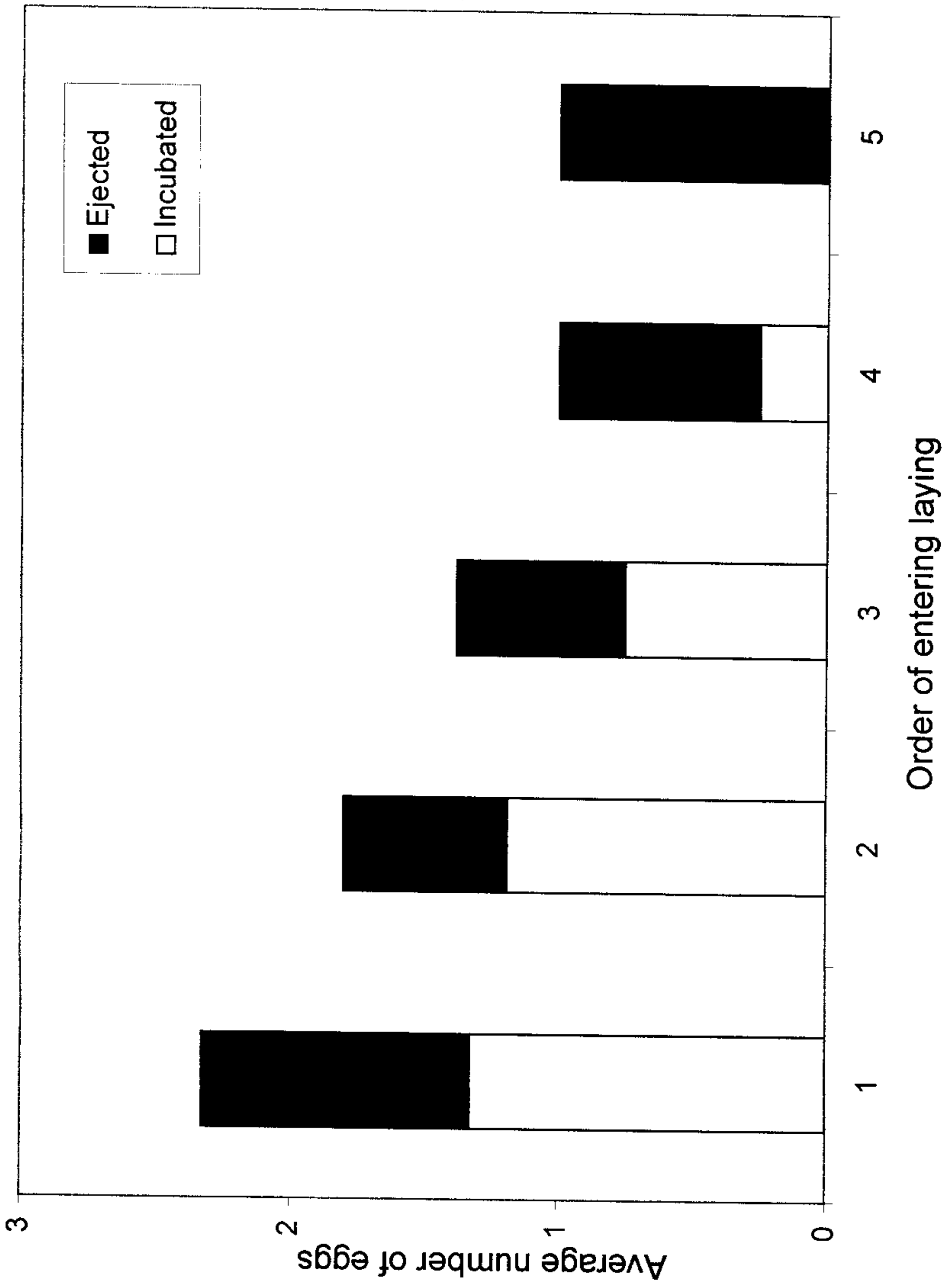
FIGURE LEGENDS

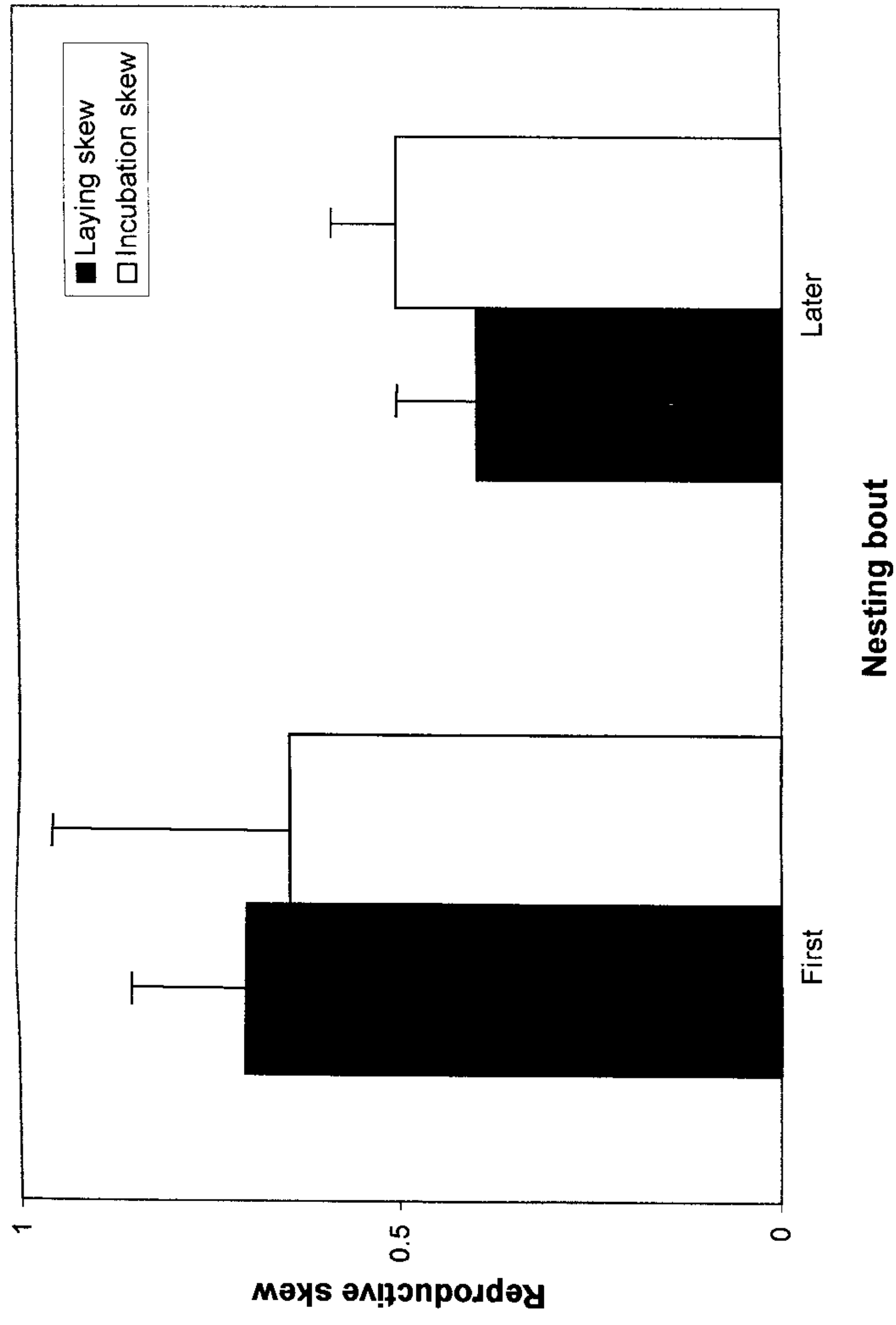
Figure 1. SDS-PAGE of yolk proteins of eggs from the second and third nesting bouts of guira cuckoo group B11, in which three of six eggs laid were analyzed from the second bout (the remaining three eggs were ejected to the ground), and the whole communal clutch of eight eggs was analyzed from the third bout. In the second bout, eggs 1, 4 and 5 had distinct banding patterns, indicating three females. These were represented in the third nesting bout, with the additional presence of two new females (banding patterns D and E). Egg yolk proteins were digested by trypsin and fragments visualized by silver staining. The migration positions of the molecular weight standards are indicated on the left.

Figure 2. Mean number of eggs laid, ejected and retained for incubation for females that entered the laying sequence in positions 1 through 5 of nesting bouts.

Figure 3. Mean reproductive skew (± s.e.) for the laying and incubation phases of first nesting bouts versus later ones for groups of guira cuckoos.


Molecular weight
(kDa)
215.9 -
120.9 -
73.0 -
46.5 -
29.5 -
20.1 -
14.6 -
Egg number 1 4 5 1 2 3 4 5 6 7 8
Nesting bout 2 2 2 3 3 3 3 3 3 3 3
Female A B C A A B A D C E D


Average number of eggs
0
1
2
3
Order of entering laying
1
2
3
4
5
■ Ejected
□ Incubated


Reproductive skew
0
0.5
1
■ Laying skew
□ Incubation skew
First
Later
Nesting bout