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Estratégias de acasalamento, sinalização da qualidade individual e deslocamento sazonal de *Sicalis citrina* Pelzeln, 1870

Mariana de-Carvalho

Orientadora: Regina H. F. Macedo

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Tese de Doutorado apresentada ao Programa de Pós-Graduação *stricto sensu* em Ecologia da Universidade de Brasília como parte dos requisitos para obtenção do título de Doutor em Ecologia

Orientadora: Regina H. F. Macedo

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TERMO DE APROVAÇÃO

MARIANA DE CARVALHO

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Tese de doutorado apresentada em 31 de agosto de 2022, junto ao Programa de Pós-Graduação em Ecologia do Instituto de Ciências Biológicas da Universidade de Brasília, sob orientação da Prof^a. Regina H. F. Macedo, com o apoio financeiro da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), como parte dos requisitos para obtenção do título de Doutor em Ecologia.

Banca Examinadora:

Prof^{a}. Dr^{a}. Regina H. F. Macedo
Presidente/Orientadora
Universidade de Brasília (UnB)

Prof. Dr. Felipe Malheiros Gawryszewski
Membro titular interno vinculado ao PPG Ecologia
Universidade de Brasília (UnB)

Prof. Dr. Fábio José Viana Costa
Membro titular externo
Instituto Nacional de Criminalística/Polícia Federal

Prof. Dr. André Guaraldo
Membro titular externo
Universidade Federal do Paraná (UFPR)

Profa. Dr^{a}. Rosana Tidon
Membro suplente
Universidade de Brasília (UnB)

Ao Cerrado,

à todos que nele vivem e

à quem o ama.

Prefácio

A tese está dividida em três grandes partes. A primeira constitui um referencial teórico, que traz o leitor para o contexto do trabalho. Esta é subdividida em introdução geral, informações sobre a espécie de estudo, objetivos gerais e detalhes sobre a área de estudo.

A segunda parte se refere aos estudos desenvolvidos durante meu doutoramento. Estes estão expostos como três capítulos em formato de artigo que em conjunto propõe preencher lacunas no conhecimento a respeito da história natural de uma ave Neotropical, o canário-rasteiro, *Sicalis citrina,* e testar hipóteses sobre coloração, qualidade individual e fonte alimentar. O primeiro capítulo trata da descrição da coloração de *S. citrina* dentro do modelo visual das aves, incluindo a descrição de estratégias reprodutivas não antes relatadas. O segundo capítulo consiste na descoberta da fonte alimentar de onde são extraídos os pigmentos que dão origem a cor de *S. citrina* por meio de uma metodologia inovadora e como estes são utilizados fisiologicamente pelas fêmeas e machos. Por fim, o terceiro capítulo identifica os padrões de movimentação sazonal de *S. citrina* associando duas metodologias de baixo custo analítico, quando comparadas às metodologias tradicionais.

A seguir estão relatados os títulos de cada capítulo e os periódicos científicos aos quais cada manuscrito será submetido:

Capítulo I: Delayed plumage maturation and breeding behavior of stripe-tailed yellow finch *Sicalis citrina* Pelzeln 1870 (Aves: Thraupidae). Submissão para Journal of Zoology (Fator de Impacto: 2.39).

Capítulo II: Trade-offs between health and carotenoid-derived color in stripe-tailed yellow finch males. Submetido para The American Naturalist (Fator de Impacto: 3.92)

Capítulo III: Seasonal movements of Neotropical stripe-tailed yellow finch inferred from citizen science and stable Hydrogen isotopes. Submetido para Animal Behaviour (Fator de Impacto: 3.03).

A terceira e última grande parte da tese integra nossos achados, por meio de uma conclusão geral, além da incluir as licenças obtidas para a coleta dos dados.

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Sumário

1. Resumo geral

As aves possuem alta diversidade de coloração da plumagem, com diversas espécies apresentando dicromatismo sexual. Além disso, a coloração dos indivíduos pode expressar informações acerca da qualidade corporal ou saúde dos mesmos. Dentre os tipos de coloração presentes em aves, a baseada em pigmentos carotenoides têm sido usualmente demonstrada como um desses sinais que refletem a qualidade individual. Entretanto, nem sempre esses sinais refletem de forma verdadeira a qualidade dos indivíduos. O canário-rasteiro *Sicalis citrina* Pelzeln 1870 é um passeriforme com dicromatismo sexual que apresenta coloração derivada de pigmentos carotenoides, configurando-se como um interessante modelo para investigação da (i) variação da coloração de indivíduos dentro do sistema visual das aves; e (ii) expressão da qualidade dos indivíduos por meio da coloração. Além disso, apesar de ser uma espécie abundante, pouco se sabe sobre seu comportamento reprodutivo e sua movimentação sazonal, sendo considerado, de forma anedótica, como migratório. Essa tese está dividida em três capítulos que buscam avançar nos objetivos acima citados, bem como contribuir com o conhecimento a respeito da história natural desta ave. O capítulo I descreve a coloração das fêmeas e machos, identifica a ocorrência de atraso da maturação da plumagem em machos e descreve facetas, não antes documentadas, do comportamento reprodutivo de *S. citrina*. Fêmeas e machos apresentam alta reflectância no espectro ultravioleta e amarelo na cabeça e no ventre, principais regiões do corpo para sinalização social. O atraso da maturação da plumagem em machos faz parte de uma estratégia reprodutiva de *S. citrina*, mas existem diferenças significativas, indistinguíveis aos olhos humanos, entre a coloração dos sexos. O capítulo II testa hipóteses que versam sobre a expressão fenotípica (coloração) e qualidade individual das fêmeas e machos. A cor das fêmeas depende de sua condição corporal, i.e., àquelas que possuem uma dieta rica em grãos C_3 e C_4 possuem o ventre marrom, e também são as que tem maior condição corporal. Já a qualidade dos machos não está associada a coloração da plumagem. Eles acessam diferentes itens alimentares que afetam sua cor (grãos C_3 e C_4) ou seu sistema imunológico (proteína), o que sugere que possam optar por investir em ornamentação ou na saúde. Por fim, o capítulo III identifica o tipo de movimentação sazonal de *S. citrina*, e diferente do registrado na literatura, *S. citrina* é uma espécie nômade. De forma integrada, os resultados desta tese contribuem para o conhecimento a respeito da coloração de um traupídeo Neotropical, testando hipóteses sobre alocamento de recursos para produção de cor e sinais que expressam a qualidade dos indivíduos, além de contribuir com a história natural desta espécie.

Palavras-chave: Carotenoides, Coloração, Comportamento animal, Condição corporal, Dimorfismo sexual, História natural, Luteína, Imunidade, Ornamentos sexuais, Reprodução, Saúde, Thaupidae

Title: Breeding strategies, signaling of individual's quality and seasonal movements of Sicalis citrina pelzeln, 1870

2. Abstract

Birds have a high diversity of plumage coloration, with several species showing sexual dichromatism. The individual´s color can express information about quality or health. Among the types of colors that occur in birds, those based on carotenoid pigments have usually been shown to be signals that reflect individual quality. However, these signs do not always truly reflect the individual's quality. The stripe-tailed yellow *finch Sicalis citrina* Pelzeln 1870 is a passerine with sexual dichromatism that presents coloration derived from carotenoid pigments, and is an interesting model for investigation of: (i) variation in the color of individuals within the visual system of birds; and (ii) expression of individual quality. In addition, despite being an abundant species, little is known about its reproductive behavior and seasonal movement, being considered, anecdotally, as a migratory species. This thesis is divided into three chapters that seek to advance the objectives mentioned above, as well as contribute to knowledge about the natural history of this bird. Chapter I describes the coloration of females and males, identifies the occurrence of delayed plumage maturation in males, and describes previously undocumented facets of the reproductive behavior of the stripe-tailed yellow finch. Females and males have high reflectance in the ultraviolet and yellow spectrum on the head and belly, the main regions of the body for social signaling. The delay in plumage maturation in males is part of a reproductive strategy of the stripe-tailed yellow finch, but there are significant differences, indistinguishable to human eyes, between the coloration of the sexes. Chapter II tests hypotheses associated with the phenotypic expression (color) and female and male individual quality. Female color depends on body condition, i.e. those in better condition have a brown hue. Male quality is not associated with a colorful plumage. Individuals access different food items that improve their color (C_3 and C_4 grains) or their immune system (protein), which suggests that they may choose to invest in ornamentation or health. Finally, chapter III identifies the type of seasonal movement of the stripe-tailed yellow finch. Different from the literature, it is a nomadic species. In an integrated way, the results of this thesis contribute to the knowledge about the coloration of a Neotropical Thraupid, testing hypotheses about the allocation of resources for the production of color and signals that express the individual's quality, in addition to contributing to the natural history of this species.

Keywords: Animal behavior, Body condition, Carotenoids, Coloring, Immune health, Lutein, Natural history, Ornaments, Reproduction, Sexual dimorphism, Thaupidae

3. Introdução geral

A seleção sexual foi proposta por Darwin (1871) para explicar a evolução do dimorfismo sexual, armamentos e ornamentos exagerados encontrados em diversas espécies animais (e.g. tamanho dos chifres, coloração de penas, etc.). Segundo a teoria, essas características surgem a partir da variância no sucesso de acasalamento dos indivíduos, por meio de dois mecanismos: seleção intrasexual, i.e., competição entre membros do mesmo sexo por acesso ao sexo oposto; e seleção intersexual, i.e., escolha diferencial de membros de um sexo pelo sexo oposto (Darwin 1871, Andersson 1994). Esta última tem sido apontada como uma forma importante de seleção para a evolução da coloração em Aves (Darwin 1871, Andersson 1994, Hill 2006). Salvo exceções (Amundsen 2000), é proposto que fêmeas avaliem os machos de acordo com seus ornamentos (particularmente cor da plumagem) favorecendo tipicamente àqueles com coloração extravagante, o que leva a um grande efeito no sucesso reprodutivo destes (Cotton et al. 2006). Enquanto isso, a coloração opaca das fêmeas pode ser influenciada pela pressão da seleção natural, que favorece uma coloração críptica que minimiza a predação dos ovos ou ninhegos (Shultz e Burns 2017). Assim, tanto componentes da seleção sexual quanto da seleção natural podem ter efeitos sobre as diferenças entre os sexos em diversas espécies (Shultz e Burns 2017).

Essa preferência das fêmeas por determinados machos pode ser influenciada por aspectos da coloração que sejam indicadores do estado de saúde e qualidade genética dos machos (Griffith et al. 2002). Uma coloração extravagante da plumagem pode ser um sinal honesto sobre a qualidade dos indivíduos (Weaver et al. 2017). Porém, para que esses sinais sejam evolutivamente estáveis, eles devem ser confiáveis de tal forma que haja uma correlação entre a expressão do ornamento e alguma qualidade que seja de interesse para o sexo oposto (Zahavi 1975, 1977, Grafen 1990, Maynard e Harper 2003). No entanto, enquanto é vantajoso para as fêmeas escolherem parceiros por aspectos que representem a qualidade destes e que permitirão aumentar seu sucesso reprodutivo (por meio da qualidade da prole gerada), é vantajoso para os machos apresentarem sinais que maximizem suas chances de acasalamento com diversas fêmeas, mesmo que seja um reflexo desonesto de qualidade (Hill 1994, Gavrilets et al. 2001). Isso acaba abrindo brechas para possíveis "trapaças" evolutivas (e.g., Senar et al. 2014, Hutton e McGraw 2016), uma vez que algumas espécies exibem colorações que não refletem a qualidade individual, mas, mesmo assim, estão sob pressão da seleção sexual (Isaksson et al. 2007, Senar et al. 2014).

Os mecanismos que produzem os padrões de cores das aves são resultado de processos fisiológicos que envolvem pigmentos carotenoides, melaninas, estrutura do tecido ou uma combinação destes (Breitwisch 2003). Um tipo de coloração que usualmente tem sido demonstrado como sinal honesto de qualidade individual é a coloração baseada em pigmentos carotenoides (Svensson e Wong 2011, Weaver et al. 2017). Os carotenoides são obtidos exclusivamente de alimentos e são responsáveis pelas cores amarela, laranja e vermelha da plumagem (Goodwin 1984, Linville e Breitwisch 1997, Grether et al. 1999). Além da produção de cor, os carotenoides também podem ser usados como antioxidantes e na função imune (Fitze et al. 2007, Simons et al. 2012). As quantidades de carotenoides adquiridas do meio ambiente e armazenadas para uso futuro são limitadas e podem não ser suficientes para permitir que indivíduos aloquem tais pigmentos tanto para a produção de cor, quanto para os processos internos (Koch e Hill 2018). Por isso, a alocação dos pigmentos carotenoides para a coloração é considerada custosa para machos de baixa qualidade (Weaver et al. 2017). É razoável supor que apenas indivíduos de alta qualidade conseguem investir numa expressiva coloração baseada em carotenoides, pois somente para eles tal sinalização proporcionaria benefícios suficientes na aptidão reprodutiva para compensar os custos de saúde (Getty 2006, Svensson e Wong 2011).

É importante frisar que existem vários indicadores de qualidade individual, como medidas de condição (e.g., índice de massa corporal), de saúde (e.g., razão heterofilo/linfócito), e de qualidade reprodutiva (e.g., tamanho da ninhada) (para mais detalhes ver Weaver et al. 2018) e essa relação entre a coloração e as várias medidas de qualidade não é consistente entre os estudos (Weaver et al. 2018). Apenas determinadas medidas de qualidade são expressas por meio da coloração dos indivíduos (Weaver et al. 2018). Por exemplo, a coloração amarela de *Parus major* (chapim-real) reflete o grau de infestação hemoparasitária e sobrevivência local (Hõrak et al. 2001), mas não o estresse oxidativo (Isaksson et al. 2007). Além disso, essa relação ainda varia entre espécies (Weaver et al. 2018).

Um dos maiores grupos de aves canoras, a família Thraupidae, possui grande diversidade na coloração da plumagem, desde espécies com padrões crípticos e opacos até outras com padrões coloridos e brilhantes (Isler e Isler 1999, Burns et al. 2014, Burns et al. 2016). Essa família apresenta tanto representantes monocromáticos até espécies altamente dicromáticas, ou seja, ambos os sexos, ou apenas um deles coloridos (Burns 1998). Aos olhos humanos, cerca de metade das espécies do grupo são sexualmente dicromáticas, mas de uma perspectiva visual das aves, cerca de 90% das espécies são dicromáticas (Burns e Shultz 2012). Medições objetivas por meio da espectrofotometria de reflectância revelaram que diversas espécies anteriormente classificadas como monocromáticas apresentam, na verdade, dicromatismo sexual (Burns e Shultz 2012). Em outras palavras, existem diferenças sexuais indetectáveis pela visão humana em diversas espécies de aves (Eaton 2005, Burns e Shultz 2012). Ainda, aquelas que já eram classificadas com dicromatismo sexual apresentam diferenças sexuais ainda mais acentuadas considerando o modelo de visão das aves (e.g. Saldívar e Massoni 2018).

Assim como a retina humana, a retina da maioria das espécies de aves contém várias classes espectrais de cones com máximos de sensibilidade espectral na região aproximada do vermelho, verde e azul (Valera et al. 1993, Chen et al. 1984). Porém, diferentemente de nós, as aves também apresentam uma quarta classe de cone com sensibilidade no ultravioleta (Valera et al. 1993, Chen et al. 1984). Assim, a maior parte das espécies de aves são tetracromáticas e enxergam cores imperceptíveis aos nossos olhos (Hill e McGraw 2006). Foi apenas na década de 90 que a quantificação da coloração da plumagem das aves, por meio da espectrofotometria, se tornou uma ferramenta padrão na ecologia comportamental (Hill e McGraw 2006). Estudos que abordam a coloração de aves dentro de seu sistema visual agora são comuns, especialmente no contexto da seleção sexual (por exemplo, Eaton e Lanyon 2003, Eaton 2005, Stoddard e Prum 2008). No entanto, ainda poucos descrevem a coloração das espécies dentro do sistema visual das aves (mas veja Eaton 2006, Seddon et al. 2010), mesmo dada sua importância para entender a variação entre e dentro de diferentes clados (por exemplo, Benites et al. 2010).

Dado esse contexto, as principais contribuições da presente tese são: (1) descrever a coloração da plumagem de um traupídeo dicromático com coloração derivada de pigmentos carotenoides, *Sicalis citrina* Pelzeln, 1870, dentro do modelo de visão das aves; e (2) testar hipóteses que versam sobre a expressão fenotípica e qualidade individual das fêmeas e machos. A tese também contribui para o conhecimento básico sobre o comportamento reprodutivo e de movimentação desta espécie que, apesar de comum, ainda é pouco conhecida (veja detalhes sobre sua história natural a seguir).

4. Espécie de estudo

Sicalis citrina (canário-rasteiro) é uma espécie granívora de cerrado aberto e que forrageia no solo. Apresenta dicromatismo sexual, os machos exibem a cabeça amareloolivácea, com ampla fronte amarela e as fêmeas pardas com leves tons amarelados (Sick 1997) (Fig. 1). A espécie se distingue de outras do gênero *Sicalis* pela presença de nódoas brancas nas retrizes externas (Sick 1997). Tem ampla distribuição na América do Sul, podendo ser encontrada em regiões montanhosas da Argentina à Colômbia, Venezuela, Suriname e Guianas (Fig. 2) (Jaramillo 2019). No Brasil ocorrem duas subespécies: *S. c. citrina* que se distribui em áreas abertas do sul do Pará até o leste de Mato Grosso e Paraná; e *S. c. browni* que se distribui no norte do Brasil (Jaramillo 2019). No Cerrado do Brasil central pode ser encontrada a subespécie *S. c. citrina*, a qual é vista facilmente em sua época reprodutiva no Parque Nacional de Brasília.

Sicalis citrina é uma espécie socialmente monogâmica, onde no Distrito Federal se reproduz entre dezembro e maio, com pico de janeiro a março (Gressler e Marini 2011). Durante a estação reprodutiva é possível avistar machos executando *displays* (comportamentos de corte) constantemente. A única descrição de seu *display* é dada por Sick (1997), que o descreve apenas como um voo ascendente seguido de um voo descendente emitindo um canto trinado até um poleiro próximo.

Seus ninhos são construídos em forma de cesto a uma distância média de 30 cm do solo sendo sustentado pela base na planta suporte (Gressler e Marini 2011). As laterais e a base do ninho são construídos com folhas largas de gramíneas e internamente são revestidos com finos talos de gramíneas (Gressler e Marini 2011). Apresenta variação natural no tamanho da ninhada, com posturas de dois a três ovos, e, até o momento, apenas as fêmeas foram avistadas realizando a incubação dos ovos e ninhadas (Gressler e Marini 2011). Estratégias reprodutivas, detalhes acerca do processo de construção do ninho e informações sobre o cuidado parental ainda não foram relatados.

Pouco se sabe a respeito da movimentação de *Sicalis citrina*, constando na literatura relatos anedóticos de que a espécie desaparece de algumas regiões, como Minas Gerais, durante alguns meses de inverno (Sick 1997). Sendo assim, alguns autores o consideram como espécie migratória (e.g. Sick 1997, Nunes e Tomas 2008), enquanto outros como espécie nômade (e.g. Jaramillo 2019).

Fig. 1. Macho (à esquerda) e fêmea (à direita) de *Sicalis citrina*. Fotos de autoria própria.

Fig. 2. Distribuição geográfica de *Sicalis citrina*. Fonte: Jaramillo 2019

5. Objetivos

O objetivo geral do trabalho é testar hipóteses que versam sobre a expressão fenotípica e a qualidade dos indivíduos, bem como descrever a coloração de *S. citrina* e contribuir com o conhecimento básico de história natural, detalhando comportamentos reprodutivos e de movimentação desta espécie.

Assim, o primeiro capítulo tem como um dos objetivos descrever a coloração de *S. citrina* no sistema visual das aves e investigar se machos atrasam a maturação da plumagem como parte de uma estratégia reprodutiva. Como fêmeas e machos subadultos apresentam o mesmo padrão de coloração aos olhos humanos, o segundo objetivo deste capítulo é determinar a capacidade dos coespecíficos discriminarem entre as plumagens das fêmeas e dos machos subadultos. Ainda, este capítulo descreve pela primeira vez algumas facetas do comportamento reprodutivo de *S. citrina*, incluindo a descrição completa do *display*, estratégias reprodutivas, tamanho e qualidade dos territórios reprodutivos e cuidado parental.

O segundo capítulo visa identificar como os pigmentos que dão origem à sua cor são adquiridos e utilizados no corpo. Para isso, foram traçados três objetivos específicos: (i) identificar os tipos de carotenoides que determinam as cores de *S. citrina* e suas fontes alimentares, (ii) identificar se a concentração de carotenoides é refletida na coloração individual, na saúde imunológica ou na condição corporal, e (iii) identificar se os indivíduos mais saudáveis e/ou com maior condição corporal (*proxies* para a qualidade dos indivíduos) expressam essas características por meio de atributos específicos de coloração e se tem acesso à fontes alimentares específicas.

Por fim, o terceiro capítulo visa identificar o padrão de movimentação sazonal desta espécie e suas áreas de uso por meio da combinação de duas metodologias, isótopos estáveis e ciência cidadã.

6. Área de estudo

Entre setembro e dezembro/2018 foram realizadas campanhas para a localização das regiões de maior ocorrência e nidificação de *S. citrina* no Parque Nacional de Brasília/DF

(15° 38' 28" S, 48° 1' 15" O). Foram identificadas oito áreas de nidificação da espécie, onde quatro apresentaram alta abundância de adultos e ninhos e grande sucesso de captura (Fig. 4). Após a identificação dos melhores locais para captura dos indivíduos, a coleta de dados ocorreu durante três estações reprodutivas (2018/2019, 2019/2020 e 2020/2021). Para mais detalhes, veja a seção de Material e Métodos dos capítulos seguintes.

O Parque Nacional de Brasília compreende uma área superior a 42.000 ha, com clima sazonal bem definido, caracterizado por verões chuvosos e invernos secos (ICMBio 2017). Abrange fitofisionomias típicas do Cerrado, bem como regiões alteradas pelo ser humano, como certas áreas de antiga extração de cascalho, que apesar de impactada, é a região preferencial de nidificação de *S. citrina* (Gressler e Marini 2011). Durante a construção da barragem Santa Maria, no interior da unidade de conservação em 1969, diversas frentes de mineração de cascalho foram abertas no parque (ICMBio 2017). A exploração destas áreas resultou em ambientes de solo exposto que compreendem cerca de 180 ha da área do parque (Martins et al. 2004). Mesmo após cerca de cinco décadas, essas áreas ainda não se recuperaram completamente, permanecendo com o solo exposto, pouca cobertura vegetal e sinais de erosão avançada (Fig. 5). Algumas espécies vegetais presentes atualmente na área pertencem às famílias Poaceae, Melastomataceae, Myrtaceae (Eucalipto) e Pinaceae. Moitas de pteridófitas (Gleicheniaceae) são visivelmente abundantes nas cascalheiras e são a planta suporte preferencial de nidificação de *S. citrina* (Gressler e Marini 2011).

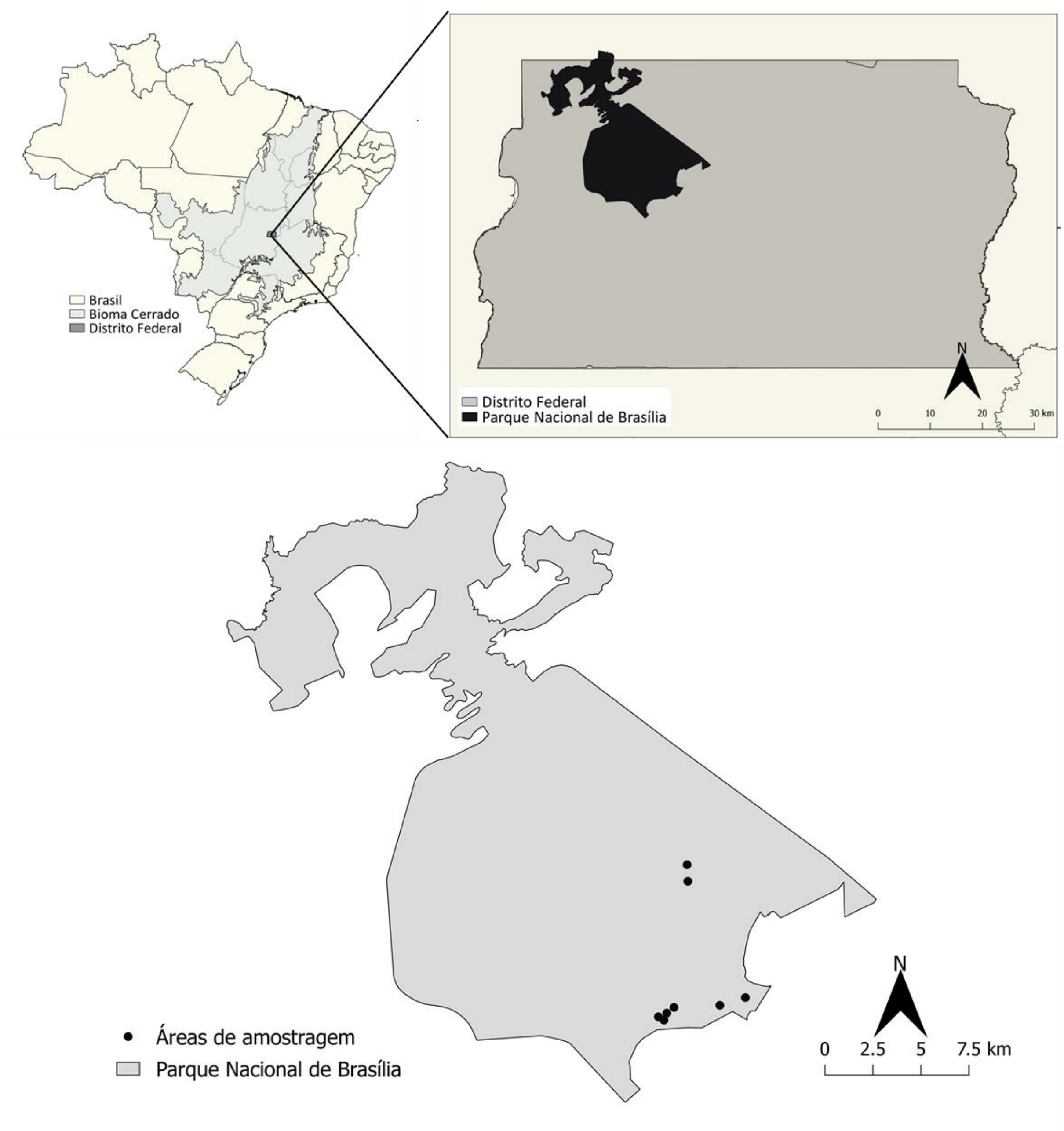

Fig. 4. Localização das áreas de maior ocorrência e nidificação de *S. citrina*

Figura 5. Cascalheira – áreas de amostragem

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Capítulo I

Delayed plumage maturation and breeding behavior of the stripe-tailed yellow finch *Sicalis citrina* Pelzeln 1870 (Aves: Thraupidae).

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Ninho de *Sicalis citrina*
Foto por Mariana de Carvalho

Delayed plumage maturation and breeding behavior of the stripe-tailed yellow finch *Sicalis citrina* Pelzeln 1870 (Aves: Thraupidae).

Mariana de-Carvalho[1], Nathalie K. Citeli[2,3], Regina H. Macedo[3]

[1]Departamento de Ecologia, Instituto de Ciências Biológicas, Universidade de Brasília, Brasília, Federal District, Brazil
[2]Departamento de Engenharia Florestal, Faculdade de Tecnologia, Universidade de Brasília, Brasília, Federal District, Brazil
[3] Departamento de Zoologia, Instituto de Biologia, Universidade de Brasília, Brasília, Federal District, Brazil.

Abstract

The stripe-tailed yellow finch *Sicalis citrina* Pelzeln 1870 is a Neotropical, sexually dichromatic thraupid: browner females and yellowish males. To human eyes, subadult males are indistinguishable from females, and these males possibly have delayed plumage maturation as part of a reproductive strategy, as do other thraupids. Here, we investigated the stripe-tailed yellow finch plumage coloration in the context of the avian visual model system and determined the ability of conspecifics to discriminate between females' and brown males' plumages. Also, we included the first description of specific aspects of its breeding behavior, with a detailed account of its display, information about size and quality of reproductive territories, and parental care. Our results indicate that delayed plumage maturation is part of the stripe-tailed yellow finch life-history strategy, and conspecifics should be able to distinguish between the color patterns of females and that of males with delayed plumage maturation. The female color is reduced in reflectance across almost all of the spectra, and they are distinguishable from brown males in the avian system model, especially in ventral color. Both brown and yellow males hold territories that vary in size and quality, but the yellow ones hold better-quality territories. However, not all males hold territories, and we observed many floater males in the area, apparently waiting for opportunities to dispute territories. Though less frequently than females, we recorded males helping with parental care by brooding and feeding the chicks, cleaning the nest, and feeding females. Evidence of differences between male and female parental care, the presence of floaters and the existence of sexual dichromatism reinforce the possible existence of extra pair fertilization in the stripe-tailed yellow finch mating system, a hypothesis that needs to be tested.

Keywords: Avian visual model, Display, Feather color, Ornamentation, Parental care, Sexual dichromatism

Introduction

Variation in levels of sexual dimorphism is generally considered a consequence of the strength of sexual selection, which differs among species, and can result in a high variance in mating success and distinct parental care strategies (Danchin et al. 2005, Dale et al. 2007). Due to the apparent low variance in mating success of monogamous avian species, a weak sexual selection was long expected in the literature (Danchin et al. 2005). However, several monogamous species exhibit marked sexual dimorphism (e.g. mountain bluebird *Sialia currucoides,* Balenger et al. 2009; blue-black grassquit *Volatinia jacarina*, Macedo et al. 2008; and saffron finch *Sicalis flaveola,* Saldívar and Massoni 2018). The conspicuous sex, usually males, are assumed to be under strong intrasexual (i.e., competition between members of the same sex) or intersexual selection (i.e., differential choice of members of one sex by the opposite sex). The duller sex, usually females, are assumed to be selected for crypsis to reduce nest predation (Darwin 1871, Andersson 1994, Shultz and Burns 2017, but see Amundsen 2000 for exception).

In the Thraupidae, the largest bird family, sexual dichromatism, i.e., differences just in coloration between the sexes, is common (Burns et at. 2012). Males are born with the same cryptic color pattern as females, but when they reach sexual maturity, they molt to a conspicuous adult plumage (Jenni and Winkler 2020). However, some passerines delay their plumage maturation and only acquire a definitive adult plumage pattern after their first potential breeding season (see Hawkins et al. 2012 for a list). In these species, the adult secondary sexually dichromatic signals, including the colorful plumage, are reduced or absent, even in reproductive males (Lyon and Montgomerie 1986, Saldívar and Massoni 2018). There is no consensus about the function of delayed plumage maturation, but it is commonly thought that this life-history strategy benefits first-time breeders by reducing the chances of agonistic encounters with older individuals (Lyon and Montgomerie 1986, Hawkins et al. 2012).

It is unknown whether delayed plumage maturation is a widespread life-history strategy in thraupids (e.g., Studd and Robertson 1985, Lyon and Montgomerie 1986, Saldívar and Massoni 2018). The stripe-tailed yellow finch *Sicalis citrina* Pelzeln 1870 is a Neotropical, monogamous and sexually dichromatic thraupid (Gressler and Marini 2011, Jaramillo 2019). Males have mostly yellow heads with an olive wash, an olive-yellow back with fine streaking while ventrally they are entirely yellow with a slight olive cast; the tail is dusky with a white terminal half on the inner web of the outer two rectrices (R1 and R2) (Sick 1997, Jaramillo 2019). Females are brownish, with a dull olive head and an olive and finely streaked back (Sick 1997, Jaramillo 2019). Their ventral plumage is pale yellow with streaked brown on the breast and the tail has the same color pattern as the males. Subadult males have a browner plumage similar to that of females (Jaramillo 2019) and to human eyes, they are indistinguishable from females. The inner webs are the diagnosis of the species and at the time of display, males show them for other individuals (Sick 1997, Jaramillo 2019). Also, it is proposed in the literature, but without testing, that these webs may be smaller in females than in males (Jaramillo 2019). It is unknown whether the colors of stripe-tailed yellow finches are different between females and brown males and whether they delay their plumage maturation, similarly to other thraupids.

This species inhabits rocky mountain slopes and open savannas in South America (Hilty and Brown 1986, Ridgely and Tudor 1989, Sick 1997). Its breeding season extends from at least late December to early May (Gressler and Marini 2011) in central regions of Brazil, when males perform a display that consists of a song in flight: the male flies up diagonally, then descends with a melodious trill to land on a perch (Sick 1997). No more details are known about its display. Clutch size varies from two to three eggs laid in open nests found on tussock grasses, shrubs, and ferns (Gressler and Marini 2011). Nests are built at an average height of 30 cm from the ground, with grass and leaves forming a basket fixed at its base to the vegetation substrate (Gressler and Marini 2011). The sides and base of the nest are built with broad grass leaves and the internal cup is lined with fine grass stalks (Gressler and Marini 2011). Details of the nest construction process and parental care are still unknown, and until now only females had been observed incubating eggs and brooding nestlings (Gressler and Marini 2011).

Here, we investigated the stripe-tailed yellow finch plumage coloration in the context of an avian visual model system, since some species have dichromatic plumage differences undetectable to human vision (e.g., Rajchard 2009, Griggio et al. 2010) and assessed whether males delay their plumage maturation. We evaluated the possibility of conspecifics to discriminate between females’ and brown males’ plumages and also contribute to information about this species' natural history, providing the first complete descriptions of its display; of size and quality of reproductive territories, identifying whether yellow males hold better reproductive territories than brown males; and parental care.

Material and Methods

Field work

We carried out field campaigns to collect data on behavior, to capture adults and search for nests during three breeding seasons (2018/2019, 2019/2020 e 2020/2021) in Brasília National Park, Federal District (15°38'28" S; 48°1'15" W). The park encompasses typical Cerrado phytophysiognomies, in addition to areas altered by humans, such as old mining pits areas, which are preferential nesting sites for stripe-tailed yellow finches (Gressler and Marini 2011). We focused our searches in these old mining pits areas that have not fully recovered, remaining with exposed soil, little vegetation cover and signs of advanced erosion. Some of the current belongs to the families Poaceae, Melastomataceae, Myrtaceae (Eucalyptus), Pinaceae and Gleicheniaceae (Gressler and Marini 2011).

To capture adults, we opened six days a week three to five 12 m x 3 m mist nets at sunrise (about 5:00h to 14:00h) at the sites with greatest activity of individuals in the first two breeding seasons. To maximize capture success, we change the placement of nets daily. We banded captured individuals with metal bands provided by CEMAVE (National Center for Research and Conservation of Wild Birds) and with a unique combination of colored plastic bands. We collected a blood sample from the ulnar vein using a 0.45 x 13 mm needle and Perfecta ® capillary tubes. The collected blood was stored in 99.7% alcohol for further molecular sexing analysis. We then collected seven to ten body feathers (head, dorsal, and ventral), which were fixed with masking tape onto a cardboard, packed in aluminum foil envelopes, and kept away from moisture and light. We took pictures of the white inner webs of the outer rectrices alongside a ruler to serve as a scale.

To confirm whether males delayed their plumage maturation, we monitored stripe-tailed yellow finch nests in the third breeding season to identify breeding males with subadult plumage. When active nests were found, we installed camera traps (Bushnell HD model 119874) to record activity across 48 hours and to identify the nesting individuals. The cameras were motion-triggered and set up to record 20 seconds of filming footage with an interval of 10 seconds before the next shooting starts. During field work, we conducted daily behavioral observations, recording details regarding the display, identifying reproductive territories, and observing the activity of individuals near the nests. We determined whether brown males could occupy reproductive territories and if these differed in size and quality (see below) when compared with territories defended by yellow males.

Molecular sexing

All individuals that could not have their sex identified through plumage were sexed by the company Endêmica Júnior of the University of Brasília. First, the blood samples were subjected to organic DNA extraction in a partnership with Laboratory of Genetics and Molecular Evolution of Birds/University of São Paulo and the National Institute of Criminalistics of the Federal Police, where we did several tests to identify the best quality extracted DNA. The best methodology consists of separating the cell’s DNA through cell lysis with a stain extraction buffer (10 mM Tris-HCl; 100 nM NaCl; 10 mM ETDA; 39 mM DTT (Dithiothreitol); 2% SDS (Sodium Dodecyl Sulfate), pH 7.5), proteinase K and DTT 1 mol/L, DNA extraction with phenol: chloroform: isoamyl alcohol (25:24:1) and was followed by concentration and purification of the DNA in concentrating membranes Amicon® Ultra-0.5 3k. Second, the company Endêmica Júnior conduced a multiplex polymerase chain reaction (PCR) and simple agarose-gel electrophoresis with the primers P2/P8 described by Griffiths et al. (1998) for sexing of individuals.

Plumage coloration analysis

We analyzed plumage color (head, dorsal and ventral feathers) of all individuals using an Ocean Optics® USB4000 spectrophotometer with a pulsating xenon light source PX-2 (Ocean Optics, Dunedin, Florida), which allows for reflectance in the ultraviolet spectrum. We used a pressed plate of $BaSO_4$ as the standard white reference and a UV-free insulating tape as the standard black reference. We obtained the measurements by positioning the optical fiber at a 45° angle in relation to the feather's surface. We placed feather samples on a black, spectrally flat surface and recorded three spectral measures (to the right of the raquis, above and to the left) of each feather to obtain the mean value using the OceanView® software. The spectra were interpolated at 1 nm intervals over the range of 300–700 nm. We used the R package pavo (Maia et al. 2013) to smooth spectral curves and quantify the brightness of feathers. We determined the hue (phi and theta angles in radians) and saturation (distance from the achromatic origin, that indicate the color purity) within a bird's visual model (Stoddard and Prum 2008). For this, we applied visual modeling considering the blue tit *Cyanistes caeruleus* visual system, i.e., an avian UV visual system, and set the models assuming illuminant conditions for open habitats, typical stripe-tailed yellow finch breeding habitat. Using the images of the rectrices, we computed the sum of the measured areas of the white inner webs using the ImageJ software (Schneider et al. 2012). We excluded all individuals which had one or both rectrices missing from analyses.

To determine the possibility of conspecifics to discriminate between females and brown males we performed an ANOVA or Kruskall-Wallis, depending on the normality of the model residuals, for RGB, UV hue and saturation in all body regions and for the size of the white inner webs. Normality was tested using a Shapiro test and from a visual evaluation of the residual graphs.

Reproductive territories

We estimated the reproductive territories of banded males based on their vocalization perches, which were georeferenced in a Garmin etrex GPS (~7m error); this process was repeated until the area of the reproductive territory was obtained (minimum of three points). The demarcated area was considered a reproductive territory only when the same male was seen singing or performing displays for at least two days in the same perches. The outermost vocalization points were linked using QuantumGis 2.18.4, creating polygons that correspond to the males' reproductive territories, and from which we were able to calculate their areas. We subsequently created an index that indicates the quality of the territory. This index included the presence of important resources for stripe-tailed yellow finch nesting, such as availability of water, the distance to feeding sites and the presence of critical plant substrates for nest support. We used the presence of gorges within the delimited territories as a proxy for the presence of water, since the region does not have natural watercourses; however, following rain downpours, these gorges are filled with water which is retained for some time. Water is an important resource both for the hydration of individuals and for the thermoregulation of eggs (Deeming 2011). The vegetation of the Cerrado (savanna) surrounding the mining pits was considered as the foraging area, since there are no records of these birds feeding within the mining pits (personal obs.; Gressler and Marini 2011). Thus, we calculated the shortest distance between the center of the territory and the adjacent Cerrado. Finally, we considered the fern *Dicranopteris flexuosa* as the main support plant for nesting, preferred by stripe-tailed yellow finches for nesting, and which is associated with a high nest success (Gressler and Marini 2011). The quality of the territory was calculated by summing water availability

(+0 for absence and +1 for presence) with the inverse of the distance from the territory center to the nearest feeding site, and the percentage of fern present in the territory (calculated through satellite images, ranging from 0 to 1).

Parental care

The footage taken by the trap cameras, along with field observations, allowed us to identify whether males delay their plumage maturation and compare males and females in terms of frequency and types of parental care behaviors.

Results

Ornament description and differences of the sexes

We identified hue in the red-green-blue (RGB) and in the ultraviolet (UV) spectra (phi and theta angles in radians), and saturation for 84 females, 87 males with yellow plumage, and 30 males with brown plumage. Females and both male plumage types showed reflectance in the UV spectra in the head and in the dorsal and ventral regions. Yellow males had the highest reflectance in UV and yellow when compared with females, especially in the head plumage (Fig. 1A-C). Females and both types of males showed the reflectance spectra expected for carotenoid pigmentation, mainly in the head and ventral area (Fig 1A-C). Yellow males had a higher reflectance in yellow in the head when compared with both females and brown males (Fig. 1A). Female and brown males did not overlap in the spectra reflectance of the head and or ventral plumage, but overlapped in the dorsal area (Fig. 1A-C).

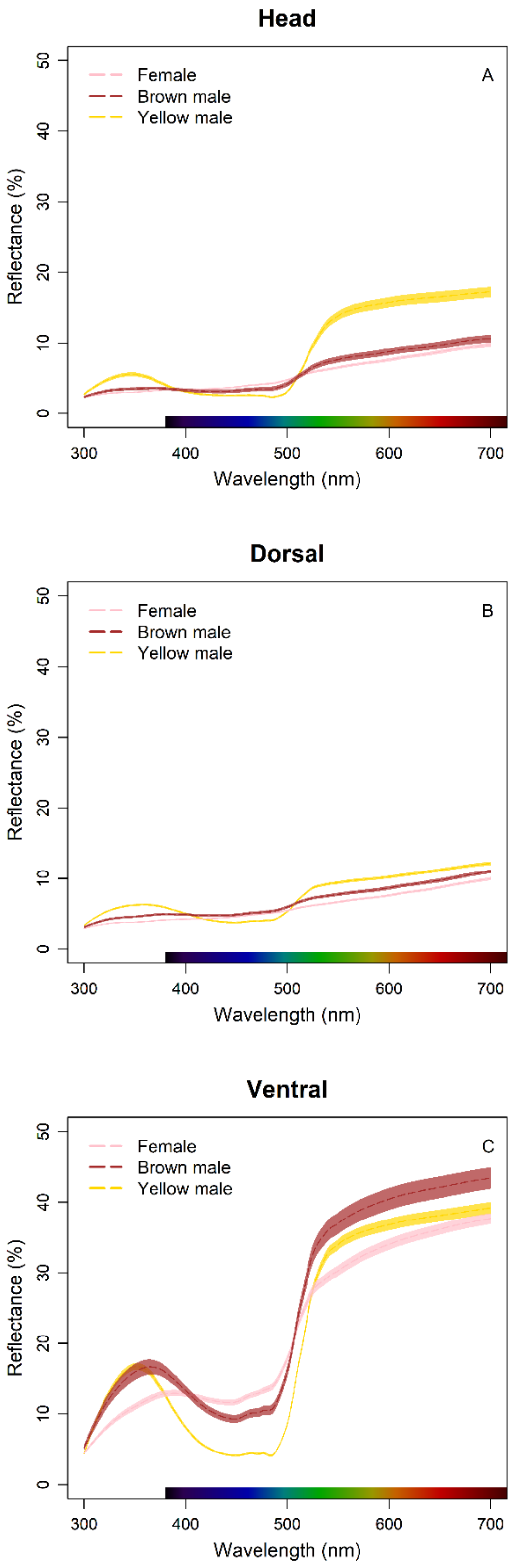

Fig. 1. Head (A), dorsal (B) and ventral (C) mean reflectance for females (pink line), brown males (brown line) and yellow males (yellow line). The rainbow strip refers to the reflective color in the given wavelength, in which UV reflectance corresponds to wavelengths below 400 nm.

Our field observations and the footage taken by the trap cameras indicate that stripe-tailed yellow finches delay their plumage maturation. The perceptual coloration of the females, brown and yellow males showed that the hue vectors for all body regions were located towards ultraviolet, medium (green) and long (red) wavelength (u-ml) regions of the tetrahedron space. The head and ventral area of brown males showed a small overlap with females, but the dorsal region was totally overlapping.

Except for the saturation of the dorsal area, females and males' colors are statistically different (Table 1). There is a slight overlap of the hues between females and brown males, wherein females' measurements have lower values (Table 1). Saturation is the measure that most overlapped between the sexes, but the medians show significant differences between females and brown males, with females being less saturated (Table 1).

We determined the area of the white inner webs of 70 females, 19 brown males and 64 yellow males. The size of the white inner webs differs among the three groups ($P < 0.001$): female white inner webs are about half the size of brown male webs and approximately one third the size of yellow male webs, on average. However, there is a high degree of overlap between the size of these inner webs for females and brown males (Table 1).

Table 1. Color description (head, dorsal and ventral areas) and size of the white inner webs (rectrices) of females, brown males and yellow males (mean ± standard deviation). The last column shows the p-value of ANOVA or Kruskal-wallis for the comparisons between females and brown males.

	Females	Brown males	Yellow males	Statistic: females vs. brown males	*P*-value: females vs. brown males
Head					
Hue in RGB	0.14 ± 0.06	0.25 ± 0.06	0.31 ± 0.03	**$H_1 = 39.44$**	< **0.001**
Hue in UV	-0.75 ± 0.09	-0.60 ± 0.11	-0.48 ± 0.04	**$F_1 = 60.37$**	< **0.001**
Saturation	0.15 ± 0.03	0.18 ± 0.04	0.25 ± 0.04	**$F_1 = 30.20$**	< **0.001**
Dorsal					
Hue in RGB	0.10 ± 0.04	0.20 ± 0.06	0.34 ± 0.04	**$H_1 = 41.74$**	< **0.001**
Hue in UV	-0.77 ± 0.05	-0.67 ± 0.11	-0.37 ± 0.07	**$F_1 = 51.18$**	< **0.001**
Saturation	0.11 ± 0.01	0.11 ± 0.02	0.14 ± 0.02	$F_1 = 0.17$	0.95
Ventral					
Hue in RGB	0.33 ± 0.05	0.39 ± 0.03	0.41 ± 0.02	**$H_1 = 35.35$**	< **0.001**
Hue in UV	-0.69 ± 0.13	-0.51 ± 0.12	-0.35 ± 0.06	**$H_1 = 33.02$**	< **0.001**
Saturation	0.17 ± 0.02	-0.19 ± 0.02	0.23 ± 0.02	**$F_1 = 10.84$**	< **0.001**
Rectrices					
area (cm^2)	172.10 ± 88.56	275.96 ± 174.47	389.8 ± 147.63	**$F_1 = 50.15$**	**0.004**

Breeding behavior

The male display consists of a diagonal flight starting from a perch, followed by a moment of gliding, and landing on another nearby perch. This movement pattern is repeated several times and different perches within the defended territory are used. The height of flight and distance between the perches is quite varied. We monitored 50 individuals performing displays, and our anecdotal observations allowed us to estimate flight height of about 3m and the distance between the starting and landing perches ranged from approximately 1m to 5m. At the maximum height of the flight during the interval where the males glide in the air, the yellow ventral plumage, and the white inner web of the rectrices are exposed in sharp contrast to the sky (Fig. 2). During the morning is common to see males performing displays, however, some individuals (one or two) also conduct displays in the afternoon, at times when the region temperature can reach up to 30°C, according to data from the Brasília weather station (INMET).

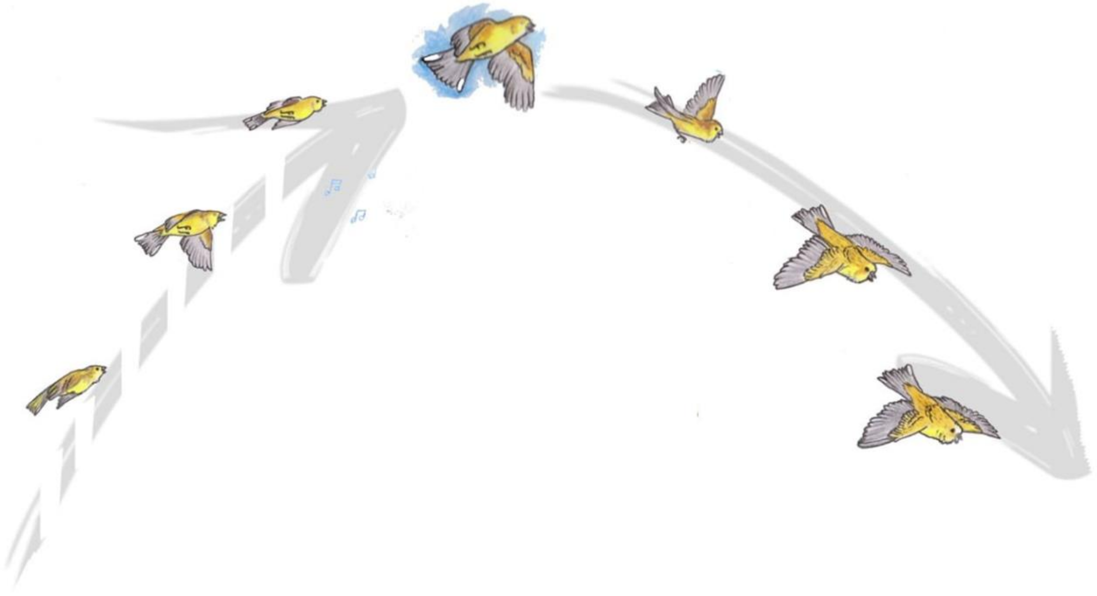

Fig. 2. Stripe-tailed yellow finch display. Note the presentation of the ventral region and white inner web when the individual is at the highest point of the flight. Illustration by Diana Monteiro

Males can change territories within and between breeding seasons. The size and quality of territories vary widely (Table 2). Most of the territories had gorges (28 with presence and 9 without), the distance from these to the feeding site was quite varied (0.05m ± 0.08m) and 12% (± 7%) of the territory on average was covered by the stripe-tailed yellow finch preferential support plant. There was no difference in territory size between brown and yellow individuals (Student test = -0.49, *P* = 0.31), but the quality of yellow male territory was higher (Student test = 1.98, *P* = 0.03).

Table 2. Brown and yellow males' territory sizes and quality, as described in the text.

		Brown	Yellow
Size (m2)			
	N	22	28
	mean	2217.66	1954.33
	standard deviation	2272.88	1467.78
	min	28.97	247.08
	max	8365.77	5782.89
Quality			
	N	15	22
	mean	0.76	1.05
	standard deviation	0.48	0.39
	min	0.10	0.04
	max	1.21	1.60

During much of the breeding season, the same banded males were observed performing displays and responding antagonistically to playbacks. In addition to these individuals holding territories, many other males were observed flying over the breeding area and sometimes engaging in disputes. These males that did not hold territories were considered as floaters.

Only females build nests, but in many cases, males were observed close to the females, perhaps guarding them, while females carried branches. We were able to observe the beginning of the construction of a nest, which began to be shaped by the edge at 06:00 h and took approximately two hours to finish (Fig. 3).

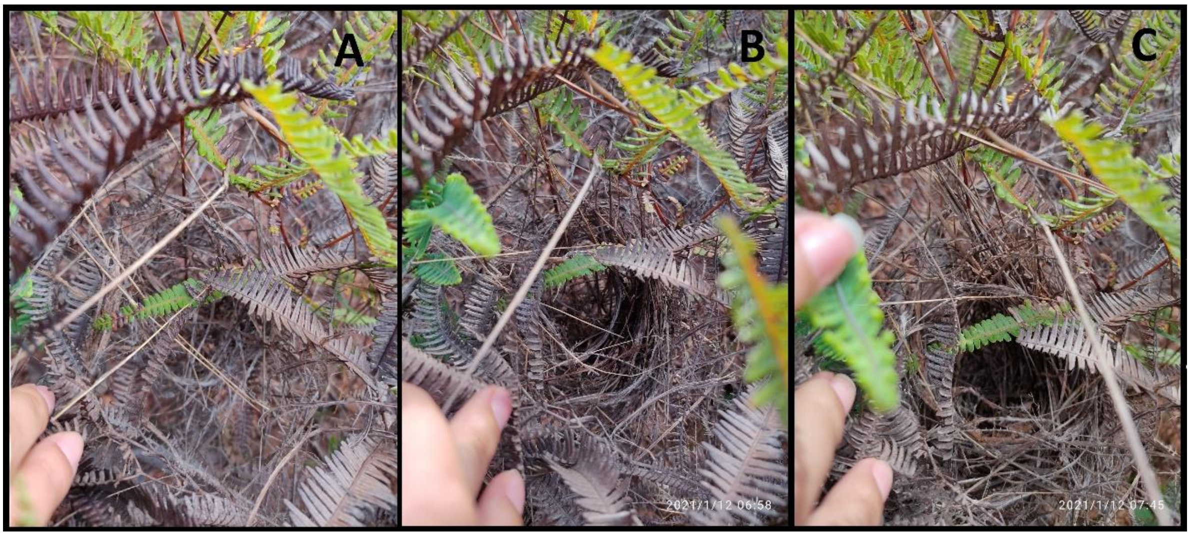

Fig. 3. Construction of a stripe-tailed yellow finch nest started before 06:06 h. At 06:06 h (A) the female had inserted materials and started to build it from the edge, but at this point the nest still had no shape. At 06:58 h (B) the nest begins to take shape and the base starts to close. At 07:45 h (C) the nest was almost finalized.

We recorded 18 active nests, two abandoned, and one preyed upon by a southern caracara *Caracara plancus* as soon as the chicks hatched. In this last nest, the female returned to the nest several times for about 2h, while the male visited the nest only once. Only females incubate the eggs, remaining in the nest even when the ambient temperature reaches high temperatures. At study region temperatures maximums of 30°C were reached during the stripe-tailed yellow finch's breeding season. This temperature does not actually correspond to the temperature at which the females are exposed in the nest, given that the cameras are installed directly under the sun, but even so in front of the nest. In these moments, they were observed breathing heavily and with their wings spread. We recorded females eating the eggshells after the nestlings hatched and documented biparental care in 12 nests, three of which were attended by males with brown plumage. Although males help with parental care, they visit the nest at lower frequencies. Our data show that males visited their nests an average of 7 times in 48 hours (staying in the nest for 2 hours on average), while females visited an average of 15 times (staying in the nest for 4 hours on average). However, male visitation frequency varied widely, with a male visiting the nest 20 times, while another male visited the nest only once. We recorded females incubating eggs, brooding nestlings, perched next to the nest (perhaps guarding it), feeding the chicks, and executing nest maintenance by removing the faecal sacs of the chicks. Females stay all night brooding nestlings and often display restless behavior. Males brood nestlings, perch next to the nest (perhaps guarding it), feed the chicks, assist in the maintenance of the nest by removing the faecal sacs, and also feed the females (i.e., allofeeding).

Discussion

Ornament description and discrimination of the sexes

We described plumage coloration of females, brown and yellow males of stripe-tailed yellow finches based on the avian visual system. The head, dorsal, and ventral plumages exhibit reflectance in the UV spectrum, but the highest UV peaks were found for the head and ventral plumages of yellow males. Moreover, these body regions are the most important for social signaling (Shultz and Burns 2017).

Another interesting finding is that males, both yellow and brown, have the white inner web larger than that of females. This may be a sexually-selected feature that is attractive to females. The yellow male web is on average twice the size when compared to that of females and brown males have webs that are slightly smaller than that of yellow males, but still larger than those of females. In the description of Jaramillo (2019), he states that the female tail has white patterning like that of the male but reduced in size. This was confirmed in our study; however, there is a very large overlap in web sizes between the sexes, which does not allow this feature to be used as a discriminatory trait for sexual identification.

In species with delayed plumage maturation, subadults have a distinctly different plumage pattern compared with that of adults of the same sex, with little or no overlap in color expression (Hawkins et al. 2012). Our observations and camera trap records confirm that the stripe-tailed yellow finch has delayed plumage maturation. Subadult and adult males in our study showed little overlap in their ornamental features, and although females are indistinguishable to human eyes from subadult males, these two groups can be distinguishable in almost every feature of their ornamentation considered the avian visual system. There are several hypotheses to explain how reproductive male can benefit by retaining subadult plumage (see Hawkins et al. 2012 for details). Most studies of delayed plumage maturation support the status-signaling hypothesis (Hawkins et al. 2012). This hypothesis suggests that males with delayed mature plumage experience less aggressiveness from mature males because the subadult plumage indicate their subordinate status (Lyon and Montgomerie 1986, Hawkins et al. 2012). Thus, former males can breed by reducing the risks associated with such aggression and their chances of surviving to the next breeding season can also increase (Lyon and Montgomerie 1986). In this case, colorful adult males perceive dull subadult males, but respond less aggressively to them (see review in Hawking et al. 2012). The patterns of ornamental coloring suggest that striped-tailed yellow finch almost certainly can discriminate between the adult and subadult male categories. This lends greater credibility to the status-signaling hypothesis as the most plausible explanation for the species delayed plumage maturation. Reproductive brown males may be viewed as subordinate males, which can lead to less aggression directed at them from more experienced males (i.e., those exhibiting yellow plumage). Despite this, brown males attract mates and hold territories (see below), which indicates that they exhibit characteristics that can attract females.

The region of the body that could best be used for differentiation by conspecifics is the ventral plumage color, especially relative to UV reflectance. The ventral area is readily apparent during the flight display and may be subject to intersexual selection. In addition, the bright yellow ventral color of the stripe-tailed yellow finch is produced by carotenoid pigments in the feathers and is also visually conspicuous in the avian visual context. In birds, UV and yellow color can be associated with some aspect of individual quality (Siitari and Huhta 2002, Griggio et al. 2010, Jones et al. 2014), but this proposal needs further empirical consolidation.

Our results show that female coloration is reduced in reflectance across almost all of the spectra when compared to brown and yellow males. Female coloration is important in various contexts, including parental care (Bulluck et al. 2017), reproductive success (Doutrelant et al. 20008, Bulluck et al. 2017), and in male mate choice (Wolf et al. 2004, Griggio et al. 2009). Female ornamentation, in dichromatic species, is frequently similar to that of males, but often reduced in intensity or extent (Amundsen 2000, McGraw 2006), which corresponds to the pattern found for the stripe-tailed yellow finch. Further study with this species can clarify whether female ornamentation influences any aspect of individual survival or reproductive success.

Notes on breeding

In some species, males with delayed plumage maturation can hold territories (e.g., Hill 1988, Landmann and Kollinsky 2010). We found that stripe-tailed yellow finch males with delayed plumage maturation and those with mature plumages can hold territories of similar sizes, but which differ in terms of quality. If highly ornamented males defend better territories, female choice of males with better territories and more expressive ornamentation are indirectly linked (Keyser and Hill 2000). In stripe-tailed yellow finches, adult yellow males are better competitors and manage to hold better quality territories when compared to subadult brown males, which therefore can be considered subordinates. Possibly, females choose their mates according to plumage features as this is associated with the quality of the territories. This hypothesis should be tested by identifying the features of males that are directly favored by females.

Our observations also allowed us to identify non-territorial individuals that live around the territories of breeders and move from one place to another. Floater males (Winker 1998) are a common feature in many songbird populations (see review in Moreno 2016). Floaters were previously thought to be young, lower quality or subordinate individuals that were unable to compete with adult males for territories or mates (Lenda et al. 2012). However, they are more mobile than territorial individuals, using more space and a greater diversity of habitats (Bylicka et al. 2010, Campioni et al. 2010). These individuals use reproductive areas as support points, opportunistic reproduction, or to challenge other males for territories (Lenda et al. 2012).

The existence of floaters may select for intense territorial behavior and competitive mate guarding tactics in territorial males (Moreno 2016). On the other hand, the attempts by floaters to attain breeding opportunities may have contributed to the propensities for female prospecting and the signaling of female dominance towards other females (Moreno 2016). It is plausible that this occurs in the stripe-tailed yellow finch mating system, since we identified high territoriality and female guarding by males while they are building the nests. Females, in turn, have different plumage patterns, ranging from brown to a yellowish pattern, which may indicate dominance between them.

Floaters may invest more in reproduction because they do not spend energy in territory defense or parental care (Lenda et al. 2012). The costs of holding territories and caring for offspring is high (e.g., Low 2006, Ryder et al. 2011), so males that hold territories could be superior to floaters, as they invest also in other aspects of reproduction, in addition to sperm production (Lenda et al. 2012). In stripe-tailed yellow finches, we also recorded paternal behavior for territorial males, including brooding and feeding nestlings, cleaning the nest, and feeding females. Other male thraupids also provide parental care (e.g., blue-black grassquit, Carvalho et al. 2006; saffron finch, Palmeiro and Massoni 2011). Biparental care is the most common pattern of care in birds (Cockburn 2006).

Although male stripe-tailed yellow finches pay a reproductive cost with parental care, it is considerably lower than that of females. This leads us to expect that there may be a high frequency of extra-pair paternity (EPP) in the stripe-tailed yellow finch mate system. EPP is expected to occur in bird species in which males play a minor role in offspring provisioning and in which attractive males provide relatively little parental care (Moller 2000). Evidence of differences between males and females in parental care, the presence of floaters and the existence of sexual dichromatism in the stripe-tailed yellow finch mating system reinforce the possibility of EPP. Genetic analyses will allow us to corroborate this hypothesis and identify the rate of extra pair fertilization in the species.

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Capítulo II

Trade-offs between health and carotenoid-derived color in stripe-tailed yellow finch males

Submetido para The American Naturalist (Fator de Impacto: 3.92)

Macho de *Sicalis citrina*
Foto por Mariana de Carvalho

Trade-offs between health and carotenoid-derived color in stripe-tailed yellow finch males

Mariana de-Carvalho[1,2], Ranne K. C. dos Santos[2], Osmindo R. Pires Júnior[3], Gabriela B. Nardoto[2], Regina H. Macedo[1]

[1] Departamento de Zoologia, Instituto de Ciências Biológicas, Universidade de Brasília, Brasília, Federal District, Brazil.

[2]Departamento de Ecologia, Instituto de Ciências Biológicas, Universidade de Brasília, Brasília, Federal District, Brazil.

[3] Departamento de Ciências Fisiológicas, Instituto de Ciências Biológicas, Universidade de Brasília, Brasília, Federal District, Brazil.

Abstract

Carotenoid-derived color is an important criterion in avian mate choice because plumage color may be an indicator of individual quality. However, recent investigations show that cheating may occur because low-quality individuals can also be colorful. To determine whether carotenoid-derived color can be a sign of individual quality, it is necessary to identify how carotenoids are acquired by individuals and subsequently used by the organism. By using carbon and nitrogen stable isotope analyses we were able to determine how carotenoid pigments are acquired and later used in stripe-tailed yellow finch *Sicalis citrina*. We determine if color is a signal of individual quality in immune health and body condition perspective. Sexes differ in how their bodies use carotenoids. Females invest their carotenoids directly in reproductive processes (e.g. egg production), while males invest indirectly, in the production of ornamentation. Female ventral plumage color is condition-dependent: browner ones have a diet rich in C_3 and C_4 grains, and the females with this diet also show better body condition. Male quality is not necessarily associated with a colorful plumage. They access different food items to improve their color (grains) or their immune health (protein), suggesting that they may choose to invest in either their ornamentation or their immune system.

Keywords: Body Condition, Females, Honest Signals, Immune health, Lutein, Stable Isotopes

Introduction

Selection of sexual partners is one of the main tenets in the process of sexual selection (Darwin 1871). Both sexes use a variety of signals, including vocalizations, behavioral displays, or morphological features, to attract their potential sexual partners (Andersson 1994, Griggio et al. 2005). Because such signals may contain information about individual quality, theoretically they should have a high cost of production and/or maintenance (Zahavi 1975, Grafen 1990). In birds, one of the signals that may have a high cost of acquisition and production and can be used as a clue of individual quality is plumage color. Both females and males could potentially benefit by choosing partners with more extravagant coloring (e.g., Griggio et al. 2005, Whittingham and Dunn 2016).

The colors of bird ornaments are the result of tissue structure combined with carotenoid pigments and/or melanins (Jawor and Breitwisch 2003). In some species, the importance of carotenoid pigments for social signaling has been highlighted (Svensson and Wong 2011, Weaver et al. 2017). Carotenoids are obtained exclusively from food, and are responsible for yellow, orange, and red plumage colors (Goodwin 1984, Linville and Breitwisch 1997, Grether et al. 1999). In addition to color production, carotenoids can also be used in other contexts, as antioxidants and in immune function (Fitze et al. 2007, Simons et al.

2012). In the latter case, when the immune system is limited by carotenoids, immunological benefits will be lost if carotenoids are used for color production (Navara e Hill 2003, Maney et al. 2008). The quantities of carotenoids acquired from the habitat and stored for future use are finite and may not be enough to allow birds to allocate sufficient carotenoids for both color production and internal processes (Koch and Hill 2018). Even if carotenoids are unlimited in the habitat, the challenges of distributing carotenoids internally could still create trade-offs in how carotenoids are allocated physiologically (Koch and Hill 2018). Thus, it is reasonable to assume that only high-quality individuals should invest in carotenoid ornamentation, because only for them would such signaling provide sufficient benefits in overall fitness to offset health costs (Getty 2006, Svensson and Wong 2011).

Carotenoids provide the basis for color differences in plumage-based sexually selected ornaments (Pagani-Núñez et al. 2019). Nonetheless, there is a gap in studies that determine how carotenoids are allocated in the body (Koch and Hill 2018). To determine whether carotenoid-derived color can be a sign of individual quality, it is necessary to identify how carotenoids are acquired and used in the body (Simons et al. 2012). In this context, stable isotope analyses of nitrogen (δ^{15}N) and carbon (δ^{13}C) can provide information on the assimilation of food items and identification of the sources of consumed carotenoids (e.g. Frincke-Craig et al. 2015, Sparrow et al. 2017). Keratin (which is also important for expressing color) δ^{15}N and δ^{13}C are incorporated into the feathers during molting and subsequently remain inert. The δ^{15}N can be used to estimate trophic position since the δ^{15}N of a consumer is enriched from its diet and basal resources (Post 2002, Dammhahn and Goodman 2014), while δ^{13}C is strongly influenced by the microhabitat used by the species, imparting information about the food source (Zhao et al. 2010, Dammhahn and Goodman 2014). Individuals that have diets based on C_3 plants will have lower δ^{13}C values, and those with diets based on C_4 plants will have higher $\delta^{13}C$ values (Fry 2006, Zhao et al. 2010, Andriollo et al. 2017).

Assimilation of carotenoids differs widely among species (e.g., in lesser goldfinches *Spinus psaltria* the acquisition of carotenoids does not result in production of color in all year, Frincke-Craig et al. 2015; in Bullock's orioles *Icterus bullockii* the use of carotenoids for color production depends on habitat-quality and molt condition, Sparrow et al. 2017); furthermore, the relationship between measures of individual quality and carotenoid-derived color is not consistent across studies (Weaver et al. 2018). We used a Neotropical bird species, the stripe-tailed yellow finch *Sicalis citrina* Pelzeln 1870, to examine these issues. Our objectives in this study were two-fold: (1) to describe the process of carotenoid pigment assimilation in stripe-tailed yellow finch; and (2) to determine whether the color conferred by such pigments is a sign of individual quality in terms of immune health and body condition. This granivorous species has ground-foraging behavior and is widely distributed in South America. Individuals exhibit sexual dichromatism, but both sexes have carotenoid-derived ornaments. Sexually mature males exhibit a yellow head, with a broad yellow forehead and bright yellow ventral plumage, while females have a dusky streak on the head and underparts but have light yellowish ventral tones (Sick 1997). Males perform a display that consists of an ascending flight of up to about 3 m, followed by a descending flight to a nearby perch (de-Carvalho, obs. pess.). During the display, males show their yellow ventral plumage, the white spots of the rectrices and emit a trilling song while descending (de-Carvalho, obs. pess., Sick 1997).

Given this theoretical background and the features of the stripe-tailed yellow finch we raised the following questions: (i) What types of carotenoids determine the stripe-tailed yellow finch colors and what are their food sources? (ii) Is carotenoid concentration reflected in individual color, immune health, or body condition? (iii) Do healthier individuals and/or those with a better body condition (proxies for individual quality) exhibit specific color attributes and could they have access to identifiable food sources? Because reproduction is costlier for females (Trivers 1972), we hypothesized that they should prioritize strengthening their immune health and body condition rather than investing in extravagant carotenoid-based color. On the other hand, we speculate that males should prioritize color production over health or body condition, given the importance of sexually-derived ornamentation in female choice for male successful breeding.

Material and Methods

Study area

The study area is located in Brasília National Park, Federal District (15°38'28" S; 48°1'15" W), Brazil, where stripe-tailed yellow finches occur in abundance. The Park comprises an area of over 42,000 ha, with well-defined seasons, characterized by rainy summers and dry winters (ICMBio 2019). The vegetation is composed of typical Cerrado (savanna) phytophysiognomies, including human-disturbed areas used for gravel extraction in the past (ICMBio 2019). These areas currently have low vegetation cover, formed mainly by plants from the Poaceae, Asteraceae and Melastomataceae families, as well as thickets of pteridophytes (Gleicheniaceae), and exposed soil. Despite these disturbances, these areas provide the preferred nesting sites for stripe-tailed yellow finches in the Brasília National Park (Gressler and Marini 2011).

Fieldwork

We carried out fieldwork to capture stripe-tailed yellow finches in the Brasília National Park, during two breeding seasons: 2018/2019, 2019/2020. We identified the sites of greatest activity of individuals and set up three to five mist nets in the morning, totaling a minimum capture effort of 2,268 h.m² per site. To maximize capture success, we changed the locations of mist nets daily. We banded all captured individuals with metal bands provided by CEMAVE (National Center for Research and Conservation of Wild Birds), and a unique combination of colored plastic bands. We identified females as those individuals with incubation patches and males by their complete yellow plumages (ventral and head). We collected five to ten ventral feathers for carotenoid analysis and to determine color. We chose ventral feathers because this is the part of the body with the most saturated yellow color and which is most apparent to other individuals during displays. Also, ventral part is the one of most important on social signaling (Shultz and Burns 2017). Feathers were attached by their calamus with tape to pieces of cardboard and wrapped with aluminum foil for protection from light and moisture. We also collected blood samples through brachial vein puncture, using a 0.45 x 13 mm needle and non-heparinized capillary tubes. To allow assessment of immune condition we used a drop of blood to produce blood smears on slides, which were fixed and stained with the rapid panopticon kit Instant Prov. To estimate body condition, we measured tarsal length with Digimess® digital calipers (300mm) and weighed all individuals with a precision spring balance (30g). Finally, we collected one wing feather (first primary remiges) to determine carotenoid acquisition with the stable isotope protocol (described below). These samples were stored in small paper bags.

Carotenoids

We identified carotenoid type and estimated its concentration using ventral feathers of females and males with complete yellow plumage. We analyzed these samples in the Toxinology Lab of the University of Brasília, Brazil. We weighed samples on an analytical balance (0.0001 g) and placed them in Falcon tubes (15 ml) containing 3 ml of a solution composed of acetone:petroleum ether:didethyl ether (1:1:1). Subsequently, we subjected these samples to aventere cell disruptor for 3 cycles of 2 min each with an interval of 1 min. After 48 h at 5 °C, each tube was centrifuged at 12000 RPM for 10 min. Then, we removed the solution and placed it in a new Falcon tube, which was subjected to vacuum drying in a speedvac system. After drying, the carotenes were resuspended in 400 µl of methanol and filtered through a 0.45 µm membrane for analysis in a high-performance chromatography system (HPLC) LC-10 (Shimadzu, Japan) with the analytical column C-18 KINETEX Phenomenex (0,45 x 25 cm), oven at 35 °C, mobile phase 100% methanol, and a photodiode detector in the range of 300-600 nm. We identified the carotenoids present in the feather samples according to the comparison with the lutein, alpha carotene and beta carotene standards of retention time and absorbance spectrum of 300-600 nm.

We quantified the concentration of the carotenoid most well represented in the samples by comparison with carotenoids that occur in cabbage. For this, we ground 50g of cabbage and extracted the carotenoids separately with a 50 ml solution of acetone:petroleum ether:diethyl ether (1:1:1). The extract was divided into two 50 ml Falcon tubes and subjected to the cell disruptor for 3 cycles of 2 min each with an interval of 1 min. After 48 h at 5 °C, tubes were centrifuged at 12000 RPM for 10 min. We removed the extracts and conditioned them in new Falcon tubes (15 ml) which were then submitted to vacuum drying in a speedvac system. After drying, the carotenes were resuspended in 1 ml of methanol and filtered with a 0.45 µm membrane for analysis in an HPLC system. We collected and dried the lutein carotenoids separately in a vacuum system. These carotenoids were quantified according to Britton (1995).

Color attribute

We measured the ventral color of all individuals using the spectrophotometer Ocean Optics® USB4000 with pulsating xenon light source PX-2 (Ocean Optics, Dunedin, Florida), which allows for reflectance in the ultraviolet spectrum. We used a pressed plate of $BaSO_4$ as the standard white reference and a UV-free insulating tape as the standard black reference. We obtained the measurements by positioning the optical fiber at a 45° angle in relation to the feathers. We placed feather samples on a black, spectrally flat surface and recorded three spectral measures (to the right of the raquis, above and to the left) using the OceanView® software. The spectra were interpolated at 1 nm intervals over the range of 300–700 nm. We used the R package pavo (Maia et al. 2013) to smooth spectral curves and quantify the brightness of feathers. We considered brightness as the mean relative reflectance over the entire spectral range measured (300–700 nm). We also determined the hue in the red-green-blue (RGB) and ultraviolet (UV) spectrum (phi and theta angles in radians) and saturation (color purity) within the avian visual model. For this, we applied visual modeling considering the blue tit *Cyanistes caeruleus* visual system, i.e., an avian UV visual system, and set the models assuming illuminant conditions for open habitats, where the stripe-tailed yellow finch breeds. The phi and theta angles are highly correlated for both sexes ($r > 0.70$, $p<0.005$), and therefore, for subsequent analyses, phi angle was disregarded.

Immune health

White blood cell counts (heterophils, lymphocytes, monocytes, eosinophils, and basophils) were conducted by the commercial Santé Veterinary Lab, Brasília, DF. White blood cells, as part of the body's immune defense system (Miesle 2011), have been used to study health parameters in several taxa (e.g., Dehnhard et al. 2011, Simons et al. 2012). Given that in the exploratory phase of our analyses we found no eosinophils or basophils, that the number of monocytes was close to the normal range (0-3%) (as suggested by Miesle 2011), and that the number of heterophils, lymphocytes and the heterophil/lymphocyte (H/L) ratio were highly correlated between males and females ($r > |0.95|$; $P < 0.001$), we chose to assess the immune health of individuals using the H/L ratio. The H/L ratio has been established as a robust measurement of physiological stress, such that an elevated H/L ratio implies a higher level of stress (Gross and Siegel 1983, Davis et al. 2008). The H/L ratio changes in response to external stressors that typically represent the risk of injury to individuals, which may include weather changes, social challenges, increased reproductive effort, or parasite infestation (Minias et al. 2019).

Body condition

Body condition results from accumulated energy in the individual's body as a result of food ingestion and can be an indicator of quality (Peig and Green 2009). A body condition index (BCI) was calculated for all individuals using the scaled mass index, due to its potential to remove covariance between body size and other body components (e.g. tarsal size) (Peig and Green 2009). We used the following calculation provided by Peig and Green (2009):

$$\widehat{M}_i = M_i \left[\frac{L_0}{L_i}\right]^{b_{SMA}}$$

where $\widehat{M}_i$ is the scaled mass index, M_i is individual i's body mass, L_i the length of individual i's tarsus, b_{SMA} the scaling exponent estimated by standardized major axis regression of the mass (M) by the length (L), and L_0 is the arithmetic mean of the tarsal length of the population studied.

Stable Isotope Analysis

We used the nitrogen (δ^{15}N) and carbon (δ^{13}C) isotope ratio to determine the food sources from which carotenoid pigments were extracted. We used only wing feather samples of females and males with complete yellow plumage. These feather samples reveal the isotopic values corresponding to the breeding season of stripe-tailed yellow finches, once they molt and then start breeding, all within the same geographic area (unpublish data, de-Carvalho).

We first cleaned the samples by leaving them submerged for 30 minutes in a 2:1 solution of chloroform and methanol to remove dust particles and oil residues (Paritte and Kelly 2009). After this step, feathers were oven-dried at 50°C for 48h, the distal part of the vane was cut, weighed (~0.8 to 2.0 mg), encapsulated in tin capsules, and sent for isotope-ratio mass spectrometry analysis. The nitrogen and carbon isotope ratios were determined by combustion using an elemental analyzer (Carlo Erba, CHN-1100) coupled to a Thermo Finnigan Delta Plus mass spectrometer at the Laboratory of Isotope Ecology of the Centro de Energia Nuclear na Agricultura (CENA/Universidade de São Paulo), Piracicaba, SP, Brazil. The results are expressed in delta notation (δ), in parts per thousand (‰), based on internationally recognized standards. We used the following equation: δ^{15}N or δ^{13}C (‰) = (Rsample – Rstandard) / Rstandard × 1.000), where Rsample and Rstandard represent the heavy/light isotope molar ratio of the sample and standard, respectively. The standard used for nitrogen analysis was atmospheric air (^{15}N:^{14}N ratio = 0.0036765) and the standard used for carbon analysis was Vienna Pee Dee Belemite (Vienna PDB; ^{13}C:^{12}C ratio = 0.01118). Internal standards (tropical soil and sugarcane leaves) are routinely interspersed with target samples during analyses. Long-term analytical error for the internal standards is of 0.2‰ for both δ^{15}N and δ^{13}C.

Statistical Analyses

We performed separate statistical analyses for each sex and descriptions below are grouped according to the three main questions of the study. To answer question (i) we performed High-Performance Liquid Chromatography to identify the types of carotenoids that produce the stripe-tailed yellow finch colors; and to assess the food sources used by the birds, we determined the stable isotopic space of δ^{15}N x δ^{13}C.

To identify whether carotenoids are associated with the color attribute, immune health, and body condition of individuals (question ii), we performed Pearson correlation analyses between the concentration of the main carotenoid in samples and (1) plumage color measurements (hue, saturation and brightness); (2) the H/L ratio (proxy for immune health); (3) the BCI (index for body condition).

To determine if female and male's color attribute is affected by their quality or food source (question iii), we first performed a model selection with generalized linear models using the colors measurements as response variable and the H/L ratio, BCI and δ^{15}N and δ^{13}C as the predictor variable. After that, we tested if female and male's quality is affected by food source. To do that, we performed a model selection with generalized linear models using the quality proxies (H/L and BCI) as response variable and the food source (δ^{15}N and δ^{13}C) as predictor variable.

All linear regressions were performed using Gaussian distribution for residuals with a normal distribution (Shapiro test $P > 0.05$) and Gamma distribution for residuals that did not conform to a normal distribution (Shapiro test $P < 0.05$). The selection of models was based on the lowest Akaike information criterion corrected for small samples (AICc), and only the models that presented Δ AICc < 2 were considered plausible. All analyses were performed in R environment (version 3.4.0, R Core Team, 2017), using the package MuMIn (Barton 2016).

Results

Types of carotenoids and food sources

We identified the carotenoids of 29 females and 35 males. We identified three types of carotenoids in the feathers of stripe-tailed yellow finches: lutein, alpha-carotene, and beta-carotene. Lutein occurred in all individuals and always in greater concentrations relative to the other carotenoids (females: N = 29, males: N = 35). Only 7% (N = 2) of females and none of the males had alpha-carotene; none of females and 6% (N = 2) of males had beta-carotene; and none of females and 6% (N = 2) of males the three types of carotenoids. The concentration of alpha and beta carotene in all individuals were below the ability to quantify. Thus, just lutein was used for subsequent analyses.

We identified the food sources of 38 females and 77 males. The main food source of both females and males of stripe-tailed yellow finch is based on C_4 grasses. Some individuals access only grains and others complement their diet with other consumers (e.g. arthropods), exhibiting a wide range of δ^{15}N (Fig. 2).

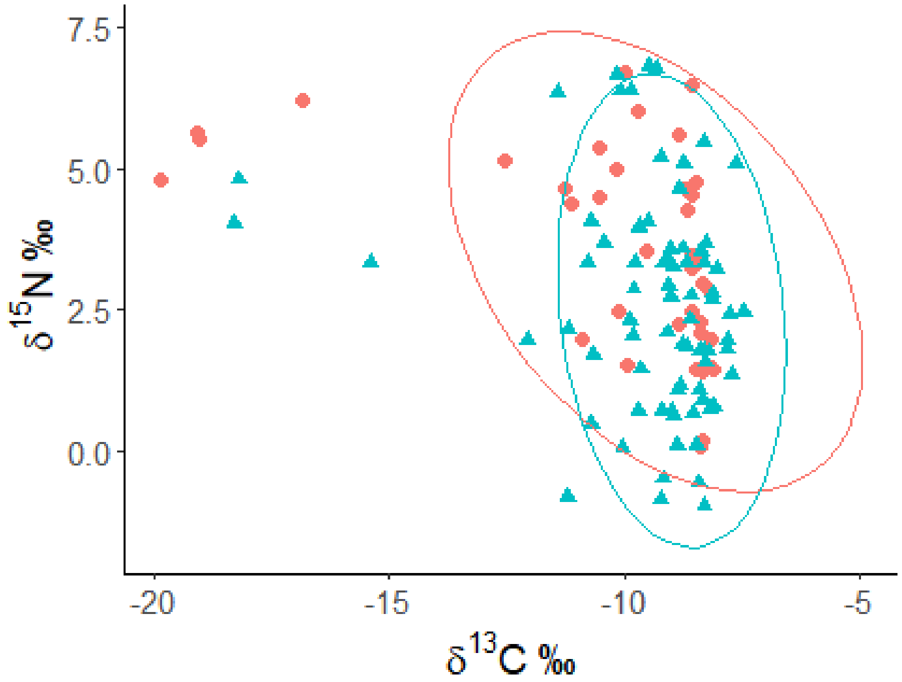

Fig. 2. Stable isotopic space of δ^{15}N x δ^{13}C for females (circles) and males (triangle) with 95% confidence ellipses.

Relation between carotenoid concentration, color, immune health, and body condition

Due to technical limitations we only identified carotenoid concentration, color, immune health, and body condition for 23 females and 26 males. We did not find any correlation between lutein concentration and color, immune health, or body condition for females (*P* >

0.05), but males had a positive correlation between lutein concentration and saturation (r = 0.48, $P < 0.05$). See Supplement Material I for details.

Color attributes and individuals' quality

Browner females, i.e., with a lower RGB, access specific food sources: they have a mixed food origin comprised of a higher proportion of C_3 plants when compared to C_4 plants, as suggested by the lower value of δ^{13}C, accessing grains directly, as shown by the lower value of δ^{15}N (Fig. 3, see details of model selection in Supplementary Material II). Males with saturated venter, in turn, had a diet rich in animal protein, suggested by the higher value of δ^{15}N (Fig. 4, see details of model selection in Supplementary Material II).

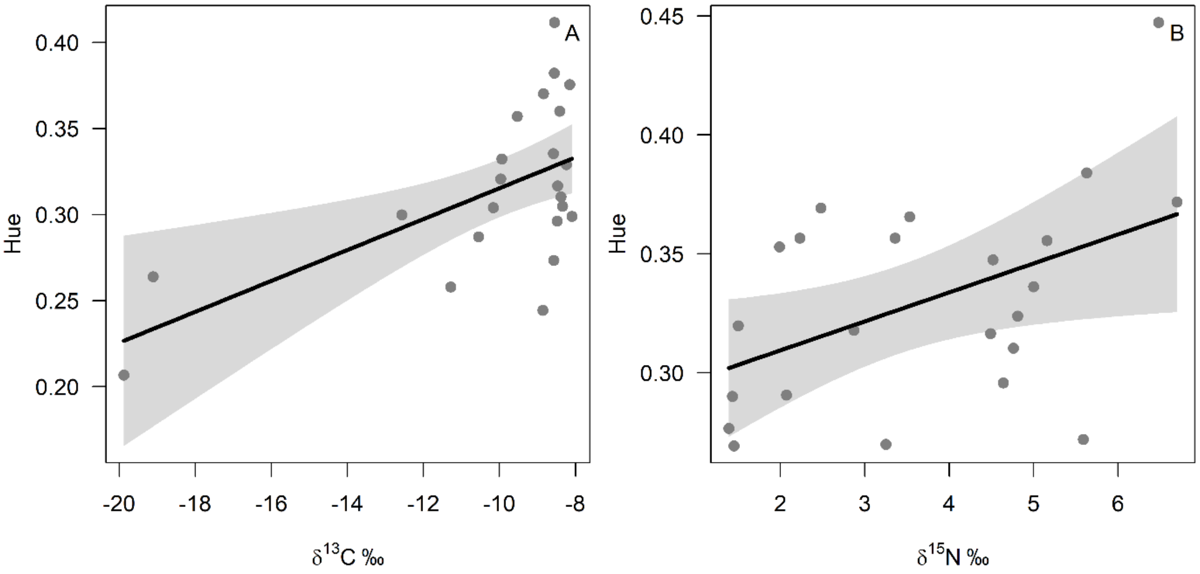

Fig. 3. Positive relationship between Hue and δ^{13}C (A) and δ^{15}N (B) for females.

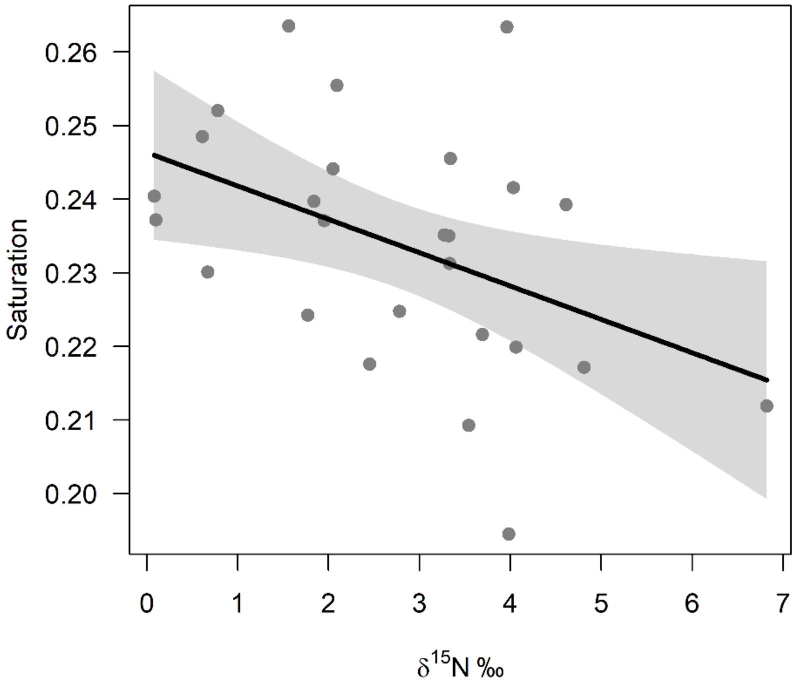

Fig. 4. Negative relationship between Saturation and δ^{15}N (B) for males.

Individual quality and food source

The diet of females also affects their body condition. Those that directly access grains based on a higher proportion of C_3 plants, have a better body condition (Fig. 5). It is these females that have a browner plumage, as seen earlier. Male quality is also affected by the food source, but the healthier ones (lower H/L values) have a diet rich in animal protein (Fig. 6). These healthier males do not correspond to those that have a more color-saturated belly, as seen earlier. See details of model selection in Supplementary Material II.

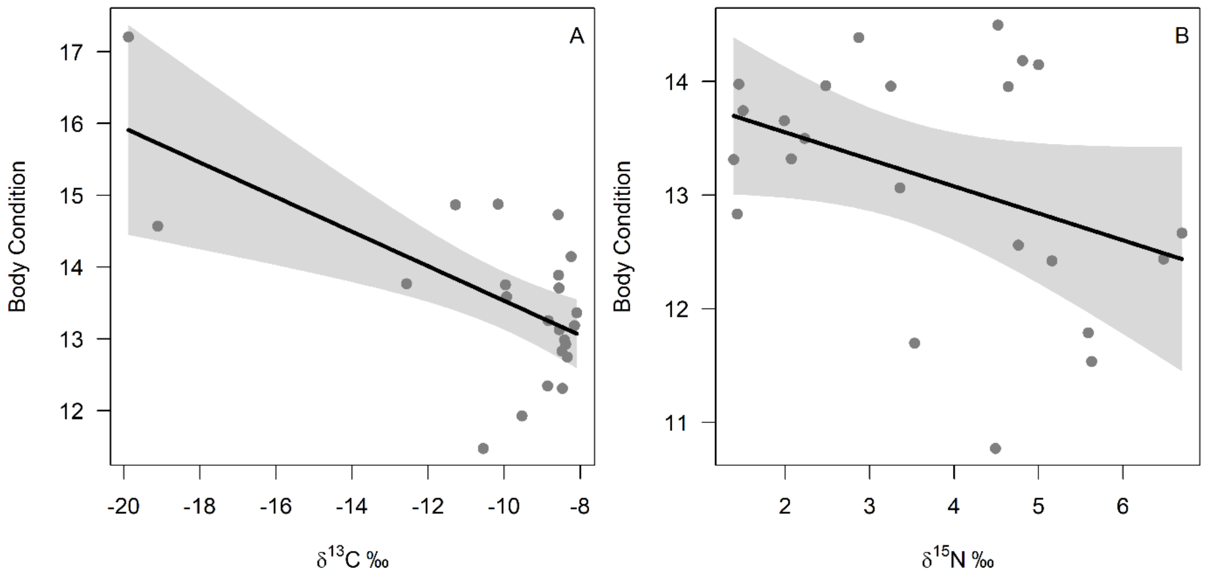

Fig. 5. Negative relationship between body condition and δ^{13}C (A) and δ^{15}N (B) for females.

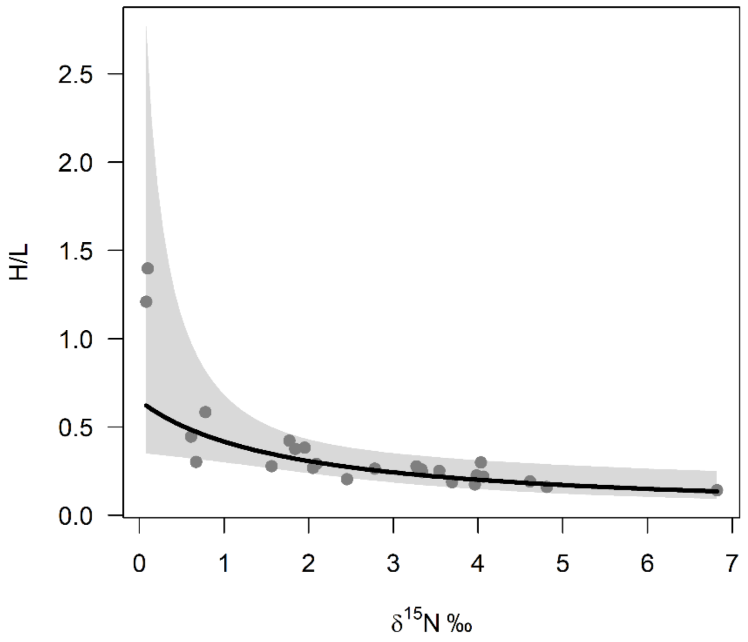

Fig. 6. Positive relationship between immune health (H/L) and δ^{15}N for males.

Discussion

Lutein occurred in feather samples of all individuals and always in greater concentration than alpha and beta-carotene, thus constituting the main type of carotenoid that determines the color of stripe-tailed yellow finches. The $\delta^{13}C$ of most individuals was in the range of -8.80‰, indicating that individuals obtain these pigments directly or indirectly manly from C_4 plants, such as *Tristachya leiostachya* and *Loudetiopsis chrysothrix,* native grasses with a high content of carotenoids and which occur in the study region in the central Brazilian savanna (Reis 2013, Filgueiras 2021).

Analyses indicated that some individuals have mixed food sources, from both C_4 and C_3 plants. In this case, a third grass that is possibly consumed is *Echinolaena inflexa*, a native C_3 grass rich in carotenoids and also found in the study region (Martins et al. 2011, Ferreira 2017, Filgueiras 2021). Feather samples from the stripe-tailed yellow finch also showed a large amplitude in the values of $\delta^{15}N$, indicating that many individuals access carotenoids directly from grains, but many others may access these pigments from secondary or even tertiary consumers, such as herbivorous and predatory invertebrates. It is common for granivorous birds to also feed on insects as a way of supplementing their diet, especially in the breeding season (Pinowski and Kendeigh 1977). Grasshoppers, sawflies, spiders, leaf-beetles, butterflies and moths and their larvae can be important foods for this granivorous bird (Wilson et al. 1999).

Females with higher body condition did not have extravagant color; on the contrary, these females exhibited a browner color. This less colorful plumage may provide information about their condition and could influence the behavior of prospective mates. Studies have shown that in some species (e.g. barn owls *Tyto alba,* Roulin et al. 2000, 2001; bearded tits *Panurus biarmicus,* Romero-Pujante et al. 2002; northern cardinals *Cardinalis cardinalis,* Jawor et al. 2004; blue-footed booby *Sula nebouxii* Morales et al. 2012) female ornamentation conveys information about specific aspects of physical condition and/or health, which differ from that of males (Roulin et al. 2000, 2001, Jawor et al. 2004, Morales et al. 2012). If female ornament expression is maintained by sexual selection acting on females, then such ornaments should provide information about female-specific reproductive capabilities (e.g., maternal effects) (Møller 1993, Romero-Pujante et al. 2002, Jawor et al. 2004). Females usually invest their carotenoids in egg production instead of extravagant plumage color (see Svensson and Wong 2011 for review), and males may be more willing to help females who invest in offspring at the expense of ornamentation (Morales et al. 2012). Our results also show that stripe-tailed yellow finch females that have a better body condition have a specific diet, i.e., they access grains directly, as shown by the lower trophic level they occupy, with a food source based on C_3 and C_4 plants. All individuals captured during the breeding season had new feathers, suggesting that stripe-tailed yellow finches molt before breeding, so it is possible that females are allocating the carotenoids in egg production.

The male finches exhibit a trade-off between their immune health and plumage color. This trade-off can be seen in the context of sexual vs. natural selection (Garland et al. 2022), i.e., male secondary sexual characters increase reproductive success but decrease survival due to higher predation risk. Those with a more saturated ventral plumage have a higher concentration of lutein in their feathers and access specific food items based upon a greater mixture of C_3 then C_4 grains, and this is independent of their immune or body condition. However, males that feed on other consumers – i.e., with a higher trophic level – and which have more C_3 then C_4 food sources, have a strengthened immune system, independent of their ventral color. Thus, male ventral color, despite being derived from carotenoid pigments, does not reflect an individual´s quality, in either body condition or immune health perspectives. Although in some studied cases the data show that carotenoid-derived color can express an individual´s quality (Weaver et al. 2017, Alonso-Alvareza et al. 2022), the exhibition of extravagant color that does not match quality is not uncommon and plumage color could be a dishonest signal for some measures of quality (Weaver et al. 2018). Lutein, in turn, is on the list of carotenoids that are unrelated to quality in a recent meta-analysis (Weaver et al. 2018). Also, in other species, no association was found between plumage color and condition or quality in lesser goldfinches *Spinus psaltria* (Frincke-Craig et al. 2015) and northern cardinals *Cardinalis cardinalis* (Maney et al. 2008). In northern cardinals, for example, the deeply saturated, redder birds are more physiologically stressed than the less colorful birds (Maney et al. 2008). This is similar to the results we found with the stripe-tailed yellow finch males that have higher concentrations of feather carotenoids could perhaps be under greater stress, due to higher energy expenditure associated with mate seeking and/or territory defense, but this link remains to be substantiated in future studies.

As expected, stripe-tailed yellow finch males appear to prioritize improving their color over their body condition or immune health. In some species individuals may consume certain items that maximize carotenoid intake and that result in the production of a colorful plumage, but this can limit the intake of other food items that would help improve health (Walker et al. 2014, Peneaux et al. 2020). Depending on foraging opportunities, stripe-tailed yellow finch males either improve their ornamental color by consuming carotenoids directly from grains, or enhance immune health by consuming a diet rich in animal protein. Males that improve their plumage color do so by accessing grains directly. Protein, on the other hand, should play a role in strengthening immune health (Klasing 2007). In general, greater δ^{15}N values result from consuming prey items that have greater nutritional quality (e.g., Forero et al. 2002).

In conclusion, our results show that female ventral color is affected by food source as well as body condition. The same food source that affects their color attribute improves their body condition, suggesting that their color may be condition-dependent, i.e., females with a better body condition have a browner color. Males, on the other hand, can cheat when expressing their quality, exhibiting a saturated color plumage, regardless of the quality of their immune system or body condition. Thus, male ventral color does not express quality, and could be interpreted as a dishonest signal. However, to evaluate these speculations concerning the honesty of female and male carotenoid-derived ventral color, future studies should test the occurrence of mate choice in the stripe-tailed yellow finch, female–female competition and consider other proxies for quality.

We have shown that stripe-tailed yellow finch females and males diverge in carotenoid usage. In many studied avian species, females invest large amounts of carotenoids in eggs, while males invest in ornamentation (review in Svensson and Wong 2011). The contrasting dynamics of carotenoid allocation of male and female stripe-tailed yellow finches certainly appear to confirm theoretical considerations in the literature indicating that sexual dimorphism goes beyond superficial plumage color, and express more deeply-seated differences in physiology and foraging ecology.

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Supplementary Material

I. Pearson’s correlation between lutein concentration, body condition index (BCI), immune health using heterophil and lymphocyte ratio as a proxy (H/L), nitrogen (δ^{15}N) and carbon (δ^{13}C) isotope ratio, venter saturation, venter hue and venter brightness

a) Females

	Lutein	**H/L**	**BCI**	**δ^{15}N**	**δ^{13}C**	**Saturation**	**Hue**	**Brightness**
Lutein	1.00	0.10	-0.02	0.03	0.16	0.15	-0.22	-0.02
H/L	0.10	1.00	0.12	0.11	-0.12	-0.12	-0.18	0.09
BCI	-0.02	0.12	1.00	-0.07	-0.51	-0.21	-0.37	0.26
δ^{15}N	0.03	0.11	-0.07	1.00	-0.41	-0.14	0.19	-0.09
δ^{13}C	0.16	-0.12	-0.51	-0.41	1.00	0.34	0.42	-0.09
Saturation	0.15	-0.12	-0.21	-0.14	0.34	1.00	0.02	0.00
Hue	-0.22	-0.18	-0.37	0.19	0.42	0.02	1.00	0.27
Brightness	-0.02	0.09	0.26	-0.09	-0.09	0.00	0.27	1.00

b) Males

	Lutein	**H/L**	**BCI**	**δ^{15}N**	**δ^{13}C**	**Saturation**	**Hue**	**Brightness**
Lutein	1.00	0.25	0.19	-0.20	0.08	0.48	-0.08	-0.04
H/L	0.25	1.00	-0.01	-0.44	0.24	0.20	0.01	-0.24
BCI	0.19	-0.01	1.00	-0.17	0.06	-0.08	0.09	-0.27
δ^{15}N	-0.20	-0.44	-0.17	1.00	-0.38	-0.44	0.38	0.01
δ^{13}C	0.08	0.24	0.06	-0.38	1.00	0.07	-0.04	0.22
Saturation	0.48	0.20	-0.08	-0.44	0.07	1.00	-0.53	0.14
Hue	-0.08	0.01	0.09	0.38	-0.04	-0.53	1.00	-0.19
Brightness	-0.04	-0.24	-0.27	0.01	0.22	0.14	-0.19	1.00

II. Model selection to identify if female and male color attributes are affected by individual's quality (Body condition index – BCI – and Heterophile/Leucocyte ratio – H/L –proxy for immune health) or food source (nitrogen isotope ratio – δ^{15}N – and carbon isotope ratio – δ^{13}C). The most plausible models and the null model are in bold and italics, respectively. AICc – Akaike information criterion corrected for small samples; Δ AICc – relative difference between the AIC of the best model and the AICc of the other defined model; K – number of parameters; LL – log likelihood; Wi – weight of evidence.

Intercept	**δ^{13}C**	**δ^{15}N**	**H/L**	**BCI**	**K**	**LL**	**AICc**	**delta**	**weight**
a) Female's saturation									
0.22	0.0037				3	46.17	-85.10	0.00	0.24
0.18					*2*	*44.80*	*-85.00*	*0.09*	*0.23*
0.27				-0.0064	3	45.32	-83.40	1.70	0.10
0.19		-0.0029			3	45.02	-82.80	2.30	0.08
0.19			-0.0122		3	44.95	-82.60	2.44	0.07
0.22	0.0036		-0.0081		4	46.25	-82.30	2.81	0.06
0.24	0.0034			-0.0016	4	46.20	-82.20	2.91	0.06
0.22	0.0037	0.0000			4	46.17	-82.10	2.96	0.05
0.29		-0.0032		-0.0067	4	45.62	-81.00	4.07	0.03
0.27			-0.0097	-0.0061	4	45.43	-80.60	4.45	0.03
0.20		-0.0027	-0.0107		4	45.14	-80.10	5.02	0.02
0.24	0.0034		-0.0079	-0.0014	5	46.27	-79.00	6.07	0.01
0.22	0.0036	0.0001	-0.0082		5	46.25	-79.00	6.11	0.01
0.24	0.0033	-0.0004		-0.0018	5	46.20	-78.90	6.20	0.01
0.28		-0.0030	-0.0079	-0.0064	5	45.68	-77.80	7.25	0.01
0.24	0.0033	-0.0003	-0.0077	-0.0016	6	46.27	-75.30	9.79	0.00
b) Female's hue									
0.36	**0.0090**	**0.0122**			**4**	**42.47**	**-74.70**	**0.00**	**0.34**
0.38	0.0063				3	40.08	-72.90	1.84	0.14
0.37	0.0088	0.0125	-0.0221		5	42.89	-72.20	2.48	0.10
0.52				-0.0149	3	39.49	-71.70	3.01	0.08
0.38	0.0086	0.0118		-0.0017	5	42.49	-71.50	3.27	0.07
0.32					*2*	*37.84*	*-71.10*	*3.64*	*0.06*
0.48	0.0047			-0.0083	4	40.51	-70.80	3.93	0.05
0.38	0.0061		-0.0183		4	40.31	-70.40	4.34	0.04
0.50		0.0045		-0.0144	4	39.83	-69.40	5.28	0.03
0.52			-0.0193	-0.0143	4	39.73	-69.20	5.48	0.02
0.30		0.0052			3	38.24	-69.20	5.50	0.02

0.33			-0.0251		3	38.20	-69.10	5.59	0.02
0.38	0.0085	0.0123	-0.0219	-0.0009	6	42.89	-68.50	6.19	0.02
0.48	0.0046		-0.0167	-0.0079	5	40.71	-67.90	6.84	0.01
0.31		0.0058	-0.0283		4	38.71	-67.20	7.52	0.01
0.49		0.0050	-0.0223	-0.0137	5	40.16	-66.80	7.94	0.01

c) Female’s brigthness

617.00					*2*	*-145.18*	*295.00*	*0.00*	*0.31*
208.70				30.2400	3	-144.38	296.00	1.06	0.18
645.40		-7.6430			3	-145.08	297.40	2.46	0.09
577.30	-3.9440				3	-145.09	297.40	2.47	0.09
603.60			37.2200		3	-145.09	297.40	2.47	0.09
240.30		-6.1380		29.5800	4	-144.31	298.80	3.88	0.05
210.90			25.2100	29.4000	4	-144.34	298.90	3.93	0.04
187.60	2.3980			33.5900	4	-144.35	298.90	3.96	0.04
596.70	-6.7650	-12.8800			4	-144.84	299.90	4.93	0.03
633.50		-8.5280	41.8600		4	-144.96	300.10	5.17	0.02
569.40	-3.5370		33.2800		4	-145.01	300.20	5.27	0.02
246.30		-6.8090	29.2600	28.5400	5	-144.25	302.00	7.06	0.01
188.00	2.6140		26.6500	33.0000	5	-144.30	302.10	7.17	0.01
233.00	0.5584	-5.6630		30.4100	5	-144.31	302.10	7.18	0.01
588.60	-6.4220	-13.4000	37.3500		5	-144.74	303.00	8.04	0.01
238.60	0.5850	-6.3120	29.2900	29.4100	6	-144.25	305.70	10.78	0.00

a) Male’ saturation

0.2463		**-0.0045**			**3**	**72.91**	**-138.70**	**0.00**	**0.34**
0.2715		-0.0048		-0.0019	4	73.31	-136.70	2.02	0.14
0.2414	-0.0006	-0.0050			4	73.08	-136.20	2.48	0.11
0.2462		-0.0045	0.0002		4	72.91	-135.90	2.81	0.09
0.2339					*2*	*70.12*	*-135.70*	*3.02*	*0.08*
0.2303			0.0120		3	70.62	-134.20	4.58	0.04
0.2666	-0.0006	-0.0052		-0.0019	5	73.48	-134.00	4.77	0.03
0.2720		-0.0049	-0.0008	-0.0019	5	73.31	-133.60	5.11	0.03
0.2462				-0.0010	3	70.20	-133.30	5.43	0.03
0.2380	0.0004				3	70.19	-133.30	5.45	0.03
0.2409	-0.0006	-0.0049	0.0008		5	73.08	-133.20	5.57	0.02
0.2424			0.0119	-0.0009	4	70.70	-131.50	7.23	0.01
0.2320	0.0002		0.0116		4	70.63	-131.40	7.37	0.01
0.2513	0.0005			-0.0010	4	70.28	-130.70	8.08	0.01
0.2667	-0.0006	-0.0053	-0.0002	-0.0019	6	73.48	-130.50	8.19	0.01
0.2447	0.0002		0.0114	-0.0010	5	70.72	-128.40	10.29	0.00

b) Male’s hue

0.3974		0.0049			3	65.80	-124.50	0.00	0.29
0.4110					*2*	*63.82*	*-123.10*	*1.39*	*0.14*
0.3891		0.0062	0.0167		4	66.37	-122.80	1.68	0.12
0.3664		0.0053		0.0024	4	66.15	-122.40	2.11	0.10
0.4041	0.0008	0.0055			4	65.97	-122.00	2.47	0.08
0.3941				0.0013	3	63.91	-120.70	3.77	0.04
0.3534		0.0066	0.0181	0.0027	5	66.83	-120.70	3.84	0.04
0.4078	-0.0003				3	63.84	-120.60	3.91	0.04
0.4108			0.0006		3	63.82	-120.50	3.96	0.04
0.3951	0.0007	0.0066	0.0160		5	66.49	-120.00	4.51	0.03
0.3729	0.0009	0.0059		0.0024	5	66.33	-119.70	4.85	0.03
0.3900	-0.0004			0.0014	4	63.94	-118.00	6.52	0.01
0.3939			0.0006	0.0013	4	63.91	-117.90	6.58	0.01
0.4070	-0.0004		0.0015		4	63.85	-117.80	6.72	0.01
0.3593	0.0007	0.0071	0.0174	0.0027	6	66.96	-117.50	7.00	0.01
0.3890	-0.0004		0.0017	0.0014	5	63.95	-114.90	9.60	0.00

c) Male’s brigthness

573					*2*	*-166.50*	*337.50*	*0.00*	*0.18*
943				-29.0900	3	-165.54	338.20	0.64	0.13
611.4			-129.1000		3	-165.77	338.60	1.09	0.10
684.9	11.4900				3	-165.84	338.80	1.24	0.10
772.1	15.3000		-168.3000		4	-164.57	339.00	1.52	0.08
1082	12.3200			-30.5700	4	-164.72	339.30	1.81	0.07
984.9			-130.5000	-29.3400	4	-164.72	339.40	1.82	0.07
1181	16.2400		-172.2000	-31.3700	5	-163.26	339.50	1.98	0.07
571.3		0.6408			3	-166.50	340.10	2.57	0.05
961.9		-3.5950		-29.8000	4	-165.52	340.90	3.41	0.03
650.2		-10.9900	-157.7000		4	-165.61	341.10	3.59	0.03
678.2	13.6000	9.9160			4	-165.71	341.30	3.79	0.03
1088		-16.9000	-174.7000	-32.7500	5	-164.33	341.70	4.13	0.02
775.4	15.0000	-1.8410	-172.3000		5	-164.57	342.10	4.60	0.02
1065	13.4900	5.6330		-29.6000	5	-164.67	342.30	4.81	0.02
1214	15.0200	-7.7460	-189.4000	-32.7800	6	-163.17	342.80	5.24	0.01

III. Model selection to identify if quality of females and males (Heterophile/Leucocyte ratio – H/L – proxy for immune health; and body condition index – BCI) is affected by food source (nitrogen isotope ratio – δ^{15}N – and carbon isotope ratio – δ^{13}C). The most plausible models and the null model are in bold and italics, respectively. AICc – Akaike information criterion corrected for small samples; Δ AICc – relative difference between the AIC of the best model and the AICc of the other defined model; K – number of parameters; LL – log likelihood; Wi – weight of evidence.

Intercept	δ^{13}C	δ^{15}N	K	LL	AICc	delta	weight
a) Female's immune health (H/L)							
0.3623			*2*	*-6.88*	*18.40*	*0.00*	*0.59*
0.239	-0.0122		3	-6.72	20.70	2.35	0.18
0.2839		0.02113	3	-6.74	20.70	2.40	0.18
0.2179	-0.0092	0.01403	4	-6.67	23.60	5.21	0.04
a) Female's body condition (BCI)							
11.96	**-0.2408**	**-0.2373**	**4**	**-30.57**	**71.40**	**0.00**	**0.51**
11.6	-0.1888		3	-32.21	71.70	0.32	0.43
13.5			*2*	*-35.73*	*76.10*	*4.71*	*0.05*
13.69		-0.0509	3	-35.67	78.60	7.25	0.01
c) Male's immune health (H/L)							
1.54		**0.8569**	**3**	**11.32**	**-15.60**	**0.00**	**0.64**
-0.81	-0.2842	0.7427	4	11.82	-13.70	1.82	0.26
-1.28	-0.5084		3	9.03	-11.00	4.58	0.06
3.36			*2*	*7.35*	*-10.20*	*5.39*	*0.04*
d) Male's body condition (BCI)							
12.72			*2*	*-44.59*	*93.70*	*0.00*	*0.56*
13.11		-0.1422	3	-44.22	95.50	1.83	0.22
12.98	0.02705		3	-44.55	96.20	2.48	0.16
13.08	-0.0037	-0.1447	4	-44.22	98.30	4.64	0.06

Capítulo III

Seasonal movements of Neotropical stripe-tailed yellow finch inferred from citizen science and stable Hydrogen isotopes

Submetido para Animal Behaviour (Fator de Impacto: 3.03)

Fêmea de *Sicalis citrina*
Foto por Mariana de-Carvalho

Seasonal movements of Neotropical stripe-tailed yellow finch inferred from citizen science and stable Hydrogen isotopes

Mariana de-Carvalho[1,2], Renata Alquezar[2,3], Emanuel M. L. Silva[2], Regina H. Macedo[1], Gabriela B. Nardoto[2]

[1] Departamento de Zoologia, Instituto de Ciências Biológicas, Universidade de Brasília, Brasília, Federal District, Brazil

[2]Departamento de Ecologia, Instituto de Ciências Biológicas, Universidade de Brasília

[3]Department of Biology, University of Western Ontario, London, Ontario, Canada

Abstract

Despite the importance of understanding animal movements, our ability to determine individual stopover histories is limited. Movements of most Neotropical bird species remain understudied, especially when assessing seasonal movements and occupation of multiple sites. Our objective was to identify the seasonal movement patterns and track the stopover sites of a Neotropical passerine, the stripe-tailed yellow finch (*Sicalis citrina*). Based on anecdotal evidence this species was considered a migrant. We captured 42 individuals (21 males and 21 females) during the 2019 and 2020 breeding seasons in the National Park of Brasília, Brazil. We measured the stable-hydrogen isotope ratio (δ^2H) in tissues with different periods of integration: claw (reflecting non-breeding season) and wing feather (reflecting the late breeding season) and performed spatial assignments using a recently established feather δ^2H isoscape for Brazil. We compared the δ^2H data obtained with occurrence information collected from citizen science databases. Our findings suggest that the stripe-tailed yellow finch individuals captured in the National Park of Brasilia spent the non-breeding season in a concentrated area in central and northeastern Brazil and during the breeding season individuals scattered over a broad geographic area. These movement patterns are typical of seasonal nomadic behavior.

Keywords: Bird, Migration, Nomadism, Stable Isotope Analysis, Thraupidae.

Introduction

Animal movement patterns are a fundamental aspect of the ecology of populations and, broadly considered, individuals typically exhibit behaviors that can be subdivided into categories of daily movements, dispersion, nomadism, and migration. Daily movements are limited to short-distance travel, usually restricted to a few hundred meters and occur within the individual's home range (Newton 2008). Dispersal corresponds to random displacements during a certain stage of life (e.g., young individuals) or by one sex (e.g., males) (Newton 2008). Nomadism is characterized by the temporary and unpredictable occupation of sites according to the availability of resources necessary for survival (Newton 2008). Finally, migration is the only type of movement that presents regularity and seasonality, involving movement between two areas: breeding sites and resting or wintering areas (Webster et al. 2002).

Generally, studies that address seasonal movements include three steps: (i) identifying the type of movements; (ii) identifying their routes; and (iii) understanding the physiological, behavioral and ecological mechanisms involved (Cavalcanti 1990). Steps (i) and (ii) are a prerequisite for step (iii) (Alves 2007). The ability to track individuals has generally been limited and has encompassed only a few species, typically those that breed in Europe and North America, or those that are economically important or large (Hobson 1999). This may be due to technological limitations related to the use of markers, radios, and satellite tags, which require a large network of study sites and researchers (Hobson 1999, review in Bridge et al. 2011). For most Neotropical bird species, the situation is even more critical. We lack even the most basic information (i.e., steps (i) and (ii); Alves 2007), resulting in large gaps in knowledge about these species' seasonal movements.

A promising approach to increase information about seasonal movements of animals may come from stable isotopes analyses (SIA) (e.g., Newsome et al. 2007, Poesel et al. 2008, Hobson et al. 2009, Navarro et al. 2022). This technique is applicable to numerous species and has shown satisfactory results (e.g., Poesel et al. 2008, Hobson et al. 2009, Hobson and Robbins 2009, Gómez et al. 2019, Guaraldo et al. 2021). Furthermore, SIA has a great advantage over other techniques due to its speed and low analytical cost (Guaraldo et al. 2016, Hobson and Wassenaar 2019). The use of this technique is based on the assimilation and fixation of isotopic markers intrinsic to the environment in the tissues of animals and based upon their food sources, which allows tracing the origin and migration route of a species (Fry 2006, Hobson and Wassenaar 2019, Imlay at al. 2019). These isotopic signatures can vary spatially according to biogeochemical processes and are associated to the environmental substrates from which they are assimilated (mainly food, water, and air) (DeNiro and Epstein 1978, Hobson and Wassenaar 2019). The stable Hydrogen isotope ratios (δ^2H), for example, varies in environmental waters due to physical processes linked to the amount of rainfall, continentality, temperature, and altitude, resulting in spatially explicit patterns (Clark and Fritz 1997, Bowen 2010, West et al. 2010, Terzer et al. 2013, Baisden et al. 2016). Thus, δ^2H measurements can provide information on movements of a variety of animals that would otherwise be impossible using other techniques (Hobson and Wassenaar 2019, Gómez et al. 2019).

When an individual drinks water at a particular site, for example, the stable isotope ratios of that environment are incorporated with a predictable fractionation in the tissues growing at that time. Individuals retain clues about where they lived for specific periods of time that depend on their tissue turnover rates (e.g. Hobson and Clark 1992). Thus, by sampling tissues with different turnover rates, we are able to make ecological inferences about associated foodwebs used by animals during different life stages (e.g. Hahn et al. 2013, Hobson and Wassenaar 2019). Tissues such as feathers and claws are metabolically inactive and thus have fixed isotopic values reflecting the period and place during which they developed (Wassenaar 2008).

Another technique that has shown promise for animal movement studies is the use of databases that record presence of different species and that are contributed by volunteers and citizen-scientists, such as the platforms eBird, Xeno-canto and iNaturalist (Silvertown 2009, Cooper et al. 2014, Lees and Martin 2014, Schubert et al. 2019). The advantage of using these platforms is the large dataset that is freely available to the scientific community, since acquiring this data in the field would be time consuming and expensive, both of which constitute barriers to scientific advancement (Dickinson et al. 2010). This strategy is widely and successfully used in studies currently conducted in North America and Europe (e.g., Audubon Society 2022, European Bird Census Council 2022), and can enable important advances in studies about bird seasonal movements, especially in knowledge-limited regions, such as the Neotropics (Lees and Martin 2014, Schubert et al. 2019).

Over a decade ago Newbold (2010) highlighted the critical need of mapping areas used by different species at any given scale, particularly in the Tropics (Newbold 2010). Brazil has the world´s third richest avifauna (BirdLife International 2022); despite this, most Brazilian bird species remain understudied, especially in the assessment of their seasonal movements and occupation of multiple sites (e.g., Somenzari et al. 2018). In this study, we focus on a common Neotropical species, the stripe-tailed yellow finch, *Sicalis citrina* Pelzeln 1870 (Aves: Passeriformes), for which there is little empirical evidence to confirm seasonal movement patterns. There are only a few records showing that individuals periodically disappear from certain regions, which suggest anecdotally that the species is migratory (e.g. Sick 1997, Nunes and Tomas 2008). Here, our objective is to identify the seasonal movements of this Neotropical species, to test the hypothesis that the stripe-tailed yellow finch is indeed a migratory species.

Material and Methods

Study species

The stripe-tailed yellow finch has a wide distribution in South America, and can be found across most of the continent and in mountainous regions from Argentina to Colombia, Venezuela, Suriname and Guyana (Jaramillo 2019). It occurs in most of Brazil, where it is subdivided into two subspecies: *S. c. citrina*, which is distributed from the south of Pará to the east of Mato Grosso and Paraná; and *S. c. browni*, which occurs in northern Brazil (Jaramillo 2019). In the Cerrado biome (savanna) of central Brazil, individuals of the subspecies *S. c. citrina* are found and easily seen in their breeding season in the Brasília National Park, Federal District.

The species is granivorous and forages mostly on the ground. It exhibits sexual dichromatism, where males have an olive-yellow head, with a broad yellow forehead, and females are brown with light yellowish tones (Sick 1997). The species can be distinguished from others of the genus *Sicalis* by the presence of white spots on the external rectrices (Sick 1997). This species has a monogamous social mating system (Gressler and Marini 2011), where a few males hold territories, but many others, considered floaters, fly over reproductive areas, or use them in some way, e.g., as support sites, or while waiting for territorial vacancies (pers. obs.). In the Federal District it breeds between December and May, with a peak from January to March (Gressler and Marini 2011). During the breeding season males defend territories and perform courtship displays, which consist of an ascending flight of approximately 3 m, followed by a descending flight to a nearby perch (de-Carvalho, pers. obs., Sick 1997). During the display, individuals exhibit the white patches of the rectrices and emit a trilling song while descending. (de-Carvalho, pers. obs., Sick 1997).

Fieldwork

We carried out fieldwork to capture stripe-tailed yellow finches in the Brasília National Park, Federal District of Brazil (15°38'28" S; 48°1'15" W; Fig. 1), during two breeding seasons: 2018/2019 and 2019/2020. We identified eight sites of greatest activity of individuals and set up three to five mist nets each morning from 05:00 h to 13:00 h, totaling a minimum capture effort of 2,268 h.m^2 per site. Females were identified by incubation patches and males by their complete yellow plumage. As brown individuals without incubation patches were possibly immature males, we disregard them from the analyzes. We banded individuals with metal bands provided by CEMAVE (Brazilian National Center for Research and Conservation of Wild Birds), and a unique combination of colored plastic bands. We also collected samples of wing feathers (first primary remiges, P1, of each wing) and about 1-2mm of claw tips, for stable isotope analysis.

Because the study region is marked by a seasonality that can be a confounding factor for determining the stripe-tailed yellow finch's movement patterns, we conducted a comparative assessment using a phylogenetically close species, but which is resident in the region, the saffron finch *Sicalis flaveola*. Fieldwork to collect the same types of data was carried out in the vicinity of Brasília National Park and at Fazenda Hotel Tabapuã dos

Pireneus, in Goiás state (ca. 95 km distance, 15°46'40.39" S; 48°49'18.98" W; Fig. 1) during breeding season 2020/2021.

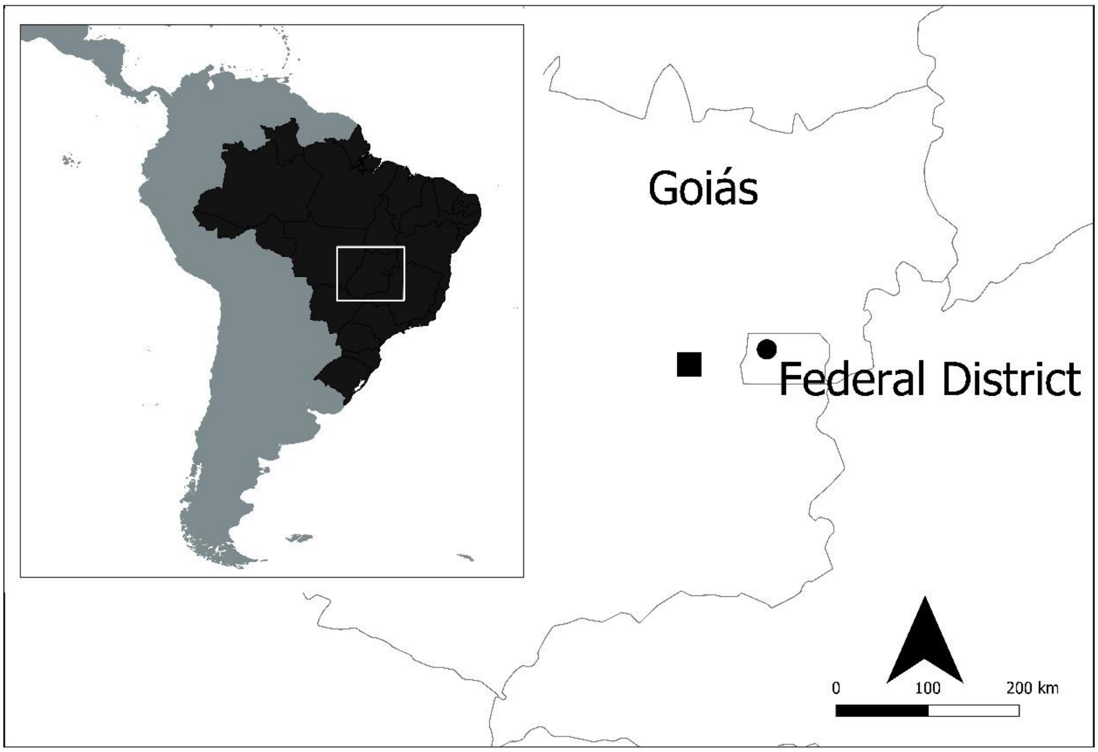

Fig. 1. Study areas, where the circle represents Brasília National Park in the Federal District, and the square represents Fazenda Tabapuã dos Pirineus in Goiás state.

Stable Isotope Analysis - SIA

We estimated the isotopic ratio (^{2}H/^{1}H, depicted as δ^2H) of Hydrogen in wing feathers ($\delta^2H_{feather}$) and claws (δ^2H_{claw}) for 21 females and 21 males of striped-tailed yellow finches and 12 females and 19 males of saffron finches. The $\delta^2H_{feather}$ of feather samples was used to indicate the site where individuals carried out their molt, just before breeding, while the δ^2H_{claw} of claw samples was used to indicate the site where individuals were some five months prior to the date of collection, corresponding to the non-breeding period (Mazerolle and Hobson 2005).

We cleaned the feathers and claws using a 2:1 chloroform: methanol solution overnight. Subsequently, we dried the samples in a fume hood and in an oven at 50°C for 24h (Hobson and Wassenaar 2019). The distal parts of the of the feather vanes and claws were cut, weighed (~0.35 mg) and enclosed in silver capsules. Samples from the striped-tailed yellow finch were sent for analysis in a mass spectrometer at the University of Western Ontario LSIS-AFAR laboratory, Canada, while the samples from the saffron finch were sent to the Center of Stable Isotopes Prof. Carlos Ducatti at the São Paulo State University “Júlio de Mesquita Filho” (CIE/Unesp).

Samples sent to the University of Western Ontario were placed into a Uni Prep carousel (Eurovector, Milan, Ital) at 60°C, evacuated and held under pressurized helium flow before being combusted in a Eurovector 3000 elemental analyzer using a glassy carbon reactor held at 1350°C. The resulting H_2 gas was analyzed in a coupled Thermo Delta V Plus (Thermo, Bremen, Germany) continuous-flow isotope ratio mass spectrometer. Stable-hydrogen isotope results are reported for the non-exchangeable H fraction using the comparative equilibration method of Wassenaar and Hobson (2000) based on within run (n=5 each) measurements of CBS (Caribou Hoof Standard: -197‰) and KHS (Kudu Horn Standard of Environment Canada: -54.1‰) keratin standards.

The samples sent to CIE/Unesp were dried in a vacuum oven at 60°C and analyzed with an Isotope Ratio Mass Spectrometer Delta V coupled with a Thermo Scientific thermal conversion elemental analyzer (TC/EA) (Thermo, Bremen, Germany) held at 1450°C. Stable-hydrogen isotope results are reported for the non-exchangeable H fraction using the comparative equilibration method of Coplen and Qi (2012) based on USGS42 (Tibetan human hair powder: -72.9 ‰) and USGS43 (Indian human hair powder: -44.4 ‰) keratin standards.

Because international keratin standards were used, both labs produce comparable results. All results are reported in standard delta notation relative to the VSMOW (Vienna Standard Mean Ocean Water) standard scale. Based on within-run replicate measurements of standards, we estimate our measurement precision to be ~ ± 2‰.

If a difference is found between the stripe-tailed yellow finch tissues it can be attributed to the geographical movement of individuals (indicative of migration) or to seasonality. Thus, it is important to compare these results with those from the saffron finch: if saffron finch tissues show a similar difference in isotopic values as that of the stripe-tailed yellow finch, we can attribute such difference to the seasonality of the region, not to geographic displacement.

Statistical Analysis

To make sure that the feather values can be assigned to the stripe-tailed yellow breeding site, we first compared the $\delta^2H_{feather}$ of stripe-tailed yellow finches with the $\delta^2H_{feather}$ of seven resident thraupids (burnished-buff tanager *Tangara cayana*, N = 11 individuals; white-banded tanager *Neothraupis fasciata*, N = 1; sayaca tanager *Thraupis sayaca*, N = 1; grey pileated finch *Coryphospingus pileatus*, N = 1; green-winged saltator *Saltator similis*, N = 1; yellow-bellied seedeater *Sporophila nigricollis*, N = 2 and saffron finch, N = 6), totaling 23 samples, captured in the same general region (Federal District and Goiás state) (data from Alquezar et al. in press). For this comparison we used the Kruskal-Wallis test, since the residuals do not have a normal distribution (Shapiro test: W = 0.93, $P < 0.001$). This comparison was used to determine whether the stripe-tailed yellow finch molts in the same region where it breeds.

To produce comparable measures in $\delta^2H_{feather}$ and δ^2H_{claw}, we performed a claw-to-feather transformation specific for adult individuals, following Gómez et al. (2019): $\delta^2H_{feather} = 24.1 + 1.3 (\delta^2H_{claw})$, which was used in all analyzes. To assess the existence of migration in the stripe-tailed yellow finch, we compared $\delta^2H_{feather}$ and δ^2H_{claw} of all individuals, using the Kruskal-Wallis test (Shapiro test: $W = 0.94$, $P < 0.001$). The same was done with all individuals of saffron finch, to confirm by visual and Kruskal-Wallis scores comparison whether the difference observed between $\delta^2H_{feather}$ and δ^2H_{claw} of the stripe-tailed yellow finch is a geographic or seasonal difference. All analyses were conducted in R environment (R Core Team 2018).

Assignment tests for the stripe-tailed yellow finch

We performed assignment tests to identify the most likely places of origin of the stripe-tailed yellow finch samples. To do this, we first determined its potential geographic distribution using the MaxEnt algorithm. We surveyed 277 geographic coordinates from citizen science platforms, such as eBird, Xeno-Canto and WikiAves. We checked all records and validated identifications. Occurrences were randomly separated into 70% for training and 30% for validating the model, with 100 bootstrap pseudo replicates. For developing the model, we selected seven of the 19 bioclimatic variables available in WorldClim (Fick and Hijmans 2017) at a resolution of 2.5 arc/min (~5km) according to the environmental requirements of the species and with low collinearity (Pearson's $P < 0.7$): precipitation of wettest month, precipitation seasonality, precipitation of warmest quarter, precipitation of coldest quarter, mean diurnal range, temperature seasonality, and max temperature of warmest month. Finally, we built the final map with Quantum GIS 2.18.1 (QGIS Development Team, 2016) applying a more conservative threshold (maximum training sensitivity plus specificity logistic threshold) for generating a smaller optimal area, but with high suitability. This distribution map was used as a mask in assignment tests (see potential geographic distribution in supplementary material I).

We excluded four $\delta^2H_{feather}$ and two δ^2H_{claw} outliers, and then assigned a location for each individual relative to each tissue (feather and claw) from a Hydrogen isoscape created for the family Thraupidae (Alquezar et al. 2022). The environmental (Hydrogen isoscape buit by feather samples from Thraupidae) and tissue (feather and claw samples) isotope model was used to rescale each cell of the environmental isoscape to a predicted tissue isotope value using ordinary least squares to fit a linear calibration (Ma et al. 2020). A threshold of 0.8 probability point was defined as the likely area of origin for each individual, that show the region within which there is an 80% chance each sample originated. Finally, we overlaid these maps of origin and identified the 20% of the area with the highest probability of origin of all feather samples, and another map for claw samples. These maps identify the most likely locations where individuals were during molting just prior to the breeding season (feather) and their location in the non-breeding season (claw). To test the quality of isotope-based assignments we use *QA* function, splitting five know-origin data and run five times of cross-validations. The assignment tests were done with assignR package (Ma et al. 2020).

Citizen science data

We searched for all occurrence data of the stripe-tailed yellow finch, the saffron finch and a third migratory species in the Brazilian central region, the fork-tailed flycatcher *Tyrannus savana* in GBIF – Global Biodiversity Information Facility – until December 2021. GBIF is a global international network and data infrastructure that provides open access to data regarding all species in the world (GBIF 2022). This platform gathers data from several citizen-science platforms, such as eBird, Xeno-canto and iNaturalist, in addition to some scientific collections. Our search was restricted to the Federal District region and we used all records found (citizen science and scientific collections data). We made circular histograms and for this we grouped monthly data and divided it by angles of 30° (i.e., 0–29° for January records, 30–59° for February, and so on). We identified the mean vector (μ), circular SD, and vector length (r) (Zar 2010) of circular histograms of each species: μ refers to the mean occurrence date of a given species in the study area, and r to data concentration around this mean, which can take on values between 0 and 1 (Zar 2010). A value close to one indicates that values of the dataset are concentrated at a single location, i.e., all data converge to the same direction. A value close to zero indicates a higher spread in values, i.e., data with uniform circular distribution (Zar 2010).

We expected that resident species' records would show a uniform (i.e. non-seasonal) circular distribution pattern, whereas migrant species´ records would show a unimodal pattern (i.e. seasonal; Zar 2010). We compared the distribution patterns of the two species with known seasonal, that is, resident and migratory behavior (i.e., saffron finch and fork-tailed flycatcher) with that of the stripe-tailed yellow finch to determine whether or not the latter also exhibited a seasonal pattern.

Results

There was no difference between the $\delta^2H_{feather}$ of the stripe-tailed yellow finch (mean ± standard error: -57.3‰ ± 10.7‰) and $\delta^2H_{feather}$ of the seven resident thraupids (-61.03‰ ± 13.28‰; Kruskal-Wallis $H_1 = 1.96$, $P = 0.16$; Fig. 2A). Thus, we could conclude that the stripe-tailed yellow finch study population breeds in the same region where it molts, that is, within the area of the Federal District. Our observation allows us to conclude that they molt before breeding, as all captured individuals exhibited new feathers.

The $\delta^2H_{feather}$ (-57.32‰. ± 10.56‰) and δ^2H_{claw} (-37.4‰. ± 9.6‰) of the stripe-tailed yellow finch were significantly different (Kruskal-Wallis $H_1 = 45.98$, $P < 0.001$; Fig. 2B). The $\delta^2H_{feather}$ (-53.4‰ ± 8.38‰) and δ^2H_{claw} (-12.4‰ ± 3.8‰) of the saffron finch also exhibited a significantly difference (Kruskal-Wallis $H_1 = 44.21$, $P < 0.001$; Fig. 2C).

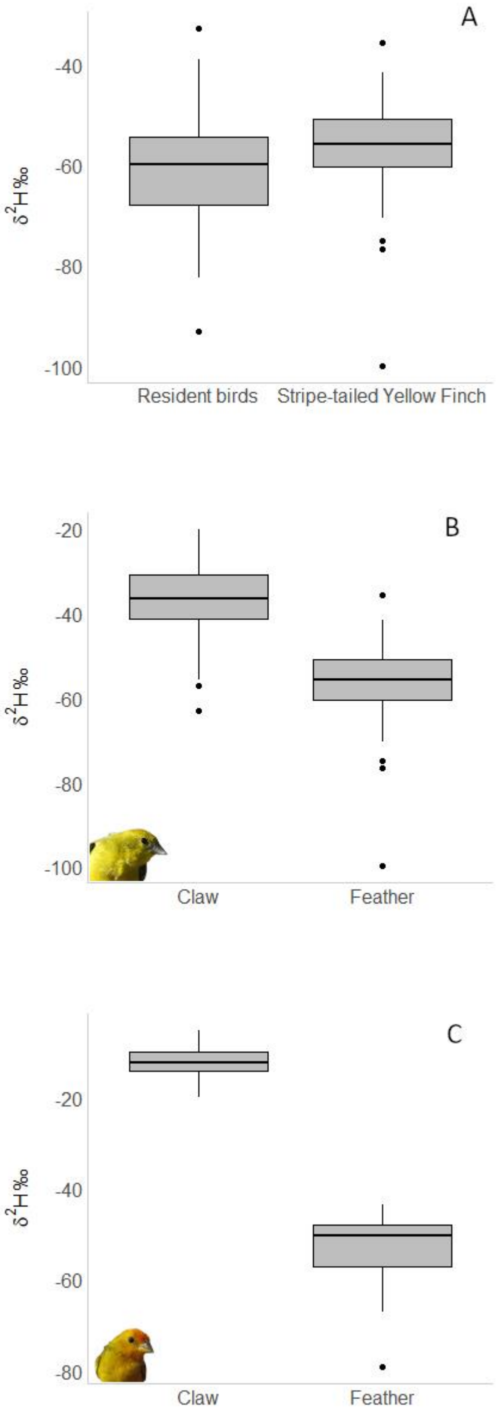

Fig. 2. Comparison between $\delta^2H_{feather}$ of the stripe-tailed yellow finch and that of resident species of the family (burnished-buff tanager, white-banded tanager, sayaca tanager, grey pileated finch, green-winged saltator, yellow-bellied seedeater, and saffron finch) showing no significant difference (A). Comparison between $\delta^2H_{feather}$ and δ^2H_{claw} of the stripe-tailed yellow finch showing a significant difference (B). Comparison between $\delta^2H_{feather}$ and δ^2H_{claw} of the resident saffron finch showing a significant difference (C).

The assignment maps indicated a high probability that feather samples had their origin in central Brazil, i.e., Federal District and Goiás (Fig. 3A-B). On the other hand, the assignment maps for the claw samples showed a lower probability of samples having their origin in central Brazil, suggesting a high probability of origin in slightly more eastern regions of central Brazil (Fig. 3C-D; see Supplementary Material II for prediction of QA function).

The migratory fork-tailed flycatcher data from citizen science show a seasonal concentration, which is not exhibited in the data from the stripe-tailed yellow finch (Table 1, Fig. 4). The latter´s occurrence data show a mean vector length < 0.40 and high SD around the mean angle.

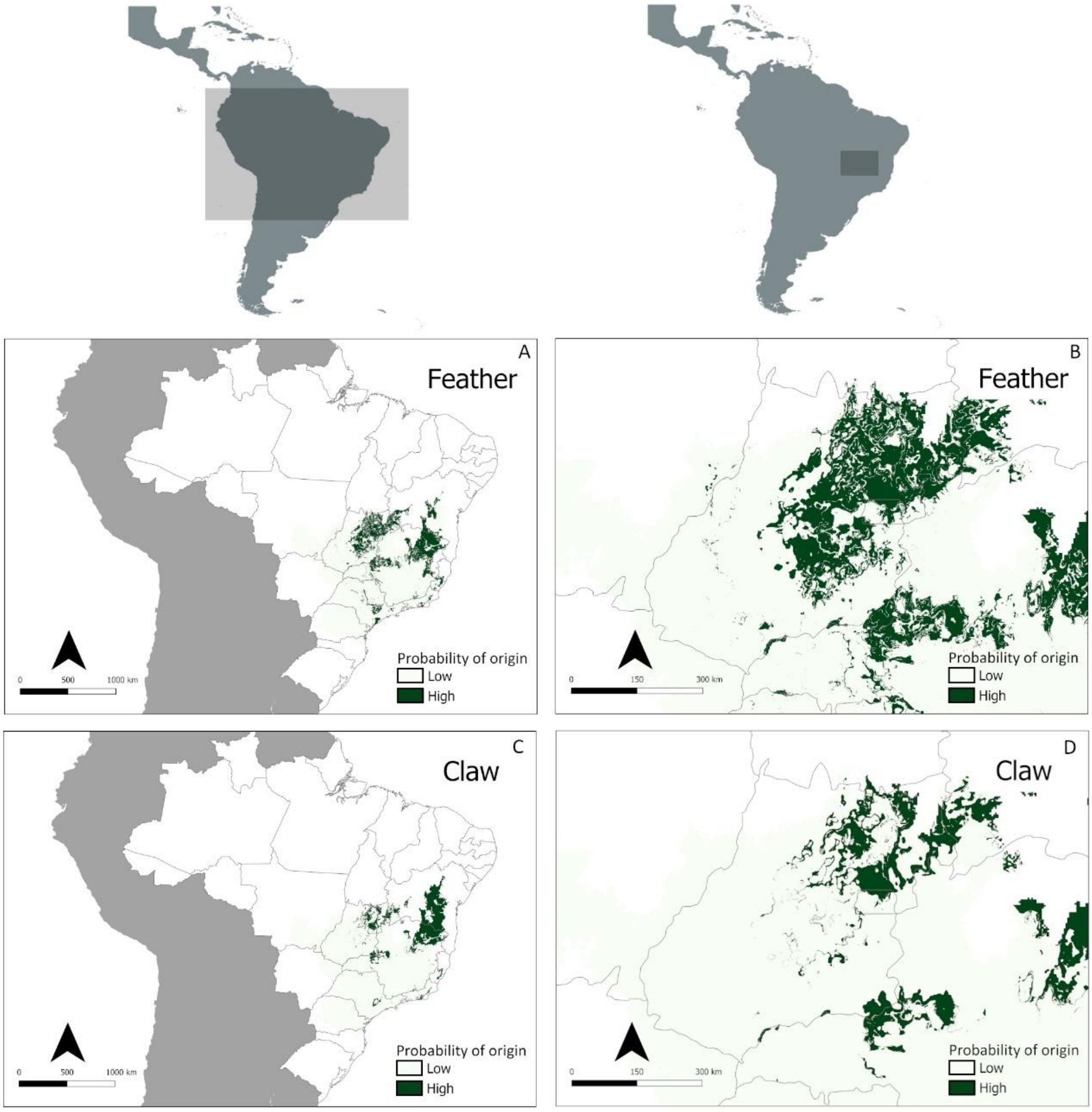

Fig. 3. Assignment for feather (A-B) and claw (C-D) samples of the stripe-tailed yellow finch. (A) shows the area of greatest occupation probability (in green) of individuals in the breeding season, (B) shows the same map, but with a zoom to the study area. (C) shows the area of greatest occupation probability (in green) of individuals in the non-breeding season, (D) shows the same map, but with a zoom to the study area.

Table 1. Descriptive circular statistics of occurrence data compiled for the striped-tailed yellow finch, the resident saffron finch, and the migratory fork-tailed flycatcher in the Federal District and Goiás. Mean vectors (μ) represent species' mean occurrence dates and concentration (r) shows the data distribution around the mean date, ranging from 0 (non-seasonal distribution) to 1 (highly seasonal). N: number of records surveyed; SD: standard deviation.

	Federal District		
Species	**N**	**μ ± SD (month)**	**r**
Striped-tailed yellow finch (*Sicalis citrina*)	105	95 ± 86° (Mar)	0.32
Saffron finch (*Sicalis flaveola*)	1691	262 ± 126° (Aug)	0.09
Fork-tailed flycatcher (*Tyrannus savana*)	752	298 ± 45° (Sep)	0.74

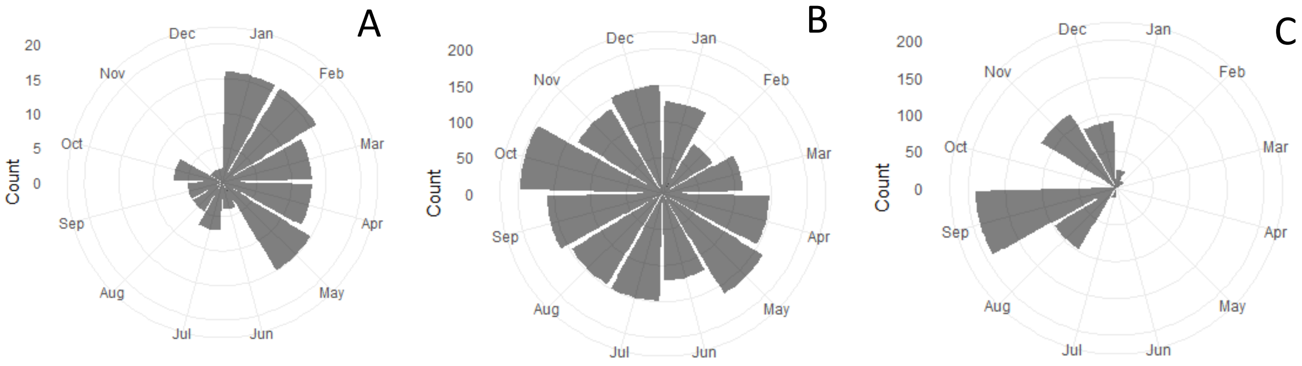

Fig. 4. Circular statistic of striped-tailed yellow finch (A), saffron finch (B) and fork-tailed flycatcher (C).

Discussion

Contrary to suggestions from the literature (e.g., Sick 1997, Nunes and Tomas 2008, Gressler and Marini 2015), our data indicate that the stripe-tailed yellow finch is not a migratory species. This can be seen by observing that the isotopic difference between feather and claw of stripe-tailed yellow finch is similar to the difference observed in saffron finch, i.e., this difference in isotopic values do not correspond to a geographic difference, but seasonal. The isotopic results show that the stripe-tailed yellow finch has a pattern of δ^2H like its resident congener, the saffron finch. The isotopic differences between feather and claw samples of the stripe-tailed yellow finch are due to the seasonality of the region and not to a geographical difference. Central Brazil is characterized by the Cerrado biome, which has a well-defined seasonal climate, with rainy summers and dry winters (ICMBio 2017). Because of this, the values of δ^2H in environmental waters can vary due to physical processes linked to the seasonality of rainfall, temperature, evaporation and moisture sources (e.g. Dansgaard 1964, Clark and Fritz 1997, Landwehr et al. 2014). This may result in different δ^2H values assimilation in the tissues of birds at these different times, i.e., the hydrogen ratio of the resources available to the species in the breeding season (December to May, rainy season) is different from the ratio in the non-breeding season (June to November, dry season).

Assignment maps, in turn, show that it is more likely that individuals spent the non-breeding season in a concentrated area in the states of Goiás, Bahia and Minas Gerais. During the breeding season individuals scattered, also occupying several areas in the Federal District to breed. The prediction of QA function was low and the standard deviation of assignments for the claw was high, suggesting that individuals may occupy other locations in non-breeding season, but in an unpredictable way. This pattern can be explained by the differences in the availability of resources or climatic features in non-breeding and breeding seasons due to the Cerrado seasonality (Marini et al. 2012). In the case of the stripe-tailed yellow finch, there is a synchronization of the breeding season with the rainy season in central Brazil, which increases the availability of food (Boang and Grant 1984, Sick 1997, Vasconcelos et al. 2007). As bird abundance may be limited by resource availability (Sinclair 1978, Johnson et al. 2005), the movement of individuals to adjacent areas in the non-breeding season can be explained by it coinciding with the beginning of the dry season, when individuals are more likely to be non-territorial and remain aggregated. On the other hand, in the rainy season there is an increase in the availability of resources throughout the Cerrado, resulting in a scattering of individuals and occupation of different areas for reproductive purposes. Bird abundance may also be limited by other climatic features (Sinclair 1978, Johnson et al. 2005). Another explanation for this pattern is that individuals may leave their breeding areas in the higher altitude of the Federal District (~1,000 m) and aggregate in warmer sites during the dry and colder season. Some birds show a tendency to seek warmer climates during their non-breeding period (Dufour et al. 2020).

Behavioral differences, such as habitat selection and mobility, can also affect δ^2H values (Magozzi et al. 2020). We can also speculate about possible social reasons for this type of movement pattern. During the breeding season, animals need more space as they pair up and hold breeding territories, which results in scattering of the population. On the other hand, aggregation during the non-breeding season may help individuals defend against predation (Lehtonen and Jaatinen 2016). The fitness of an individual that is currently in a non-reproductive state depends on its survival until the next breeding season, which can depend on group size even in the absence of specific defensive behaviors by the prey, due to dilution of predator pressure (Lehtonen and Jaatinen 2016). Moreover, some species may move through a considerable part of the breeding range and visit multiple breeding areas to increase their chances of successful reproduction (Kempenaers and Valcu 2017, Budka et al. 2021), showing a scattered pattern of site occupancy during the breeding season.

Data from citizen science agree with these isotopic results. A closer look at results shows that there is a small concentration of occurrences ($r < 0.50$; Schubert et al. 2019) around March. This value does not indicate a highly seasonal distribution pattern, such as with fork-tailed flycatchers. Instead, the small concentration of occurrences in March can be explained by the fact that the period corresponds to the peak of the stripe-tailed yellow finch breeding season (Gressler and Marini 2011), when individuals are more active, increasing human encounter rates. Our results exemplify the value of data available on citizen science platforms, which allow relatively simple analytical approaches to corroborate other types of studies.

Our results, taken together, indicate that the stripe-tailed yellow finch has a seasonal nomadic behavior, returning to the same nesting areas in consecutive years for breeding. Our behavioral data show that stripe-tailed yellow finches are faithful to their breeding areas: of the 24 individuals that held territories in the 2020/2021 breeding season, seven were banded in the previous seasons (24%); many other banded individuals were seen flying over the breeding areas (de-Carvalho, pers. obs.). This type of movement occurs when individuals move nomadically during the non-breeding season but maintain the same home ranges during the breeding season (Newton 2008, Teitelbaum and Mueller 2019). Although there is a predictable element to the occurrence of seasonal nomadism, the direction, frequency, and intra-season timing of the movement are variable (Teitelbaum and Mueller 2019).

The ability to track movements of individuals in nature is a challenging task in ecology, especially due to technological limitations and the high costs of traditional tools (e.g., markers, radios, and satellites). Our analytical approach combining SIA and citizen science data shows congruent results, indicating that low-cost tools can also be useful to elucidate species' seasonal movements.

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Supplementary material

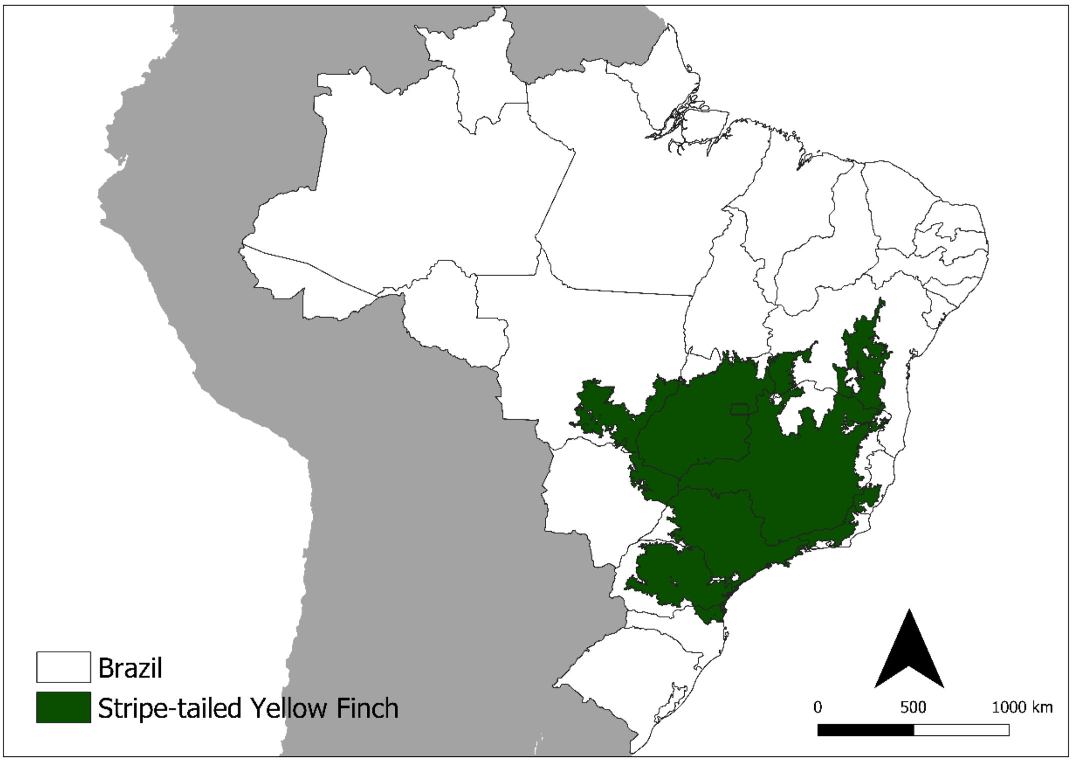
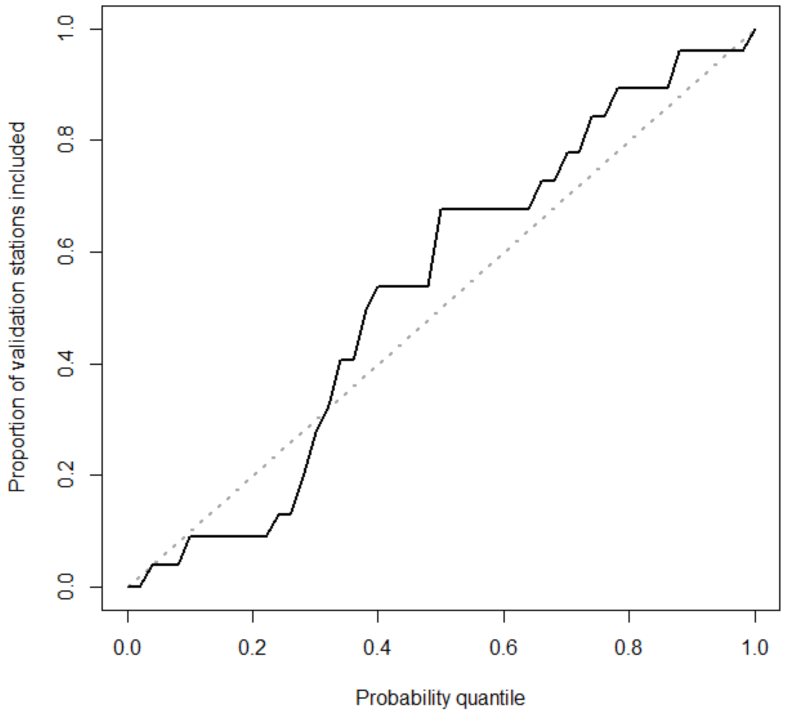


Fig. 1. Stripe-tailed Yellow Finch potential distribution area used as mask for δ^2H assignment.



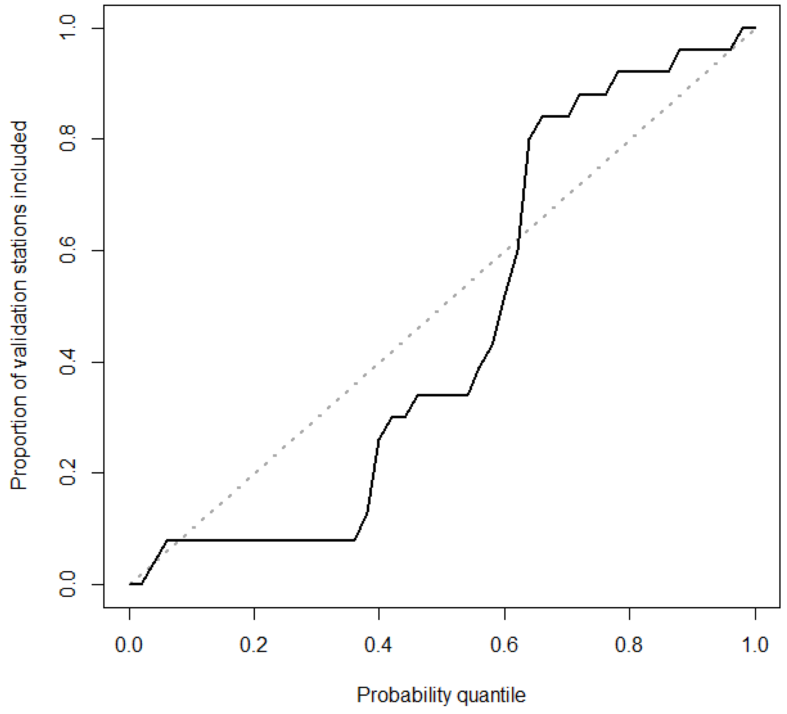


Fig. 2. Quality evaluation using five known-origin data. Proportion of validation samples correctly assigned for feather (top) and claw (down). Values should fall along the 1:1 line for feather, showing a better fit, i.e., little bias, but for claw values deviated from the 1:1 line, showing a high bias.

11. Conclusão Geral

Os resultados da tese contribuíram para esclarecer os padrões de plumagem de fêmeas, machos jovens reprodutores, e machos adultos de um traupídeo dicromático com coloração derivada de pigmentos carotenoides. A cabeça e ventre de ambos os sexos, regiões do corpo mais importantes para a sinalização social (Shultz e Burns 2017), são as que mais refletem ultravioleta. Descobrimos que machos atrasam a maturação da plumagem como parte de uma estratégia de acasalamento, porém apesar de imperceptível aos olhos humanos, há uma grande diferença entre os padrões de coloração das fêmeas e machos reprodutores com atraso de plumagem, o que sugere que os coespecíficos podem diferenciar entre os fenótipos dessas duas categorias. A hipótese mais plausível para este atraso da plumagem em *S. citrina* é a hipótese da sinalização de status, i.e., machos reprodutores menos experientes podem manter a plumagem dos machos subordinados como uma estratégia para minimizar a competição com machos adultos experientes, aumentando as chances de sobrevivência para as estações reprodutivas subsequentes, mas conseguindo cópulas dentro das possibilidades (Hawking et al. 2012).

O pigmento carotenoide que dá origem a esta coloração em *S. citrina* é a luteína, também presente em espécies congêneres (Hill e McGraw 2006). Fêmeas e machos se diferenciam em como alocam esses carotenoides. A coloração da fêmea é dependente de sua condição corporal, i,e., àquelas que possuem uma dieta rica em gramíneas C_3 e C_4 possuem a matiz do ventre no marrom, e também são as que tem maior condição corporal. As fêmeas de algumas espécies investem seus carotenoides na produção de ovos em vez de uma cor extravagante da plumagem (veja Svensson e Wong 2011 para revisão), esta pode ser uma suposição a ser testada com *S. citrina* em estudos futuros. Podemos também supor que os machos podem estar mais dispostos a ajudar essas fêmeas que investiram na prole às custas da ornamentação (Morales et al. 2012), hipótese que também seria interessante testar. Os dados coletados mostram que os machos exibem um trade-off entre a saúde imunológica e a saturação do ventre. Aqueles que investem na coloração se alimentam diretamente dos grãos C_3 e C_4, enquanto aqueles que investem na saúde imune apresentam uma dieta rica em proteínas, acessando consumidores primários e secundários. Assim, a coloração do ventre dos machos não expressa sua qualidade no que diz respeito à condição corporal ou saúde imune.

Nossas observações comportamentais permitiram detectar machos auxiliando na manutenção do ninho, provendo alimento para os filhotes e para as fêmeas, comportamento de cuidado paternal não antes documentado para *S. citrina*. No entanto, a frequência em que os machos realizam o cuidado paternal é menor quando comparado às fêmeas. Isso, somado com a presença de dimorfismo sexual, é mais um indício de que cópulas extra-par fazem parte da estratégia reprodutiva dessa espécie monogâmica, e que a taxa de fertilização extra-par pode ser alta e se igualar à de seu congênere, com cerca de 50% das ninhadas com pelo menos um filhote extra-par (Saldívar et al. 2019).

No que diz respeito ao padrão de movimentação sazonal da espécie, diferente de algumas suposições na literatura, *S. citrina* não é uma espécie migratória, e sim nômade na estação não reprodutiva, comportamento comum em diversas espécies de aves (Newton 2008). Durante a estação reprodutiva, *S. citrina* se espalha ocupando territórios em diversas áreas no Distrito Federal, Goiás, Minas Gerais e Bahia. Ao final da estação reprodutiva, eles mantêm um padrão agrupado, ocupando pequenas áreas nos mesmos estados. Esses achados contribuem diretamente para o conhecimento básico desta espécie comum, mas até o momento não documentado.

12. Referências Bibliográficas

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13. Bioética e Licenças

O presente estudo foi protocolado pela Comissão de Ética no Uso Animal (CEUA) do Instituto de Ciências Biológicas da Universidade de Brasília (Processo 23106.035081/2018-13). O Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) através do Sistema de Autorização e Informação em Biodiversidade (SISBIO) emitiu a licença para a realização do projeto (nº 62488). As atividades de captura e anilhamento estão sob a autorização expedida pelo Centro Nacional de Pesquisa e Conservação de Aves Silvestres (CEMAVE) (nº 4318).