

ROSANNETTE QUESADA HIDALGO

Pais dedicados são *sexy*: inter-relação entre cuidado paternal e seleção sexual em um opilião Neotropical

Devoted daddies are sexy: interplay between paternal care and sexual selection in a Neotropical harvestman

SÃO PAULO

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Tese apresentada ao Instituto de Biociências da Universidade de São Paulo como parte dos requisitos para obtenção do título de Doutor em Ciências, na área de Ecologia de Ecossistemas Terrestres e Aquáticos.

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(Orientador)

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Pietrico: muitas idas ao bandeirão, vários conselhos sobre comida saudável e sobre como não gastar tanto dinheiro, um curso sobre memes brasileiros que você nunca deu, uma festa eletrônica sensacional, um show na República, um Congresso de Aracnologia onde você foi parte do meu cérebro, várias conversas científicas e pessoais sobretudo no bandeirão, um baritour pelo Butantã, uma festa da USP com café da manhã no McDonald's, uma sobremesa que dividimos perto da casa da Cris, uma ida a um templo budista, muitos "olha essas costas, Rosa", várias terapias com o Bruno e várias tabelas no excel. Tudo de bom para você que, mesmo chato, soube ganhar meu coração.

Lu: um congresso sensacional na Suécia, com neve E flocos de neve, tour pela cidade e uma janta vegetariana super romântica, muitas idas no bandeirão com muitas conversas, um milhão delas sobre tabelas do excel, um assalto na Vila Indiana (porém um sorvete delicioso em uma das vezes que fomos fazer o BO) e um pote de feijão, a mesma festa da USP com café da manhã no McDonald's e muitas tardes-noites saindo da lab falando do bonito e do não tão bonito da academia. Você é uma coisa linda Lu, continue levando sua luz para onde você for.

Andresito: un millón de idas para la casa como guardaespaldas, un espagueti con frijoles, cerdo y maíz en Belo Horizonte después de una mojada, un Congresso de Aracnologia con ida a Ouro Preto, una pizza siempre que llegué "al Brasil", varias portuñoladas, un par de idas a Veragua, un par de mudanzas de casa, un Año Nuevo en Monteverde, una fiesta con Karaoke en Canadá, una visita al Castillo Ra-tim-bum y varias tortillas y gallo pinto para "matar saudade". Mae, qué tuanis tener un tico en Brasil.

Bruno: MUITAS conversas extendidas no lab durante o trabalho mas que sempre gostei muito, uma tarde de vídeo-game de nave espacial, muitas dicas sobre o português, vários "miércoles de español", um tour pelo centro de São Paulo e almoço no clandestino com a Cami, várias idas na casa de vocês para comer sushi e uma moqueca deliciosa, umas empanadas chilenas, uma ida na Feira de Aqueropita e muitas idas no Cris. Bruninho, você é um outlier mas gostamos muito de você. Cami você é um amor de pessoa.

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super curso de campo muito frio com a Helena, muitas conversas sobre comida e exercício, aulas de yoga no CEPE, uma tarraxinha (sólo porque usted me enseñó esa palabra), vários churras latinos, uma pizza feita em casa com a Helena quando eu morava na Eiras e mais uma perto do metrô junto com um café só, que conseguimos fazer juntas ha ha. Meninas, adorei conhecer vocês e espero que consigamos fazer muitos cafés a mais juntas! Obrigada por me inspirar tanto!

Isa: um vídeo sobre estrelas com a galera da Disperciência, uma janta no bandeirão da física com café no puxadinho, mais uma janta na sua casa com aquele outro brócolis, a receita do pão de queijo!, muitos lanches durante os fins de semana na Eco, aquela festinha famosa da USP com café da manhã no MacDonald's, um par de rolês pelo nosso bairro o Butantã, seu bilhete único e a sua bike entre mais muitas coisas que não dá para escrever aqui. Sempre lembrarei das coisas felizes. Tudo de bom para você, gatinha.

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Resumo

QUESADA-HIDALGO, R. Pais dedicados são *sexy*: inter-relação entre cuidado paternal e seleção sexual em um opilião Neotropical. 2018. Dissertação (Doutorado) – Instituto de Biociências, Universidade de São Paulo, SP.

Em espécies que exibem cuidado paternal exclusivo, a qualidade esperada do comportamento paternal pode influenciar as decisões de acasalamento das fêmeas e determinar o sucesso de acasalamento dos machos. Nesta tese, investigamos a inter-relação entre o cuidado paternal e a seleção sexual utilizando o opilião *Quindina limbata* como organismo modelo. Os machos nesta espécie constroem ninhos na forma de taça que são visitados pelas fêmeas na busca de um sítio de oviposição. No primeiro capítulo, nós experimentalmente avaliamos a eficiência do atendimento aos ovos provido pelos machos e testamos se os ninhos desatendidos são adotados por fêmeas e/ou por machos não relacionados com os ovos. As conclusões mais importantes deste capítulo são: (1) a proteção do macho é crucial para a sobrevivência dos ovos porque ninhos desatendidos são prontamente atacados por predadores; (2) a compensação do cuidado parental por fêmeas é rara, provavelmente porque elas estão associadas aos machos e não aos ninhos; (3) os machos adotam ovos não relacionados com eles e protegem eles tão eficientemente quanto os machos originais, provavelmente porque o cuidado dos ovos é um comportamento selecionada sexualmente. No segundo capítulo, nós testamos a existência de duas táticas alternativas de acasalamento nas fêmeas: *residentes*, na qual as fêmeas permanecem perto de um ninho, repelem fêmeas conespecíficas e copulam preferencialmente com um único macho dono de ninho, e *visitantes*, na qual as fêmeas não permanecem espacialmente associadas a ninhos, não repelem conespecíficas e copulam com vários machos donos de ninhos. Também investigamos se a monopolização de ninhos por fêmeas afeta o sucesso reprodutivo dos machos. As conclusões mais importantes deste capítulo são: (1) o comportamento das fêmeas parece ser uma tática reversível, na qual as fêmeas podem mudar de residentes a visitantes durante sua vida, provavelmente em resposta à condição corporal, e (2) a monogamia social imposta pelas fêmeas residentes pode afetar negativamente o grau da promiscuidade dos machos, mas não sua taça de acasalamento. Em conclusão, nós provemos evidência de que a preferência das fêmeas por machos que provem cuidado pode ter favorecido a manutenção do cuidado paternal. Além disso, demonstramos que a monopolização dos melhores machos ou ninhos pode ter favorecido a evolução das táticas alternativas de acasalamento nas

fêmeas.

Palavras-chave: adoção de ovos, predação de ovos, compensação flexível do cuidado paternal, ninho, seleção sexual, táticas alternativas de acasalamento em fêmeas, conflito sexual

Abstract

QUESADA-HIDALGO, R. Devoted daddies are sexy: interplay between paternal care and sexual selection in a Neotropical harvestman. 2018. Dissertação (Doutorado) – Instituto de Biociências, Universidade de São Paulo, SP.

In species exhibiting exclusive male care, the expected quality of paternal behavior can influence female mating decisions and thus determine male mating success. In this thesis, we investigated the interplay between paternal care and sexual selection using the harvestman *Quindina limbata* as model organism. Males of this species build cup-like mud nests that are visited by females in search of an oviposition site. In the first chapter, we experimentally evaluated the efficiency of egg-attendance provided by males and tested if unattended nests are adopted by females and/or unrelated males. The most important conclusions of this chapter are: (1) male protection is crucial for egg survival because unattended nests are promptly attacked by predators; (2) flexible compensation of parental care by females is rare, probably because they are associated to males and not to the nests; (3) males adopt unrelated eggs and protect them as efficiently as original owner males, probably because egg-attendance is a sexually-selected behavior. In the second chapter, we tested the existence of two reproductive tactics in females: *resident*, in which females remain close to a nest, repel conspecific females, and mate preferentially with a single nest-owner male, and *wanderer*, in which females are not spatially associated with nests, do not repel conspecific females, and mate with different nest-owner males. We also investigated whether nest monopolization by females may affect males' reproductive success. The most important conclusions of this chapter are: (1) females' behavior seems to be a reversible tactic, in which females can switch from resident to wanderer during their lifetime, probably in response to body condition, and (2) the social monogamy imposed by resident females may negatively affect the degree of male promiscuity, but not male's mating rate. In conclusion, we provide evidence that female preference for parental individuals may have favored the evolution and maintenance of paternal care. Moreover, we show that the monopolization of the best males or the best nests may have favored the evolution of alternative reproductive tactics in females.

Key-words: egg adoption, egg predation, flexible compensation of paternal care, nest, sexual selection, alternative reproductive tactics in females (ARTs), sexual conflict

Introdução geral*

- Então, você é bióloga?
- Sou.
- Você da aula?
- Por enquanto, não.
- Mas você é bióloga marinha, né?
- Não, sou bióloga “geral”.
- E então você faz o quê?
- Eu trabalho com comportamento animal, com uns bichos parecidos com aranhas.
- Nossa, você gosta de aranhas? Nunca vi isso antes!
- Pois é...
- E você faz o que com as aranhas? Estuda o veneno?
- Não. Bom, na verdade, eu trabalho com aracnídeos sem veneno.
- Aracnídeos?
- Sim, dentro do grupo dos aracnídeos estão as aranhas. Mas também existem outros animais, como os escorpiões e os carrapatos, que são os mais conhecidos. Existem ainda outros aracnídeos que são pouco conhecidos. Por exemplo, os opiliões, que são os que eu estudo. Eles parecem com aranhas, mas não têm veneno e não produzem seda para construir teias. Alguns deles são bem coloridos e bem bonitos. Pelo menos eu acho isso... Quer ver uma foto?
- Quero.

* Esta introdução é inspirada no formato do "Box 1. A synopsis: two gobies chatting" apresentado no artigo intitulado "Parental investment, sexual selection and sex ratios" de autoria de Hanna Kokko e Michael Jennions publicado no *Journal of Evolutionary Biology* em 2008 (21:919–994). O texto simula diálogos que tive com várias pessoas não-biólogas sobre o tema da minha tese de doutorado.

— Este é um opilião da Costa Rica. Ele ocorre em um lugar chamado Veragua Rainforest, que foi onde eu trabalhei durante meu doutorado.



— Nossa, que legal! Mas se eles não têm veneno, o que você estuda deles? Eles produzem alguma outra substância importante? Tipo, alguma coisa que possa ser usada para fazer remédios?

— Eu estudo comportamento, lembra? Então, na verdade, eu uso opiliões como modelo para estudar alguns comportamentos interessantes.

— Quais comportamentos???

— Estudo cuidado paternal exclusivo, ou seja, aquele no qual só o macho cuida dos filhotinhos. Para isso, eu trabalho com uma espécie de opilião em que os machos constroem um ninho de barro e matéria orgânica. As fêmeas visitam esses ninhos e, se elas gostam, copulam com o macho dono do ninho e colocam ovos dentro dele. Depois elas vão embora e quem cuida dos ovos é o macho.

— Nossa, muito interessante mesmo! Deveria ser assim nos humanos, né?

— Deveria... Quer ver uma foto de um dos ninhos?

— Claro!

— Olha, aqui neste ninho tem um macho do lado esquerdo e uma fêmea do lado direito. O nome desse opilião que eu estudo é *Quindina limbata*. Se você prestar atenção, vai ver uns pontinhos brancos no piso do ninho. São os ovinhos.



— Isso de ser o pai quem cuida dos filhos acontece também nos cavalos marinhos, né?

— Sim, mas não só neles. Muitos outros animais têm cuidado paternal exclusivo. Nos vertebrados, por exemplo, acontece também em algumas espécies de rãs, algumas aves (como a avestruz) e em muitas outras espécies de peixes além dos cavalinhos marinhos. Já nos invertebrados, existem alguns casos em insetos, como as baratas d'água, e também em piolhos de cobra. Mas nos aracnídeos só algumas espécies de opiliões apresentam cuidado paternal exclusivo.

— Legal. Mas deixa eu te perguntar uma coisa: nessas espécies com cuidado paternal, as fêmeas abandonam totalmente a prole e não cuidam dos filhos? Os machos são tão bons assim para cuidar dos ovos sozinhos?

— Sim, as fêmeas vão embora e deixam os ovos com o macho. Mas, em muitas espécies com cuidado paternal, os machos podem deixar a prole desprotegida por um tempinho, enquanto eles vão procurar comida. No caso da espécie de opilião que eu estudo, por exemplo, os machos podem sair também para procurar material necessário para o reparo do ninho,

deixando os ovos temporariamente expostos à predação. O mais legal é que, em algumas espécies de insetos, rãs e peixes, as fêmeas podem assumir o cuidado dos ovos enquanto o macho está longe. Os biólogos chamam esse comportamento de "compensação flexível do cuidado parental".

— Será que essa tal compensação acontece no opilião que você estuda?

— Bom, as fêmeas vão embora depois de colocar os ovos. Mas uma coisa que eu não te contei é que em *Quindina limbata*, a espécie de opilião que eu estudo, existem algumas fêmeas que ficam perto dos ninhos onde elas entram e parecem criar uma associação com eles. Além disso, essas fêmeas parecem atacar outras fêmeas que chegam perto do ninho, como se elas estivessem ajudando o macho a proteger o ninho. A gente chama essas fêmeas de "residentes". Mas a maioria das fêmeas não fica associada a nenhum ninho e a gente chama essas fêmeas de "visitantes". Elas entram no ninho, copulam com o macho, deixam seus ovos e vão procurar outro ninho.

— Que legal!!!

— Então, precisamente as duas perguntas que eu queria responder no primeiro capítulo da minha tese eram: (1) quão eficiente são os machos para cuidar dos ovos e (2) se as fêmeas residentes cuidam dos ovos quando os machos estão ausentes.

— E aí, o que você descobriu?

— Calma, vou fazer suspense. Antes vou te contar outra coisa legal que pode acontecer quando os machos que cuidam saem do ninho: outros machos podem roubar o ninho e começar a cuidar dos ovos no ninho.

— Sério? Tipo, adotando os ovos?

— Isso mesmo.

— Mas por que um macho faria isso? Caridade?

— Vamos fazer um teste. O que você acha desse cara aqui da foto?



— Opa, achei gato, ainda mais com essa criancinha no colo. Super fofo!

— Bom, é isso mesmo! A teoria diz que cuidar dos ovos é um comportamento *sexy* para as fêmeas. Quando um macho está cuidando dos ovos ele está dando uma prova da sua capacidade como pai. Por isso, as fêmeas preferem machos que têm ovos em seus ninhos.

— Já entendi! Os machos adotam desovas por eles acabarem se tornando mais *sexy* para as fêmeas.

— Exatamente. Já sabemos que isso acontece em outras espécies de opiliões e também em algumas espécies de peixes com cuidado paternal. Para uma espécie de peixe, em particular, sabemos que os machos adotivos cuidam dos ovos tão bem quanto os machos originais. Porém, não sabemos se o mesmo acontece com os invertebrados, como os opiliões. Essa era a terceira questão que eu queria responder no primeiro capítulo da minha tese.

— Miga, fiquei interessada nessa história. Quer dizer que tem uns machos nesse tal opilião que roubam ninhos em vez de construírem o próprio ninho deles?

— Sim, existem machos que são ladrões de ninhos. A gente sabe que as fêmeas só copulam com machos que têm um ninho e, para conseguir copular, alguns machos fazem um ninho e

outros roubam ninhos feitos por outros machos. Essa diferença de comportamento reprodutivo tem um nome chique: táticas alternativas de acasalamento. Isso acontece bastante na natureza entre os machos, que geralmente precisam competir entre si pelas fêmeas. Por exemplo, os machos grandes geralmente brigam ativamente com outros machos e monopolizam fêmeas ou os lugares que as fêmeas gostam de usar para colocar ovos. Já os machos menores, que não têm nenhuma chance de ganhar as brigas, inventam outras táticas para conseguir cópulas. Por exemplo, eles podem entrar sorrateiramente no território de um macho grande sem que sejam percebidos e copular com uma fêmea que esteja dentro do território. Olha, mas a tática que eu acho mais sensacional é usada por alguns machos pequenos que se parecem com fêmeas. Os machos grandes se confundem, deixam esses machos "afeminados" entrarem em seus territórios e eles acabam copulando com as fêmeas.

— Uau!!!

— Pois é, cada um se vira como pode.

— Mas, peraí. Você não tinha me falado que, no opilião que você estuda, algumas fêmeas brigam e outras não? Isso também poderia ser uma táticas alternativa. Só que, nesse caso, entre as fêmeas e não entre os machos.

— É uma excelente ideia. Vou te chamar para trabalhar comigo, pois você leva jeito para pesquisa. O único problema é que as táticas alternativas de acasalamento são muito mais estudadas em machos do que em fêmeas. Por isso, conhecemos muito pouco sobre as táticas utilizadas pelas fêmeas e os fatores que podem fazer com que as fêmeas tenham dois ou mais comportamentos reprodutivos diferentes.

— Isso é machismo!

— Pode ser. E, para tentar entender melhor o assunto da perspectiva das fêmeas, o segundo capítulo da minha tese está focado em responder uma questão: será que de fato existem duas táticas alternativas de acasalamento nas fêmeas do opilião *Quindina limbata*?

— Mas, de acordo com o que você já me contou, a resposta parece óbvia. É claro que existem

duas táticas.

— Bem, as coisas podem não ser tão simples quanto parecem. O comportamento das fêmeas pode variar de forma contínua, ou seja, como se fosse um “dégradé”. Entre uma fêmea que fica o tempo todo perto de um ninho repelindo intrusas e uma fêmea que nunca se associa a nenhum ninho, podemos encontrar uma grande variação no comportamento das fêmeas. Nesse caso, não dá para dizer que existem duas táticas de acasalamento entre as fêmeas. Para poder dizer isso, a gente precisa mostrar que as fêmeas podem ser classificadas facilmente nas duas categorias: residentes e visitantes.

— E como se faz isso?

— Primeiro, você precisa ficar muito tempo no mato observando o comportamento dos bichos...

— Argh! Ficar no meio do mato... Você não tem medo de cobras?!

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Depois de uma breve digressão sobre cobras e outros perigos do trabalho noturno em campo (pois os opiliões são noturnos), a conversa sobre as táticas alternativas das fêmeas é retomada.

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— Bem, como eu estava te dizendo, depois de ficar muito tempo observando as fêmeas, a gente pode caracterizar vários comportamentos interessantes, incluindo quanto tempo elas passam perto de um mesmo ninho, com que frequência elas atacam outras fêmeas e quantos ninhos diferentes elas visitam. Se depois que a gente fizer gráficos e analisar os dados eles mostrarem que existem dois grupos de fêmeas, é possível dizer que existem táticas alternativas de acasalamento nas fêmeas do opilião que eu estudei. As fêmeas residentes deveriam ficar muito tempo perto de um mesmo ninho, deveriam atacar outras fêmeas que

se aproximam do ninho e não deveriam visitar outros ninhos. Seria como um tipo de monogamia. Por outro lado, as fêmeas residentes não deveriam ficar perto de nenhum ninho, não deveriam atacar outras fêmeas e deveriam visitar vários ninhos. Nesse caso, seria algo como uma poligamia.

—Olha, que interessante. Então pode ser que uma fêmea fique copulando com um macho só e que outras copulem com muitos machos.

— É isso que eu esperava encontrar em campo.

— E se as fêmeas residentes são ciumentas e repelem outras fêmeas que se aproximam do ninho delas, então os machos donos ninhos acabam copulando só com elas.

— Exatamente. A outra questão que eu explorei no segundo capítulo da minha tese foi justamente o efeito que o comportamento das fêmeas tem sobre o comportamento sexual dos machos. Assim como você já percebeu, pode ser que um macho cujo ninho tem uma fêmea residente copule só com esta fêmea residente enquanto um macho cujo ninho não têm uma fêmea residente consiga copular com muitas fêmeas diferentes.

— E para o macho, tanto faz? Copular com uma fêmea só ou copular com muitas fêmeas diferentes? Nunca tinha parado para pensar nisso. Será que ele ganharia mais ovos se copulasse com muitas fêmeas diferentes?

— Segundo a teoria, sim. Os machos se beneficiariam em copular com muitas fêmeas. O mais interessante dessa história é que existe um conflito entre os machos que querem copular com muitas fêmeas e as fêmeas residentes que parecem querer acesso exclusivo aos machos.

— Nossa, tem muita coisa acontecendo nesses bichos! Achava que biólogo só ia no mato e anotava tudo o que via... Não sabia que vocês tinham que medir tanta coisa!

— Meus pais também acham isso. Mas como você viu, tem muita coisa acontecendo e, para conseguir entender direito toda a história, a gente precisa observar, filmar, medir, anotar... É um trabalho de detetive.

— Tipo CSI!

— Escuta mais essa: pode ser que, da perspectiva de um macho, ter uma fêmea residente não seja algo tão ruim assim. Se uma fêmea copula com vários machos, não dá para saber quem vai ser o pai dos filhotes. Portanto, se um macho se acasala principalmente com fêmeas que copulam com muitos machos, é muito difícil para ele ter certeza de que os ovos dos quais ele está cuidando são de fato filhos dele. Por outro lado, a certeza da paternidade é maior entre os machos que se acasalam principalmente com fêmeas monogâmicas.

— Ou seja, estar associado com uma fêmea residente traz tantas coisas boas quanto ruins para os machos.

— Na verdade, quase todos os comportamentos que estudamos podem ser encarados de uma perspectiva de custos e benefícios. O que importa sempre é o saldo.

— Parece economia.

— Ecologia e economia são ciências irmãs. Em ecologia comportamental, que é a minha área de pesquisa, as pessoas estão sempre "roubando" ideias dos economistas.

— Nossa, nunca pensei que um bichinho desconhecido pudesse ser tão interessante. Estou super-curiosa para saber o que você descobriu depois de quatro anos de pesquisa. Você conseguiu responder todas as suas perguntas?

— Bom, se você quer saber todos os detalhes técnicos, pode ler os dois artigos científicos que escrevi para a minha tese. O problema é que eles estão escritos em inglês e estão cheios de palavras complicadas, que são o jargão específico da área de ecologia comportamental. Se você não estiver com tempo ou paciência para encarar os dois artigos, eu posso te contar rapidinho o que eu descobri. Basta ir direto para as conclusões gerais desta tese.

Efficiency of exclusive paternal care and the role of foster parents as egg protectors in a Neotropical arachnid[†]

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The evolution of exclusive paternal care in arthropods is influenced by both natural and sexual selection. Male care may simultaneously increase egg protection against natural enemies and male attractiveness to ovipositing females. When caring males desert or die, their clutches may be adopted either by females that provide flexible compensation of parental care or by males that may increase their own attractiveness caring for unrelated eggs. Whether foster parents are as efficient as the original owner males in protecting the clutch is a question that has rarely been addressed. Here we experimentally evaluated the efficiency of egg-attendance provided by males of the mud-nest harvestman *Quindina limbata*. We also tested if unattended nests are adopted by females and/or unrelated males, and compared the efficiency of the protection provided by foster parents with that provided by original owner males. We found that when males are present (either the original owner or a foster individual), nest visitation by egg predators was much lower than in unattended nests. Even when females have had laid eggs on the nests, they adopted unattended nests less frequently than unrelated males. Foster males were as efficient as the original owner males in decreasing nest visitation by egg predators. The most important conclusions of our study are: (1) male protection is crucial for egg survival because unattended nests are promptly attacked by predators; (2) flexible compensation of parental care by females is rare, probably because they are associated to males and not to the nests; (3) males adopt unrelated eggs and protect them as efficiently as original owner males, probably because egg-attendance is a sexually-selected behavior.

Keywords: egg adoption, egg attendance, egg predation, flexible compensation of parental care, harvestman, nest, offspring survival, sexual selection

Parental care is a widespread behavior in nature. Regardless of the taxonomic group and the identity of the caring sex (female, male or both), parental care is expected to evolve only when the benefits of increasing offspring survival are higher than the costs paid by the parents (Smiseth, Kölliker, & Royle, 2012). The main benefits received by the offspring include food provisioning, attenuation of unfavorable abiotic conditions, such as hypoxia and extreme temperatures, and protection against natural enemies, such as predators, parasitoids, and pathogens (reviewed in Alonso-Alvarez & Velando, 2012). Among arthropods, for instance, a recent meta-analysis performed with 45 terrestrial species distributed worldwide showed that the main benefit of uniparental care is the reduction of offspring mortality imposed by predators and parasitoids (Santos et al., 2016). However, only three of the 45 species included in the meta-analysis exhibit exclusive paternal care, so that the general patterns reported in the paper result mainly from arthropods that exhibit exclusive maternal care. Considering that exclusive paternal care has evolved many times independently in arthropods (Requena, Munguía-Steyer, & Machado, 2013), we are still in need of experimental studies evaluating the efficiency of males as egg protectors.

In many species with exclusive paternal care, including both vertebrates and invertebrates, males temporarily leave their clutches unattended to forage or shelter, exposing the eggs to the action of natural enemies (e.g., arthropods: Machado et al., 2004; fish: Rangeley & Godin, 1992; frogs: Chen, Yu, & Kam, 2007; Cheng & Kam, 2010; Consolmagno et al., 2016). In some of these species, females may attend the offspring during periods of temporary male absence, a behavior known as flexible compensation of uniparental care. For instance, females of the frogs *Eleutherodactylus johnstonei* (Eleutherodactylidae) and *Allobates femoralis* (Dendrobatidae) care for the offspring when egg-tending males are experimentally removed (Bourne, 1998; Ringler et al., 2015). In the assassin bug *Rhinocoris tristis* (Reduviidae), most of the clutches are attended by females after

the experimental removal of egg-tending males (Beal & Tallamy, 2006). Despite empirical evidence of flexible compensation of male care by females of some species, the factors that lead them to protect temporarily unattended clutches are still not clear. In arthropods, in particular, it is also not clear whether females that temporarily care for the eggs are as efficient as egg-tending males in defending the offspring against possible predators.

When males leave their clutches temporally unattended or desert permanently, eggs may also be adopted by conspecific males (arthropods: reviewed in Requena, Munguía-Steyer, & Machado, 2013; fish: reviewed in Wisenden, 1999). In some species, other males can even usurp clutches from egg-tending males (arthropods: e.g., Mora, 1990; Thomas & Manica, 2005; fish: e.g., Unger & Sargent, 1988; Bisazza, Marconato, & Marin, 1989; Sargent, 1989). Egg adoption and clutch usurpation occur in several species with exclusive paternal care probably because egg attendance is a sexually-selected behavior, with egg-tending males being preferred by females as sexual partners (arthropods: e.g., Nazareth & Machado, 2010; Gilbert, Thomas, & Manica, 2010; Requena & Machado, 2015a; Ohba, Okuda, & Kudo, 2016; Ohba et al., 2018; fish: e.g., Unger & Sargent, 1988; Forsgren, Karlsson, Kvarnemo, 1996; Lindström, St. Mary, & Pampoulie, 2006). Thus, even though foster males are not necessarily genetically related to the eggs, egg adoption and clutch usurpation may increase their attractiveness and their chances of achieving copulations. In fact, active egg protection after adoption has already been reported for some species of arthropods (reviewed in Tallamy, 2001 and Requena, Munguía-Steyer, & Machado, 2013) and fish (reviewed in Wisenden, 1999) with exclusive paternal care. In the fathead minnow *Pimephales promelas* (Cyprinidae), for instance, the efficiency of foster males in preventing nest visits by potential egg predators was as high as nest-owner males (Unger & Sargent, 1988). To our knowledge, though, the efficiency of offspring protection provided by the original owner and by foster males has never been investigated in arthropods with exclusive paternal care.

A good study system to explore the efficiency of exclusive paternal care and the potential role of conspecific females and males in offspring protection are harvestmen of the genus *Quindina* (Opiliones: Nomoclastidae). *Quindina* males build cup-like mud nests on fallen trunks in rainforests of Central America (Pinto-da-Rocha & Bragagnolo, 2017). Nests are visited by several females that may copulate with the nest-owner males and lay eggs that are entirely or partially buried in the nest floor (Mora, 1990; Fig. 1a). Males usually stay inside the same nest for several weeks or months, repairing it from damages (promoted mostly by rainfall) and protecting eggs and newly hatched nymphs from predators (including conspecifics) and fungi infestation (Mora, 1990; Requena & Machado, 2015b). Owner males defend their nests from usurpation by conspecific males without nests (Mora, 1990). Males that usurp a nest are known to feed on some eggs (Mora, 1990), but there is no information on whether they protect the remaining eggs. In *Q.* (= *Zygopachylus*) *albomarginis* and *Q.* (= *Poassa*) *limbata*, some females seem to be spatially associated with certain nests that are regularly used as oviposition site (Mora, 1990; Fig. 1b). These females (hereafter, "resident females") are highly phylopatric and may aggressively attack wandering conspecific females that approach the nest with which they are associated (Mora, 1990; see *Study Species* below). However, it is not known whether resident females have a role in protecting the offspring against predation during temporary or permanent male desertion.

In this study, we first investigated the efficiency of egg-attendance provided by nest-owner males of *Q. limbata*. A field experiment with the closely-related species *Q. albomarginis* showed that male presence inside the nest is crucial for offspring survival because unattended nests were promptly invaded by ants and conspecifics that preyed upon the eggs (Mora, 1990). Given that both *Quindina* species occur in rainforests of Central America, where nests are probably subjected to intense predation (Santos et al., 2016), we predict that the presence of nest-owner males of *Q. limbata* will decrease nest visitation by egg predators.

Second, we investigated whether resident females play a role in protecting eggs when nest-owner males are absent. We predict that resident females may exert flexible compensation of paternal care when nest-owner males are experimentally removed, because these females probably have laid eggs inside the experimentally unattended nest. Consequently, after the experimental removal of nest-owner males, nests with resident females should have lower visitation by egg predators when compared with nests without resident females. Finally, we investigated whether wandering males adopt nests in which the owner males are absent. Given that females seem to copulate exclusively inside the nests, we predict that males should adopt experimentally unattended nests. Assuming that the presence of eggs may indicate the quality of a male as egg protector, we also predict that foster males inside nests should decrease nest visitation by egg predators as the nest-owner males would do.

METHODS

Study Species

Individuals of *Q. limbata* reproduce all year long, and males use mainly fallen logs as substrate to build their nests. Nest construction usually takes from 2 to 4 weeks and is performed exclusively by males. The nest is composed of a circular floor (ca. 1-2 mm thick) surrounded by a circular wall (ca. 5-15 mm high; Fig. 1). Males regularly clean the interior of the nest (including the eggs) from fungus growth, repair both nest floor and wall from physical damages promoted mostly by rainfall, and stay inside the nest during the day and night. Although males stay most of the time inside their nests, they occasionally leave to feed or to bring material to repair nest damages. Because individuals are mainly nocturnal, male-male fights for nest usurpation and male-female sexual interactions occur during nighttime.

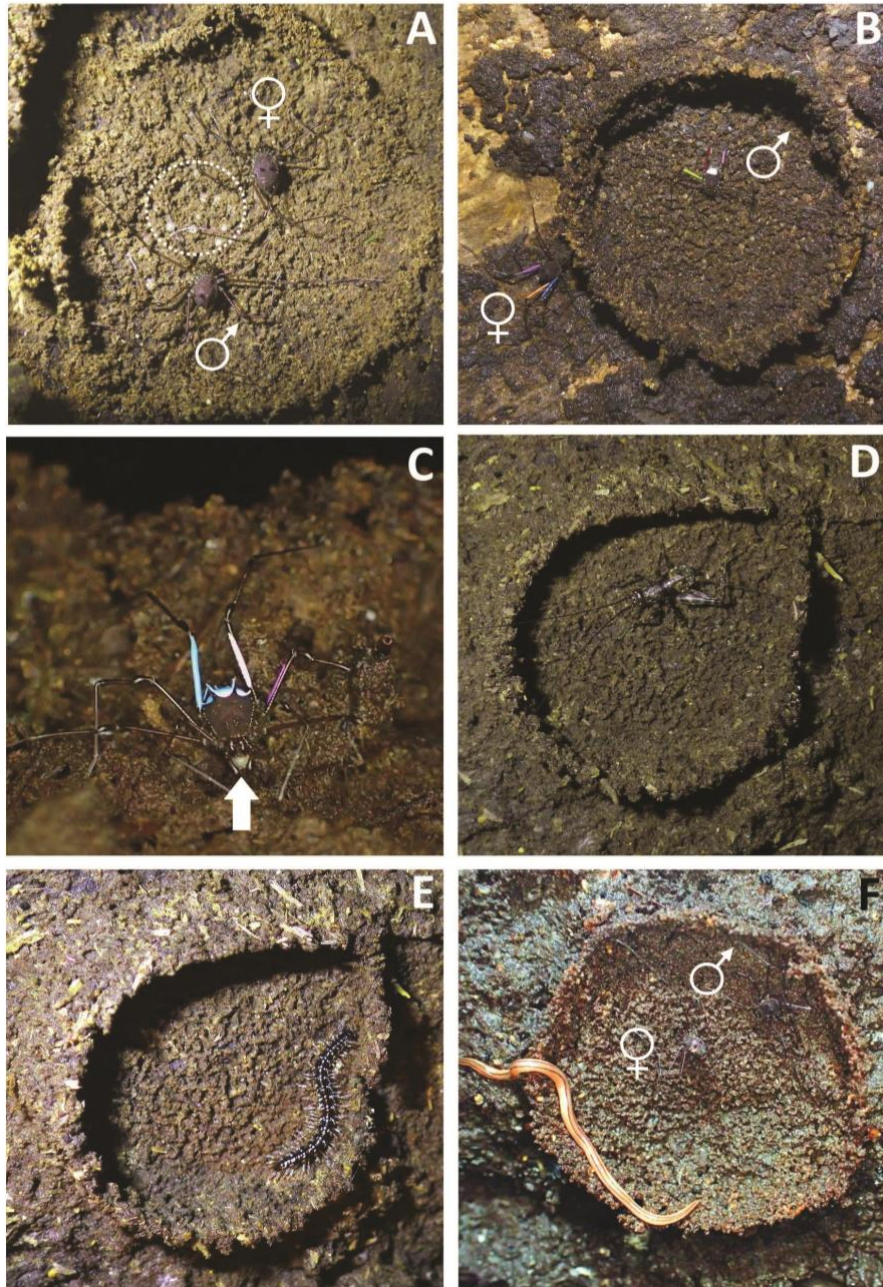


Figure 1. (A) Male and female of *Quindina limbata* inside a cup-like mud nest on a fallen log in Costa Rica. The dotted circle shows recently laid white eggs partially buried in the nest floor. (B) Two marked individuals of *Q. limbata*: the male is inside the nest and the resident female is in close contact with the external wall of the nest. Note that the female extends the second pair of sensorial legs and touches the nest wall. (C) Female of *Q. limbata* cannibalizing an egg (white arrow) inside an experimentally unattended nest. (D) A phalangopsid cricket and (E) millipede inside experimentally unattended nests. Both the cricket and the millipede feed on the nest floor and eventually consume eggs buried in it. (F) A flatworm invading a nest while the nest-owner male of *Q. limbata* is courting a female. Although no flatworm was observed visiting nests during our experiments, they are very common in the study area, and have already been observed in several occasions inside nests.

When a female visits a nest, she spends the first minutes inspecting its floor and wall (Toscano-Gadea & Rojas, 2014). Short visits occur when females leave the nest after this inspection, while long visits may also include mating and oviposition. This means that females may abandon the nest without mating or ovipositing (Toscano-Gadea & Rojas, 2014). Nest-owner males may attack visiting females by aggressively biting them and chasing them away from the nest. Male aggression to females may occur before or after any phase of the male-female interaction, including pre-copulatory courtship, copulation, and oviposition (Toscano-Gadea & Rojas, 2014). However, the factors influencing this aggression are still not clear (but see DISCUSSION for a possible explanation).

Study Area

We conducted fieldwork at Veragua Rainforest (9°55'35.7"N; 83°11'27.9"W; 400 m above sea level), which is located in the province of Limón, Caribbean slope of Costa Rica. The area consists of a primary tropical rainforest that receives an annual precipitation between 4000 and 4500 mm, and has a mean annual temperature of 23 °C (Holdridge, 1967). The climate has no well-defined seasons, so that the mean monthly temperature is always above 19 °C and the mean monthly precipitation is always above 140 mm (data obtained from a meteorological station located 20 km from the study site).

Marking Procedure

Between January and March 2016, we performed two fieldtrips of eight days each. In the first fieldtrip, we conducted our observations in two sites containing a total of three fallen logs. In the second fieldtrip, we conducted our observations in one site containing two fallen logs. Each fallen log had between one and 15 nests. Combining both fieldtrips, we

individually marked a total of 53 nests and their corresponding owner males. We also individually identified a total of 142 non-nesting individuals found on the five fallen logs: 18 wandering males, 100 females, and 24 individuals that could not be sexed (see “*Sex Determination of Marked Individuals*” below). We marked all individuals with exclusive color codes by painting their dorsal scute and/or femur of legs III and IV with enamel paint (Figs. 1b-c). This procedure has already been used with the congeneric *Q. albomarginis* and apparently does not interfere with the individuals’ behavior (Mora, 1990; Requena & Machado, 2015b). After the marking procedure, we photographed each individual for body measurements (see below), and released them at the same place where they were captured.

Sex Determination of Marked Individuals

It is very difficult to distinguish males from females of *Quindina* spp. in the field because there is no evident sexual dimorphism in species of the genus, except that females are slightly larger than males (Pinto-da-Rocha & Bragagnolo, 2017; Fig. S1 in Supplementary Material). Thus, to identify the sex of the marked individuals, we used the following criteria: (1) all individuals found alone inside a nest were recorded as males, and (2) all individuals that visited a nest without being attacked, or that copulated or oviposited in a nest where a nest-owner male was present were recorded as females. At the end of the experiments, all individuals found on the sampled fallen logs were collected and their sex was determined by dissection in the laboratory (see below). This procedure corroborated that classifications (1) and (2) were always correct. The sex of the remaining individuals that were not collected and/or for which we did not have any behavioral information was inferred based on body measurements obtained from photographs in dorsal view taken after the marking procedure. Each marked individual was photographed close to a scale and measurements (to the nearest

0.01 mm) were taken using the software *ImageJ* (Rasband, 2003). Voucher specimens of *Q. limbata* are deposited at Museu de Zoologia da Universidade de São Paulo (MZSP), Brazil.

First, we measured the width of the dorsal scute at its widest point and the total area of the dorsum (including both the dorsal scute and the free tergites) of a large sample of individuals (120 females and 139 males) collected in the study area and sexed by dissection in the laboratory. There was little overlap between the distribution of these two measurements for males and females (Fig. S2 in Supplementary Material). Thus, using a protocol presented in the Supplementary Material, we were able to estimate with little uncertainty the sex of 38 individuals (3 males and 35 females) for which we did not have any behavioral information and that were not collected at the end of the study. We excluded from the analyses described below the individuals whose sex could not be determined based on their body measures ($N = 24$).

Preliminary Nest Inspections

During the first four days of each fieldtrip, we performed four inspections per day in each nest: one during the day (14:00 hours) and three during the night (19:00, 21:00, and 23:00 hours). In each inspection, we recorded: (1) the presence/absence of the nest-owner males inside their respective nests, (2) the identity and distance of all individuals within a radius of 1 m from each nest, and (3) any relevant behavior observed near or inside the nests, with special attention to female visits, mating interactions, oviposition events, and aggressive interactions with conspecifics (males or females). We used the data on the identity and distance of females close to each nest to classify them as "residents" or "wanderers". For each female, we divided the number of inspections in which she was observed less than 20 cm from a given nest by the total number of inspections we performed in that nest. We classified as wanderers all females recorded close to a nest in less than 30% of the inspections, whereas

females recorded in more than 30% of inspections were classified as residents (Fig. S3 in Supplementary Material).

Male Removal Experiment

After four days of preliminary nest inspections, we randomly assigned the nests into one of two experimental groups: (1) without owner males (treatment nests), in which we permanently removed the owner males from their nests ($N = 33$ nests), and (2) with owner males (control nests), in which we removed the owner males but immediately returned them to their nests ($N = 20$ nests). Because eggs of *Q. limbata* are partially or entirely buried in the nest floor, it is difficult to count the total number of eggs inside the nests (see Fig. 1a). However, we only used in the experiment nests in which we could identify at least a few eggs inside, so that potential egg predators could be attracted to them. We then monitored all nests for the following four days. During these four days (hereafter, "experimental period"), we performed from 26 to 57 inspections per nest (median = 51 inspections per nest) in two periods of the day: afternoon (between 14:00 and 16:00 hours) and night (between 19:00 and 00:00 hours). In each inspection, we observed each nest for 2 min and recorded: (1) the presence and identity of any individual of *Q. limbata* inside the nest, (2) the presence and identity of any potential egg predator inside the nest, (3) egg predation events and the identity of the predator, and (4) any relevant behavior observed inside nests, with special attention to female visits, mating and oviposition events, and defensive behaviors performed by the individuals inside the nests against potential egg predators.

Given that it is not possible to count the initial and final number of eggs inside nests because some of them are buried in the nest floor, we used the visitation frequency of potential egg predators (heterospecifics and conspecifics) during the experimental period as our proxy for the intensity of predation on eggs. In the case of heterospecifics, we considered as egg predators only individuals of taxa that we have already observed consuming eggs of

Q. limbata in the field, such as ants (excluding leaf-cutters of the tribe Attini), crickets (Orthoptera: Phalangopsidae), assassin bugs (Hemiptera: Reduviidae), beetles (Coleoptera: Carabidae), harvestmen (Opiliones: Cosmetidae), millipedes (mainly Platydesmida and Polydesmida), earthworms (Annelida: Lumbricidae), and flatworms (Platyhelminthes: Tricladida) (Fig. 1d-f). In the case of conspecifics, we considered as egg predators only those individuals (males or females) observed inside the nest that have not adopted the nest (see below). For both conspecifics and heterospecifics, we recorded a nest visit when one or more individuals were observed completely inside a nest, but not necessarily feeding on eggs. If a heterospecific nest visitor was observed inside the same nest for two or more consecutive inspections, we considered it as a single prolonged visit. The same criterion was applied to conspecific visitors, but in this case, we were sure about their identity because they have all been individually marked before. We calculated nest visitation frequency as the number of inspections in which we observed any potential egg predator inside a nest considering the total number of inspections we performed in that nest during the experimental period.

We considered that a nest was adopted when a conspecific individual (male or female) was observed inside it in four or more inspections per night in at least three nights, consecutive or not (i.e., in at least 40% of the inspections). Moreover, we interpreted any aggressive response of individuals that adopted nests (hereafter, "foster parents") against potential egg predators (conspecifics or heterospecifics) as instances of nest and/or egg defense. Therefore, we also considered a nest adoption when an individual was observed defending a nest, regardless of how long it had been observed inside the nest. To compare the efficiency of egg attendance by nest-owner males and foster males, we calculated nest attendance frequency as the number of inspections in which a male was observed inside the nest divided by the total number of inspections conducted at that nest since the first inspection in which the male was observed inside the nest.

Data Analyses

We tested our hypotheses that nest-owner males, resident females, and foster males would decrease nest visitation frequency by egg predators using a two-step approach. First, we evaluated how nest visitation frequency by potential egg predators was affected by the presence or absence of different types of adults inside the nests. Then, we evaluated how nest visitation frequency by potential egg predators was affected by nest attendance frequency of the biological and foster individuals.

In the first step, we classified the nests according to the presence and identity of the tending males in three alternative ways. Nests were first classified into two groups (hereafter, "experimental groups"): (1) treatment nests, in which the owner males were experimentally removed from their nests, and (2) control nests, in which the owner males were allowed to stay inside their nests. According to this classification, we made no distinction whether treatment nests were adopted or not by foster males. Then, nests were classified into two other groups (hereafter, "male presence"): (1) nests with males, which included nests attended by their owner males, as well as nests adopted by foster males, and (2) nests without males, which included nests that were not attended by either their owner males or foster males during the experimental period. Finally, nests were classified into three groups (hereafter, "nest status"): (1) nests attended by their owner males, (2) nests attended by foster males, and (3) nests not attended by any male. These three alternative classifications consider the protective effect of foster males as being: (a) non-existent, similar to no male attending the nest ("experimental groups" classification); (b) similar to the owner

males ("male presence" classification); or (c) distinct from no male attending the nest and also from the owner males ("nest status" classification).

We also classified nests according to the presence of resident females before experimental male removal (hereafter, "female residency") in: (1) nests with resident females and (2) nests without resident females. Based on the data gathered during preliminary nest inspections, we observed 17 nests with resident females, from which 7 were later allocated to the control group and 10 to the treatment group. We also observed 36 nests that had no resident females, from which 12 were later allocated to the control group and 24 to the treatment group. To avoid multi-collinearity problems in our analyses, we performed chi-square tests to check if female residency was concentrated in any particular group of the three nest classifications based on the presence and identity of tending males described above. Since no association was found between the variables (Table S1 in Supplementary Material), we conducted a first analysis in which we built a set of 11 generalized linear mixed models (GLMMs), each of them representing an alternative hypothesis. The response variable was the nest visitation frequency by potential egg predators and the predictor variables were: (a) experimental groups, (b) male presence, (c) nest status, and (d) female residency. The list of candidate models included a null-effect model, models considering the isolated effect of each predictor variable, and models considering either the additive or the interactive effect between female residency and one of the three categorical classifications of nests based on the presence and identity of tending males. All models assumed a beta-binomial distribution of errors to account for the over-dispersion observed in the data.

In a second analysis, we used a subset of the data containing only nests that were attended by a male at least in one of our observations, classifying them according to their male status as: (1) nests attended by owner males and (2) nests attended by foster males. Then, we created a set of five GLMMs, each of them representing an alternative hypothesis.

The response variable was the nest visitation frequency by potential egg predators and the predictor variables were: (a) nest attendance frequency and (b) male status (owner or foster). The list of candidate models included a null-effect model, models considering the isolated effect of each predictor variable, and models considering either the additive or the interactive effect between the predictor variables. Each model assumed a binomial distribution of the errors. We centered and standardized the values of all predictor variables (Schielzeth, 2010) and, because we performed observations in two fieldtrips and in five fallen logs, we added fieldtrip and fallen log as random effects in both the first and second statistical analyses.

For each analysis, we compared the fit of the alternative models to the observed data using the Akaike Information Criteria for small samples (AICc), and considered the model with the lowest AICc value as the most plausible model. Models that differed from the best model by less than two units of AICc were considered equally plausible to explain the observed data (Burnham & Anderson, 2002). When two or more models met this criterion, we considered the simplest one to be the best explanation for the observed data and dismissed the models with additional parameters (following Burnham & Anderson, 2002 and Arnold, 2010). All models were created using the package *glmmADMB* (Fournier et al., 2012) and compared using the package *bbmle* (Bolker & R Core Team, 2016) in the software R 3.3.1 (R Core Team, 2016).

Regarding the first set of models, we expected that the models including both male presence inside nests and female residency would be selected as the most plausible, with a negative effect of these variables on nest visitation frequency by potential egg predators. Moreover, if foster males were as efficient as owner males in defending their nests, we would expect the models containing the "male presence" classification to be selected instead of the other two classifications. Regarding the second set of models, if foster males were as efficient as owner males in defending their nests, we would expect that only nest attendance

frequency and not male status (owner or foster) to explain nest visitation frequency by potential egg predators.

RESULTS

Nest Visitation by Potential Egg Predators

The most common potential egg predators that visited nests were conspecifics (56.2%), followed by ants (18.5%), crickets (8.2%), millipedes (6.1%), earthworms (5.5%), and other taxa (5.5%), including carabid beetles, assassin bugs, and a cosmetid harvestman (Figs. 1c-f). Nest visits of potential egg predators lasted from one to seven consecutive inspections (median = 1 inspection). In four visits performed by heterospecifics, we observed at least one egg being consumed. Males accounted for 15% ($N = 9$ visits) of all conspecific visits, and were observed consuming eggs in only one visit. Females accounted for 74% ($N = 61$ visits) of all conspecific visits, with 37 visits performed by wandering females (which consumed eggs in at least six visits) and 16 visits performed by resident females (which consumed eggs in only two of these visits, both performed by the same female). Finally, 11% ($N = 9$ visits) of the conspecific visits were performed by individuals whose sex could not be determined.

Nest Adoption

Ten conspecific individuals (two females and eight males) adopted nests during the experiment. The two females stayed in the adopted nests in 45% and 56% of the inspections performed in those nests, and females were observed consuming one egg each from the nest they adopted. Both foster females were classified as wanderers because they were observed close to a nest in 8% and 26% of the preliminary nest inspections. One of the foster females bit another wandering female on her legs when she tried to enter the adopted nest.

Five of the eight males that adopted a nest were wanderers that have not owned a nest on the fallen log during the preliminary inspections, with two of them being marked in the same fallen log, and one marked in another fallen log, approximately 1 m away. The remaining two males were unmarked, meaning they were probably not present at the studied fallen logs during the preliminary inspections. The other three foster males were owner males that abandoned their nests to adopt a nest that was 6, 32, and 41 cm away from their original nests. Two of these foster males were observed consuming, respectively, one and five eggs from the adopted nests. One foster male defended the adopted nest from an ant and another one from a conspecific male. After nest adoption, four foster males were visited by one female each, while two foster males were visited by two and three females each. In one of those visits, we observed copulation and oviposition.

Efficiency of Paternal Care and Role of Resident Females

According to our classification, there were 17 nests with resident females ($N = 10$ females in treatment nest and $N = 7$ females in control nests) among the 53 nests included in the experiment. The frequency of inspections in which these resident females were observed close to their nests ranged from 30% to 70% of all inspections (Fig. S2 in Supplementary Material). After male removal in the treatment group, five resident females (50%) were no longer observed close to their nests. In the control group, in which owner males were maintained in their nests, two resident females (28.5%) were no longer observed close to their nests. In both experimental groups, none of the females that remained in the proximities of their nests was observed repelling potential egg predators (conspecifics or heterospecifics).

We found that the three most plausible models to explain nest visitation frequency by potential egg predators included: (i) the isolated effect of male presence inside the nest, (ii)

the isolated effect of nest status, and (iii) the additive effect of male presence inside the nest and female residency (Table 1). However, according to our criterion that the simplest model would best explain the observed data, we interpret the model that best predicted nest visitation by potential egg predators as the one that only included the effect of the presence of males inside the nest (Table 1). The median visitation in nests attended by a male (owner or foster) was three times lower (ca. 5% of the inspections) than in unattended nests (ca. 15% of the inspections; Fig. 2a). Thus, according to the most plausible model, we conclude that: (i) the presence of resident females had no relevant effect on nest visitation frequency by potential egg predators, and (ii) when an owner or a foster male was inside the nest, nest visitation frequency by potential egg predators was lower when compared to nests without any male (Fig. 2a).

Efficiency of Foster Males as Egg Protectors

When we used a subset of the data containing only nests that were attended by a male, the three most plausible models to explain nest visitation frequency by potential egg predators contained: (i) the isolated effect of nest attendance frequency; (ii) the additive effect of nest attendance frequency and male status, and (iii) the interaction between nest attendance frequency and male status (Table 2). According to our criterion that the simplest model would best explain the observed data, we interpret the model that best predicted nest visitation by potential egg predators in nests with owner males and nests with foster males as the one that only included the effect of nest attendance frequency (Table 2). In nests with owner and foster males, visitation by egg predators decreased as nest attendance frequency increased (Fig. 2b).

Table 1. Summary of the model selection analysis performed to investigate the factors influencing nest visitation frequency by potential egg predators in the harvestman *Quindina limbata*. "Experimental group" is a categorical variable with two levels: nests with or without the owner male. "Male presence" is a categorical variable with two levels: nests with or without any male (owner or foster). "Nest status" is a categorical variable with three levels: nests with the owner male, with a foster male, or with no male. "Residency" is a categorical variable with two levels: nests with or without a resident female. The models are ranked according to the value of their Akaike Information Criterion corrected for small samples (AICc). ΔAICc is the difference in AICc between each model and the most plausible model (k = number of parameters; w = Akaike weight). The asterisk denotes interactive effects and plus symbol denotes additive effects between variables. The best model is highlighted in bold and the competitive models with uninformative parameters are in italics. See Table S2 in the Supplementary Material for estimates of the coefficients of the models.

Predictor variables	AIC comparison coefficients			
	K	AICc	ΔAICc	w
Male presence	5	251.0	0.0	0.410
<i>Male presence + Residency</i>	6	252.0	1.0	0.254
<i>Nest status</i>	6	253.0	2.0	0.152
Nest status + Residency	7	253.9	2.9	0.096
Male presence * Residency	7	254.5	3.5	0.068
Nest status * Residency	9	258.5	7.5	0.009
Experimental group	5	259.1	8.1	0.007
Experimental group + Residency	6	260.9	9.9	0.003
Experimental group * Residency	7	263.4	12.4	<0.001
Null model	4	273.3	22.3	<0.001
Residency	5	275.7	24.7	<0.001

Table 2. Summary of the model selection analysis performed to investigate the factors influencing the visitation frequency by potential egg predators in nests with the owner males and nests with foster males of the harvestman *Quindina limbata*. "Nest attendance frequency" is a continuous variable and "Male status" is categorical variable with two levels: nests attended by the owner or by a foster male. The models are ranked according to the value of their Akaike Information Criterion corrected for small samples (AICc). ΔAICc is difference in AICc between each model and the most plausible model (k = number of parameters; w = Akaike weight). The asterisk denotes interactive effects and the plus symbol denotes additive effects between variables. The best model is highlighted in bold and the competitive models with uninformative parameters are in italics. See Table S3 in the Supplementary Material for estimates of the coefficients of the models.

Predictor variables	AIC comparison coefficients			
	k	AICc	ΔAICc	w
<i>Nest attendance frequency + Male status</i>	5	96.8	0.0	0.440
Nest attendance frequency	4	97.1	0.3	0.380
<i>Nest attendance frequency * Male status</i>	6	98.6	1.8	0.180
Male status	4	112.3	15.5	<0.001
Null model	3	111.6	14.8	<0.001

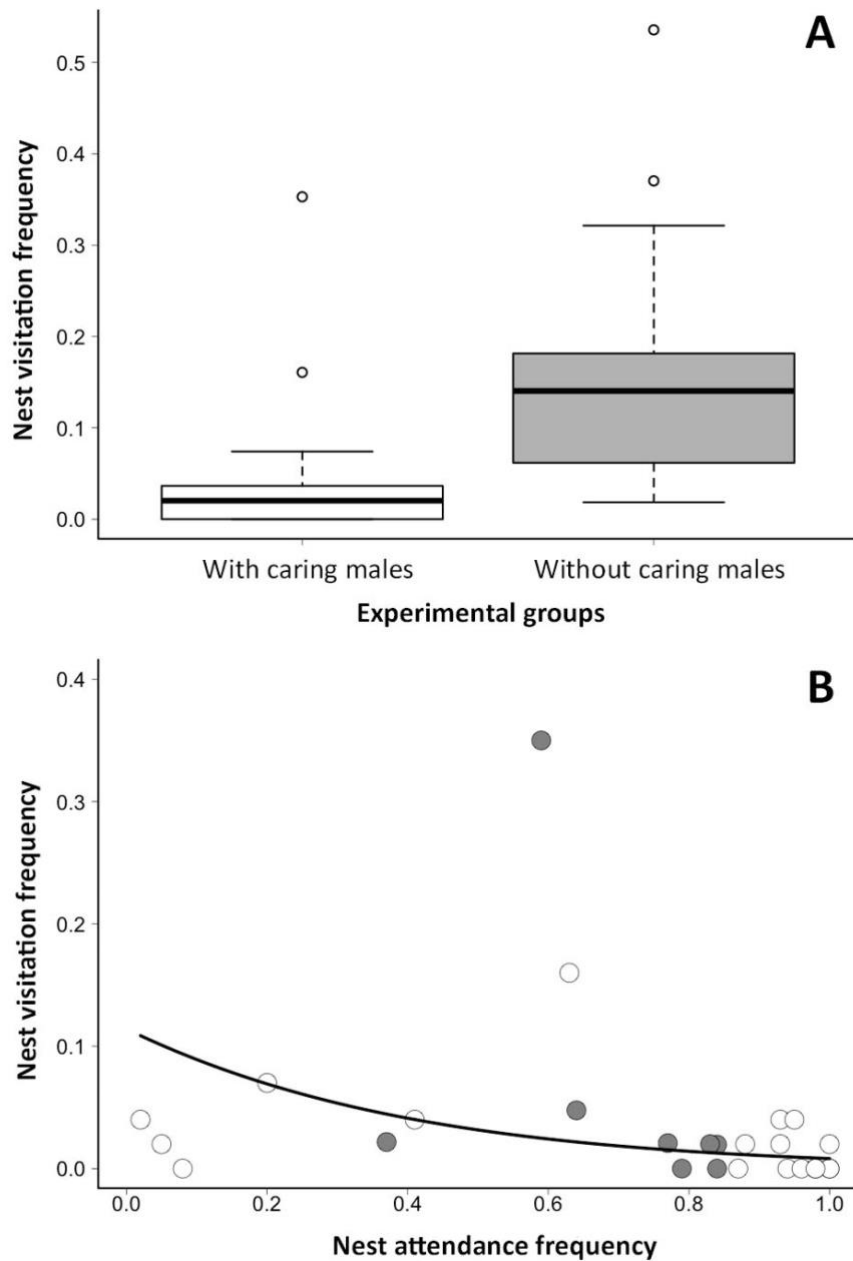


Figure 2. Frequency of observations in which a potential egg predator approached nests of the harvestman *Quindina limbata*. (A) Nest visitation frequency in nests with or without the owner males. The bold line represents the median, boxes represent the values between the lower (25%) and upper (75%) percentile, whiskers indicate the range, and dots represent outliers. (B) Relationship between nest attendance frequency by males and visitation frequency. White circles represent nests with owner males and grey circles represent nests adopted by foster males. The line represents the predicted values calculated by the best model (Table 2 and Table S3 in the Supplementary Material).

DISCUSSION

Our findings show that the intensity of predation was lower in nests where owner males of the harvestman *Q. limbata* were present when compared with nests where they were experimentally removed. We also showed that some unattended nests were adopted by conspecific males, and that foster males were as efficient as the nest-owner males in preventing nest visitation by potential egg predators. Only two females adopted unattended nests — both of them were categorized as wanderers and cannibalized eggs from the nest. This finding rejects our prediction that resident females would perform flexible compensation of paternal care when nest-owner males were absent. Moreover, we also rejected the prediction that the presence of resident females close to unattended nests would decrease nest visitation by potential egg predators. In what follows, we integrate these results and discuss the interplay between parental care and sexual selection in *Q. limbata* and other species with exclusive male care.

Exclusive paternal care has evolved independently at least 16 times among arthropods (Requena, Munguía-Steyer, & Machado, 2013), but only a few experimental studies have tested the efficiency of males as egg protectors under field conditions. In the assassin bug *Rhinocoris tritis* (Gilbert, Thomas, & Manica, 2010), as well as in the harvestmen *Iporangaia pustulosa* (Requena et al., 2009) and *Q. albomarginis* (Mora, 1990), male presence reduces egg mortality promoted by parasitoids, predators, and/or fungi. Here we found that male presence is also crucial for egg survival in *Q. limbata* because nest visitation frequency by potential egg predators was much higher in nests in which males were removed. Similar to several arthropod species studied so far (reviewed in Santos et al., 2016), conspecifics, especially females, constitute the main source of egg predation in *Q. limbata*, which may explain why males attack some females before or even after copulation (Mora, 1990; Toscano-Gadea & Rojas, 2014). Males of some arthropod species are able to recognize female

body condition (e.g., Engqvist, & Sauer, 2003; Aisenberg, Costa, & González, 2011) and reject potential mating partners in poor condition. Thus, female rejection performed by egg-tending males of *Q. limbata* could be partially explained by natural selection favoring egg protection because female in poor condition may represent high risk of egg predation.

A recent meta-analysis showed that the benefits of uniparental egg attendance are higher in tropical climates, where predation and parasitism are more intense (Santos et al., 2016). This pattern, however, is found only when post-ovipositional parental care is not associated with additional lines of egg defense, such as nests, chemical deterrents, mucus and debris coats. Although the number of arthropod species with exclusive paternal care included in the meta-analysis was limited, in all of them the eggs had additional lines of defense (Mora, 1990; Requena et al., 2009; Gilbert, Thomas, & Manica, 2010). This is also the case of *Q. limbata*, in which eggs are protected inside mud nests. First, nest walls prevent the entrance of some potential egg predators, such as flat-backed millipedes. Second, females bury their eggs in the nest floor, so that they are not readily available to predators (Fig. 1a). We suggest that additional lines of egg defense are common in arthropod species showing exclusive paternal because they may keep the eggs protected even when egg-tending males leave their clutches temporarily unattended to forage or shelter (Requena et al., 2009; Chelini & Machado, 2014). Considering the importance of additional lines of defense for egg survival, we also suggest that females of arthropod species with exclusive male care should evaluate the quality of these defenses to make their mating decisions and increase their reproductive success.

No resident female adopted unattended nests, and the only two females that did so were considered wanderers. One of these wandering females repelled a conspecific female from the nest, but we cannot assure she was actually defending the eggs. An alternative explanation is that the female was just protecting a food resource from a cannibalistic

individual. Females of the harvestman *M. neptunus* visit unattended nests and may remain inside them for several hours consuming eggs. However, two or three females can be found inside the same unattended nest and no aggressive interaction among them has been recorded (Nazareth & Machado, 2010). Nevertheless, even if the wandering female of *Q. limbata* was actually defending the eggs, we can still conclude that, overall, egg adoption by females is rare in this species and that resident females do not perform flexible compensation of paternal care in the absence of owner males. An additional possibility is that resident females are socially associated with the owner males and not spatially associated with the nests, which could also explain why 50% of the resident females abandoned their nest after male removal. In many species with exclusive paternal care, only males with large body size or in good condition are able to defend the best nesting sites, to build and maintain nests that maximize offspring protection against biotic and abiotic factors, and to provide high quality care (examples in Schaedelin & Taborsky, 2009). If this is the case of *Q. limbata*, females would benefit by being associated with high quality males than with high quality nests, and should also seek for alternative males if their regular partner deserts or dies. However, the reasons why some males are apparently monopolized by resident females remain to be explored in future studies.

Contrary to what we found for females, egg adoption by males was relatively frequent. In both *Q. albomarginalis* and *Q. limbata* females have only been observed mating and laying eggs inside nests (Mora, 1990; Toscano-Gadea & Rojas, 2014). Given that nest construction is probably costly, some males may benefit by adopting unattended nests without paying the costs of nest construction. Moreover, foster males consumed only a few eggs and 75% of them received female visits one or two days after nest adoption. If wandering females, which are neither spatially associated with nests nor socially associated with males, prefer to lay eggs with males that already provide egg care, the benefits of nest adoption for males of *Q.*

limbata may be even higher if the unattended nest contains eggs. This seems to be the case for several arthropod species with exclusive paternal care (Nazareth & Machado, 2010; Gilbert, Thomas, & Manica, 2010; Requena & Machado, 2015a; Ohba, Okuda, & Kudo, 2016; Ohba et al., 2018). In fact, similar results have been reported for several fish species in which the presence of eggs in the nest enhances male's attractiveness (reviewed in Wisenden, 1999). In some of these fish species, males can even aggressively evict resident males from their nests (e.g., Unger & Sargent, 1988; Bisazza, Marconato, & Marin, 1989; Sargent, 1989), a behavior also recorded in both *Q. limbata* and *Q. albiormarginalis* (Mora, 1990; Rojas et al., 2017).

Nest adoption in fish and arthropods with exclusive paternal care may be regarded as a parasitic tactic because some males exploit the investment of other males that spend time and energy to build a nest and court females (Taborsky, Oliveira, & Brockmann, 2008). In these two animal groups, parasitic tactics are usually exhibited by small males or males in poor condition (reviewed in Taborsky, 2008 and Buzatto, Tomkins, & Simmons, 2014). If foster males are in poor condition, we would expect intense cannibalism of adopted eggs and low efficiency in keeping predators away from the nest. However, this was not the case for *Q. limbata* and for the fathead minnow *Pimephales promelas* (Unger & Sargent, 1988), which suggests that foster males in these two species with exclusive paternal care are not necessarily individuals in poor condition and unable to pay the costs of building their own nest. We argue that nest adoption in *Q. limbata*, in particular, is an opportunistic behavior exhibited by males without nests, as well as by males with nests that are unsuccessful in attracting females. Given that foster males do not need to build a nest, they probably have enough energy to invest in activities that may increase their attractiveness and future mating success, such as egg protection, nest repair, and high-quality female courtship. Thus, as already suggested for fish species with exclusive paternal care (reviewed in Wisenden, 1999), egg adoption may impose low costs to *Q. limbata* males when compared with high benefits.

In conclusion, the presence of owner males seems to be crucial for egg survival in the mud-nest harvestman *Q. limbata*. Females do not show flexible compensation of parental care in unattended nests probably because they are associated to males and not to the nests per se. The reasons why some females show the so-called resident behavior and the benefits they derive from monopolizing the access to some owner males deserve further investigation. Males are willing to adopt unrelated eggs and protect them as efficiently as original owner males. We argue that egg adoption is probably an opportunistic behavior that allows some males to avoid the costs of building a nest and receive the benefits of access to females and increased attractiveness when compared with males without eggs in their nests. These findings reinforce that the evolution of exclusive paternal care in arthropods is influenced by both natural and sexual selection.

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SUPPLEMENTARY MATERIAL

1. Protocol to Infer the Sex of the Individuals Using Body Measurements

To infer the sex of the individuals for which we did not have any behavioral information and/or that were not collected at the end of the experiment, we compared their individual measures of the dorsal scute width at its widest point and the total area of the dorsum (including both the dorsal scute and the free tergites) to the distribution of these traits measured from photographs of 120 females and 139 males (Fig. S1).

First we plotted the distribution of each of the two traits measured for all males and females that were collected and sexed by dissection in the laboratory. These plots confirmed that for both traits males are smaller than females (Fig. S2). Then, based on the sex of the identified individuals and on the distribution of the measured trait, we determined three zones in each distribution (Fig. S2): (1) Male zone: values up to the 85th percentile of males' distribution; (2) Female zone: values above the 15th percentile of females' distribution; and (3) Undetermined zone: values between the 85th percentile of males' distribution and the 15th percentile of the females' distribution.

For the dorsal scute width, the male zone included 114 males and 6 females (whose measurements were lesser than 2.44 mm), the female zone included 3 males and 102 females (whose measurements were higher than 2.54 mm), and the undetermined zone included 22 males and 12 females (whose measurements were between 2.44 and 2.54 mm). For the total area of the dorsum, the male zone included 114 males and only 3 females (whose measurements were lesser than 6.54 mm²), the female zone included 3 males and 98 females (whose measurements were higher than 7.26 mm²), and the undetermined zone included 22 males and 19 females (whose measurements were between 6.54 and 7.26 mm²).



Figure S1. Dorsal view of a female of the harvestman *Quindina limbata* showing the two measurements used to infer the sex of some individuals that were not collected at the end of the field experiment and for which we had no behavioral information. The horizontal dotted line indicates the dorsal scute width at its widest point and the dotted silhouette indicates the total area of the dorsum. Back square on the background = 5 mm².

Based on the combined information of the distribution of both traits (Fig. S2), we classified three individuals of unknown sex as males because their values of dorsal scute width and total area of the dorsum were both located in the male zone. Likewise, we classified 35 individuals of unknown sex as females because their values of dorsal scute width and total area of the dorsum were both located in the female zone. We could not unequivocally classify 24 individuals of unknown sex as either male or female because their values of dorsal scute width and total area of the dorsum were both located in the undetermined zone or because one value was located in the male zone and the other one in the female zone. These individuals whose sex could not be unequivocally determined were excluded from our analyses (see main text).

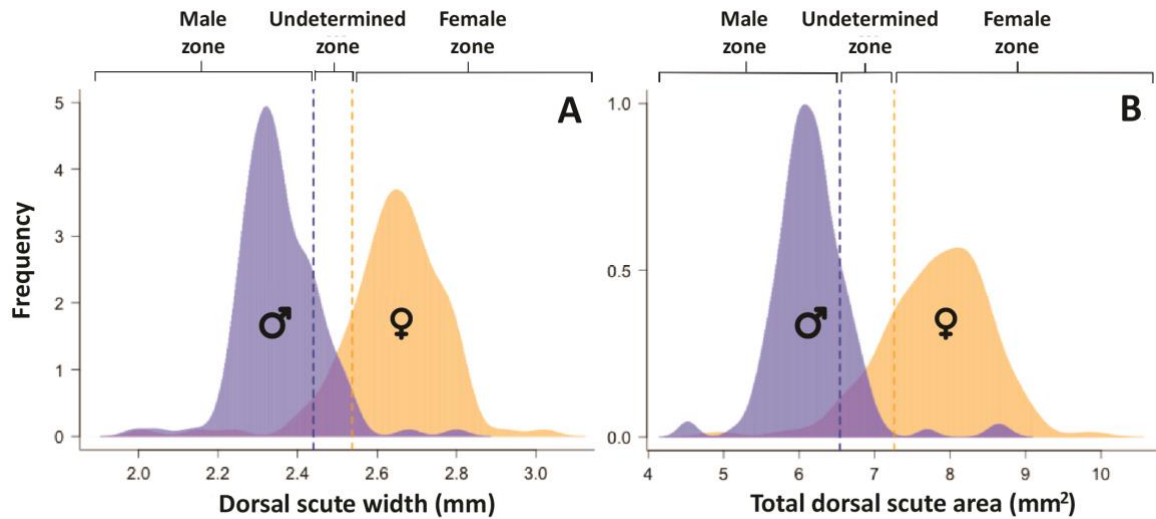


Figure S2. Distribution of the (A) dorsal scute width and (B) total area of the dorsum of 259 adults of *Quindina limbata* (120 females and 139 males) for which we have information on their sex. The blue shade represents the distribution of males and the orange shade represents the distribution of females. The male zone is the area containing 85% of all males in the sample, the female zone is the area containing 85% of all females in the sample, and the undetermined zone is the area where there is great overlap in the distribution of male and female values for both traits we measured.

2. Classification of Females as Residents or Wanderers

To classify females as resident or wanderers, we divided the number of inspections in which each female was observed less than 20 cm from a given nest by the total number of inspections we performed in that nest. We classified as wanderers all females observed close to a nest in less than 30% of the inspections, whereas females observed in more than 30% of inspections were classified as residents (Fig. S3). According to this classification, resident females were observed close to a nest from 4 to 8 inspections in two or more days. All wandering females were observed close to a nest less than 4 inspections in two or less days.

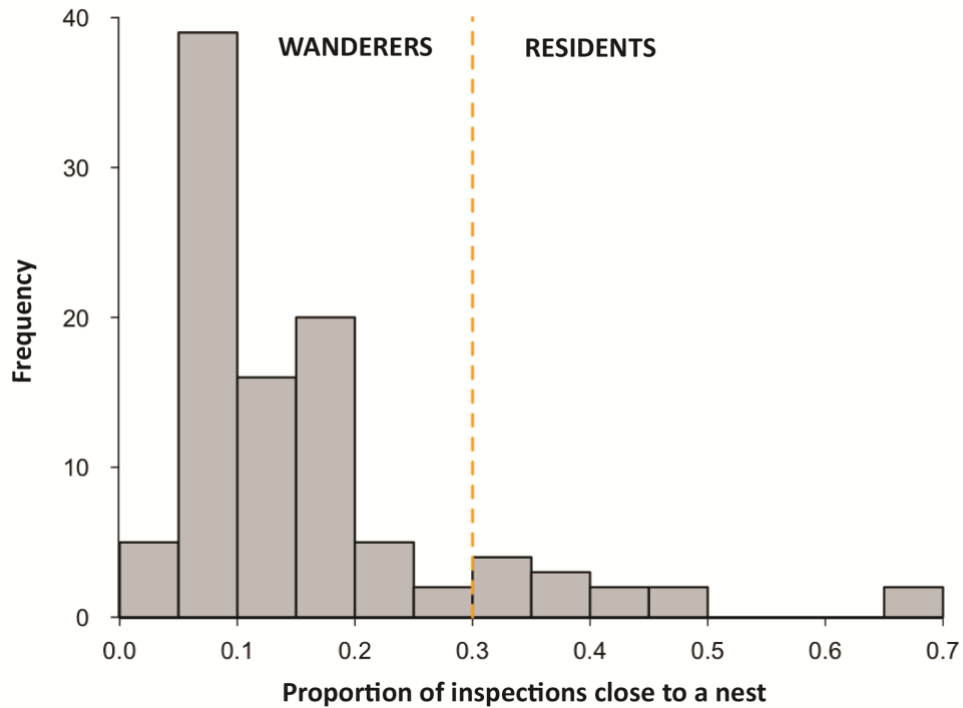


Figure S3. Frequency of inspections each female of the harvestman *Quindina limbata* was recorded close (less than 20 cm) from a given nest divided by the total number of inspections performed in that nest. Resident females are those recorded close to a nest in more than 30% of the inspections, whereas wanderers are those females recorded close to a nest in less than 30% of inspections.

3. Multi-collinearity Tests for Predictor Variables

To avoid multi-collinearity problems in the first step of our analyses, we performed chi-square tests to check whether nests with and without resident females (i.e., "female residency") were concentrated in any particular group of the predictor variables. For the predictor variable "experimental groups", nests were classified into two groups: treatment and control. For the predictor variable "male presence", nests were classified into two groups: with and without males. Finally, for the predictor variable "nest status", nests were classified into three groups: nests attended by their owner males, nests attended by foster males, and nests not attended by any male. No significant association was found between female residency and any particular group of the three predictor variables (Table S1).

Table S1. Results of the chi-square tests to check if nests of the harvestman *Quindina limbata* with and without resident females were concentrated in any particular group of the predictor variables based on the presence and identity of tending males. df = degrees of freedom.

Predictor variable	X ²	Df	P
Experimental groups	0.06	1	0.803
Male presence	0.47	1	0.499
Nest status	0.96	2	0.619

4. Coefficient estimates

Table S2 shows the summary of the coefficient estimates for the eleven candidate models built to predicted nest visitation frequency by potential egg predators (first step of our statistical analyses). Table S3 shows the coefficient estimates for the five candidate models built to predict the effect of male status (owner or foster) and frequency of nest attendance on nest visitation frequency by potential egg predators (second step of our statistical analyses). Table S4 shows the coefficient estimates for the best models to predict nest visitations by potential egg predators in the first and second steps of our statistical analyses.

Table S2. Summary of the coefficient estimates for models built to investigate the factors influencing nest visitation frequency by potential egg predators in the harvestman *Quindina limbata*. "Experimental group" is a categorical variable with two levels: nests with or without the owner male. "Male presence" is a categorical variable with two levels: nests with and without a male (owner or foster). "Nest status" is a categorical variable with three levels: nests attended by their owner males, nests attended by foster males (adopted), and nests not attended by any male (unattended). "Residency" is a categorical variable with two levels: nests with and without a resident female. The best model is highlighted in bold and the competitive models with uninformative parameters are in italics.

Predictor variables	Coefficient estimate (95% confidence interval)									
	Intercept	Experimental group	Male presence	Nest status (adopted)	Nest status (unattended)	Residency	Experimental group *	Male presence *	Nest status (adopted) *	Nest status (unattended) *
							Residency	Residency	Residency	* Residency
Experimental group	-3.32 (-3.96, -2.68)	1.32 (0.65, 2.00)	—	—	—	—	—	—	—	—
Male presence	-3.31 (-3.84, -2.78)	—	1.49 (0.91, 2.08)	—	—	—	—	—	—	—
<i>Nest status</i>	-3.44 (-4.09, -2.79)	—	—	0.39 (-0.61, 1.39)	1.62 (0.93, 2.31)	—	—	—	—	—
Residency	-2.26 (-2.78, -1.73)	—	—	—	—	-0.11 (-0.72, 0.50)	—	—	—	—
Experimental group + Residency	-3.17 (-3.88, -2.46)	1.36 (0.68, 2.03)	—	—	—	-0.27 (-0.83, 0.29)	—	—	—	—

Table S2. Continued.

Predictor variables	Coefficient estimate (95% confidence interval)									
	Intercept	Experimental group	Male presence	Nest status (adopted)	Nest status (unattended)	Residency	Experimental group *	Male presence *	Nest status (adopted) *	Nest status (unattended)
							Residency	Residency	Residency	* Residency
Experimental	-3.30	1.52	—	—	—	-0.06	-0.27	—	—	—
group * Residency	(-4.29, -2.31)	(0.42, 2.62)				(-1.27, 1.16)	(-1.64 to 1.10)			
<i>Male presence</i> +	-3.12	—	1.55	—	—	-0.35	—	—	—	—
<i>Residency</i>	(-3.72, -2.52)		(0.96, 2.13)			(-0.88, 0.18)				
Male presence *	-3.07	—	1.48	—	—	-0.43	—	0.12	—	—
Residency	(-3.81, -2.33)		(0.58, 2.38)			(-1.40, 0.54)		(-1.04, 1.27)		
Nest status +	-3.25	—	—	0.44	1.69	-0.37	—	—	—	—
Residency	(-3.95, -2.56)			(-0.56, 1.43)	(1.00, 2.39)	(-0.89, 0.16)				
Nest status *	-3.41	—	—	1.00	1.81	-0.11	—	—	-1.00	-0.21
Residency	(-4.37, -2.43)			(-0.43, 2.42)	(0.71, 2.90)	(-1.34, 1.13)			(-2.99, 0.99)	(-1.59, 1.18)
Null model	-2.33	—	—	—	—	—	—	—	—	—
	(-2.67, -1.99)									

Table S3. Summary of the coefficient estimates for models built to investigate the factors influencing the frequency of visitation by potential egg predators in nests with owner males and in nests with foster males of the harvestman *Quindina limbata*. "Nest attendance frequency" is a continuous variable and "Male status" is a categorical variable with two levels: nests attended by the owner male or by a foster male. The best model is highlighted in bold and the competitive models with uninformative parameters are in italics.

Predictor variables	Coefficient estimate (95% confidence interval)			
	Intercept	Nest attendance frequency	Male status	Nest attendance frequency * Male status
Male status	-3.51 (-4.87, -2.13)	—	-0.57 (-1.33, 0.19)	—
Nest attendance frequency	-4.00 (-5.34, -2.66)	-0.88 (-1.33, -0.43)	—	—
<i>Nest attendance frequency + Male status</i>	-3.59 (-5.08, -2.09)	-0.96 (-1.43, -0.49)	-0.74 (-1.50, 0.02)	—
<i>Nest attendance frequency * Male status</i>	-3.72 (-5.33, -2.12)	-1.66 (-2.87, -0.46)	-0.65 (-1.44, 0.14)	0.76 (-0.43, 1.96)
Null model	-3.80 (-4.91, -2.70)	—	—	—

Alternative reproductive behaviors in females of a Neotropical arachnid with exclusive paternal care

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Alternative reproductive tactics have been described for males in many species, but there are few reported cases for females. Previous studies with the harvestman *Quindina limbata* suggest two female reproductive tactics. *Residents* remain close to a nest, repel conspecific females, and mate preferentially with the nest-owner male, which cares for the eggs. *Wanderers* are not spatially associated with nests, do not repel conspecific females, and mate with different nest-owner males. Here we quantified females' behavior to formally test the existence of these two tactics, and investigated whether nest monopolization by females may affect males' reproductive success. The distribution of the proximity index (PI), which refers to the time spent by a female in the vicinity of a given nest, shows a unimodal rather than a bimodal distribution. As the PI increases, the probability of a female attacking other females increases. There is no relationship between the PI and (i) the number of nests visited by a female and (ii) female body size. From the males' perspective, the higher the PI, the lower the number of different females that visited a nest. However, the number of visits received by a male was not affected by the PI. In conclusion, females' behavior may be a reversible tactic, in which females can switch from resident to wanderer during their lifetime in response to body condition. Moreover, the social monogamy imposed by resident females may negatively affect the degree of male promiscuity, but not male's mating rate.

Key words: female fight, female rejection, male mating opportunity, polyandry, sexual conflict

Discrete variation in male reproductive tactics is a widespread pattern, with many reported cases in both invertebrates and vertebrates (examples in Shuster and Wade 2003 and Oliveira et al. 2008). Because male reproductive success depends mostly on the access to receptive females or critical resources needed for females, male alternative reproductive tactics (ARTs) are usually the result of direct or indirect intrasexual competition for access to mates (Henson and Warner 1997). Thus, large males or males in better body condition frequently exhibit reproductive tactics based on the monopolization of females or resources by means of agonistic interactions with other males, whereas small males or males in poor body condition frequently exhibit reproductive tactics that do not involve physical contests, such as sneaking copulations, acting as satellites, or even mimicking females (e.g. Brockmann 2008; Taborsky 2008; Shuster 2008). Although female ARTs have already been reported for some species of arthropods, fish, frogs, lizards, and birds (examples in Alonzo 2008, Zamudio and Chan 2008, and Johnson and Brockmann 2012), they are certainly less intensively studied than male ARTs. This bias may occur because female ARTs are less easily detected than male ARTs and/or variation among female reproductive behavior is more often continuous than discrete (Alonzo 2008).

Besides scarce empirical evidence, there are also few theoretical studies focused on understanding the factors that may favor the evolution of discrete variation in female reproductive behaviors (but see Brennan et al. 2008). Based on what we know on male ARTs, female ARTs should evolve when female access to high-quality mates is limited and alternative behaviors may circumvent this limitation (Johnson and Brockmann 2012). In the salamander *Lissotriton vulgaris*, for instance, females attempt to displace courting females in the beginning of the breeding season, when males with spermatophores are scarce. Later in the breeding season, when males with spermatophores are abundant, agonistic interactions between females no longer occur and they engage in typical courtship behaviors (Waights

1996). Female ARTs may also evolve as a condition-dependent behavior when the costs and benefits of reproduction change with resource availability (Alonzo 2008). Examples come from intraspecific brood parasites, in which some females avoid the costs of nest construction and offspring provisioning by exploiting the investment of conspecific females. Intraspecific brood parasitism in insects and birds is interpreted as a female ART conditional on the availability of food, nest sites or host nests, as well as on female age or body condition (reviewed in Field 1992 and Brockmann 1993). Female polymorphism in damselflies, in which some females exhibit the typical female wing color patterns whereas others exhibit wing color patterns that resemble conspecific males, is also considered as a case of female ART, which apparently has evolved as way to avoid harassment by males (e.g., Forbes 1994; Andres et al. 2002). Finally, more subtle examples of female ARTs include context-dependent mate choice, such as female responses to predation risk (Sih and Krupa 1992) or the identity and frequency of male morphs (Alonzo and Sinervo 2001; Johnson and Brockmann 2012).

Although male ARTs have already been described for several species of arachnids (see examples in Buzatto and Machado 2014), to our knowledge, there is no case of female ART formally documented in this major arthropod group. In harvestmen of the genus *Quindina* (Opiliones: Nomoclastidae), however, there are anecdotic reports that suggest the existence of two female reproductive tactics in natural populations. The reproductive behavior of *Quindina* species is unique among arachnids because males build a cup-like mud nest where females lay eggs and where males care for eggs and early hatched nymphs (Mora 1990; Quesada-Hidalgo et al. 2018). Some females may remain in the close vicinity of a nest for several months (Figure 1a) and, during this period, they visit the nest and copulate with the caring male multiple times. Moreover, the so-called *resident females* are sometimes observed aggressively repelling conspecific females that approach the nest with which they are associated (Mora 1990). Some females, however, are not consistently found close to any

particular nest (Mora 1990; Quesada-Hidalgo et al. 2018). The so-called *wandering females* usually visit several nests, copulate with two or more males, and rarely show aggressive behaviors against other females (Mora 1990).

The first goal of this study is to provide a quantitative characterization of female behaviors in the Neotropical harvestman *Q. limbata* to understand whether resident and wandering females indeed exhibit a discrete variation in their reproductive tactics or whether this variation is better described as continuous. More specifically, we answer the following questions: (1) What is the distribution of time spent by females in the vicinity of nests? A bimodal distribution would indicate the existence of two discrete female reproductive tactics, whereas a unimodal distribution would indicate a continuous variation in this particular female behavior. (2) Are females associated with nests more aggressive against conspecific females? Among all factors that may favor the evolution of female ARTs, we hypothesize that monopolization of high-quality males and/or nests is the best explanation for the behavior of resident females, which should aggressively repel other females to maintain exclusive access to an owner-male and/or his nest. Even if female association with nests is a continuous behavioral trait, aggression against conspecific females should be more likely to occur among females with longer associations. (3) Are females associated with nests less promiscuous than females not associated with nests? If females are defending high-quality males, they should mate preferentially with the defended males. In turn, females with ephemeral associations with nests are expected to visit more nests and be more promiscuous. (4) Is female association with nests coupled with discrete differences in female body size? In many arthropod species in which male ARTs are coupled with intrasexual dimorphism individuals exhibiting the dominant tactic are usually larger than individuals exhibiting the subordinate tactic (examples in Brockmann 2008 and Buzatto et al. 2014). Thus, females with longer associations with nests should be larger than females with

ephemeral associations.

When female ARTs evolve, they are expected to have important implications for the sexual selection acting on males and also on the conflict between males and females (Svensson et al. 2005; Alonzo 2008). Thus, the second goal of this study is to understand how female behavior may affect male reproduction in *Q. limbata*. More specifically, we answer the following questions: (5) Does female association with a male limit his mating opportunities? Assuming that some females may aggressively repel conspecific females from the proximity of the nests with which they are associated, we hypothesize that males in these nests should be less promiscuous than males associated with no female associated to them. (6) Do nest-owner males discriminate between females with longer or ephemeral associations with them? Nest-owner males may attack some visiting females and reject them even before copulation, as occurs in other harvestman species with exclusive paternal care (reviewed in Requena et al. 2013 and Machado et al. 2015). If females associated to a nest-owner male are less promiscuous, sperm competition risk would be low and the costs of caring for unrelated eggs are reduced. Moreover, given that females are the main source of egg predation in *Q. limbata* (Quesada-Hidalgo et al. 2018), females that regularly oviposit in a nest should represent lower risk of egg predation. Thus, we hypothesize that males should attack less frequently females exhibiting high association with them.

METHODS

Study species

Males of *Q. limbata* build their nests mainly on fallen logs using wood debris and saliva. The entire process of nest construction may last from 2 to 4 weeks. The final nest structure is composed of a circular floor (1-2 mm thick) with a spongy texture where females insert their eggs. The floor is surrounded by a circular wall (5-15 mm high), which prevents that some

walking arthropods, such as millipedes, enter the nest (Rojas et al. submitted). Nest-owner males frequently clean the floor and the wall of their nests, preventing fungus growth and egg mortality due to fungi infection. Moreover, nest-owner males usually repair their nests from physical damages promoted mostly by rainfall (Rojas et al. submitted). Nests are found all year long, with no clear seasonal variation in the number of new nests built by the males. Because individuals are mainly nocturnal, female-female agonistic interactions and male-female sexual interactions occur predominantly at night.

There are two different types of female visits to the nests in *Quindina* (Mora 1990). A short visit occurs when a female spends a few minutes inspecting the floor and wall of the nest with the tip of her first two pairs of legs, which probably bear chemo- and mechanoreceptors (Gainett et al. 2016), and then leaves the nest without mating and ovipositing. A long visit, in turn, comprises nest inspection, copulation, and oviposition of 1 to 5 eggs, a process that may last from 20 min to 4 hours. In both types of visits, when a female enters the nest, the owner male presses his body against the internal wall of the nest, allowing the visiting female to perform nest inspection (Figure 1b). However, nest-owner males may attack visiting females by aggressively biting them and chasing them away from the nest during nest inspection, pre-copulatory courtship, and before or after oviposition (Toscano-Gadea & Rojas, 2014).

In a previous study devoted to evaluate the efficiency of egg-attendance provided by males of *Q. limbata*, we showed that resident females were found in 17 of the 53 nests included in the experiment. For the purposes of that study, we classified as *wanderers* all females recorded close (< 20 cm) to a nest in less than 30% of the inspections we conducted in each nest. Females recorded close to a nest in more than 30% of inspections were classified as *residents*. After male removal, half of the resident females were no longer observed close to their nests, and none of them adopted unattended nests, even when they have had laid eggs

inside them. Moreover, none of the resident females that remained close their nests after male removal was observed repelling potential egg predators (Quesada-Hidalgo et al. 2018). These findings suggest that resident females may be socially associated with the caring males and not spatially associated with the nests. However, the reasons why some males are apparently monopolized by resident females remain poorly understood.

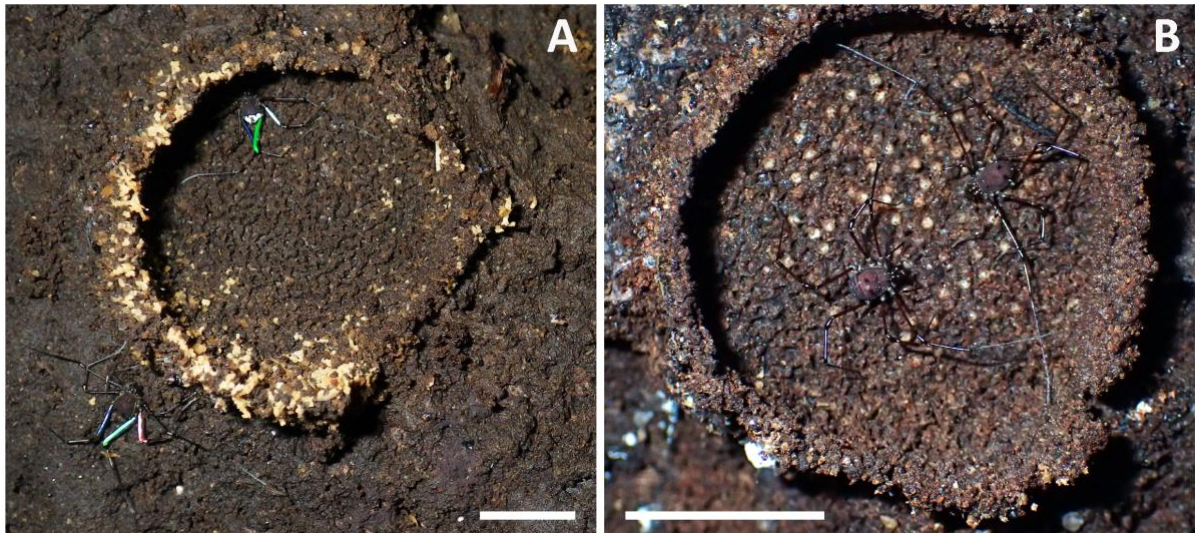


Figure 1. A. Two marked individuals of *Q. limbata*: the male is inside the nest and the resident female is in close contact with the external wall of the nest. Note that the female extends the second pair of sensorial legs and touches the nest wall. B. Visiting female (left) and nest-owner male (right) of *Q. limbata* inside a nest. Note that the owner male presses his legs against the internal wall of the nest, allowing the visiting female to inspect the nest floor.

Study area

We conducted fieldwork at Veragua Rainforest (9°55'35.7"N; 83°11'27.9"W; 400 m above sea level), a biological station located in the province of Limón, Caribbean slope of Costa Rica. The area consists of a primary tropical rainforest that receives an annual precipitation of nearly 3000 mm, and has a mean annual temperature of 23 °C (Holdridge 1967). The climate has no well-defined seasons, so that the mean monthly temperature is always above 19 °C and the mean monthly precipitation is always above 140 mm (data obtained from a meteorological station located 20 km from the study site).

Procedures to mark and sex individuals

Between August 2014 and April 2015, we conducted 11 fieldtrips lasting from 2 to 6 days each (median = 4 days). The intervals between fieldtrips ranged from 2 to 59 days (median = 23 days). In these fieldtrips we monitored two fallen logs containing a total of 61 nests. In June 2015, we performed two more fieldtrips lasting 4 and 6 days each, with an interval of 1 day between them. In both fieldtrips we monitored four fallen logs containing a total of 18 nests. Finally, in January and March 2016, we performed two fieldtrips lasting 9 days each. In the first of these fieldtrips, we monitored four fallen logs containing a total of 37 nests. In the second fieldtrip, we monitored two fallen logs containing a total of 27 nests. Combining all 15 fieldtrips, we individually marked a total of 143 nests and their corresponding owner males. We also individually identified a total of 425 non-nesting individuals (males and females) found on the monitored fallen logs.

We marked all individuals with exclusive color codes by painting their carapace and/or femur of legs III and IV with enamel paint. This procedure has already been used with at least two *Quindina* species in previous studies, and apparently it does not interfere with the individuals' behavior (Mora 1990; Requena and Machado 2015; Quesada-Hidalgo et

al. 2018). After the marking procedure, we photographed the individuals for further body measurements and released them at the same place where they were captured. From each photograph, we measured the width of the dorsal scute at its widest point as a proxy of body size of the individuals.

Given that there is no evident sexual dimorphism in species of the genus *Quindina* (Pinto-da-Rocha and Bragagnolo 2017), we used the same procedure we used in a previous study to identify the sex of all individuals we marked and measured. All individuals found alone inside a nest were recorded as males, whereas all individuals that visited a nest and were not attacked, and that copulated or oviposited inside a nest where a nest-owner male was present were recorded as females. Some of these individuals were collected and dissected in the laboratory to confirm the sexing procedure, and this checking indicated that our classification in the field was always correct (Quesada-Hidalgo et al. 2018).

Nest monitoring

During each day of each fieldtrip, we performed four inspections per day in each nest: one during the day (14:00 h) and three during the night (19:00, 21:00, and 23:00 h). In each inspection, we recorded: (1) the presence and identity of nest-owner males inside their respective nests, (2) the identity and distance of all individuals within a radius of 1 m from each nest, and (3) the presence and identity of females inside each nest. Every time a female was found inside a nest we called this event *female visit*, regardless of the female copulated with the owner male or oviposited inside the nest. When we observed a female visit, we recorded any relevant behavior observed near or inside the nest, with special attention to mating interactions, oviposition events, and aggressive interactions between females. We also recorded if the female left the nest by herself or if she was attacked and repelled from the nest by the nest-owner male. When a female was attacked by the nest-owner male before

oviposition we called this event *female rejection*. Both visiting males and females may be attacked by nest-owner males when they enter an occupied nest, but only females inspect the nest using the first pair of legs. Visiting males, in turn, try to attack the nest-owner males using their pedipalps. Based on these distinctive behaviors, we could unequivocally identify the repelled individuals as males or females. Throughout the 15 fieldtrips we accumulated approximately 342 hours of observations and, whenever possible, we videotaped male-female and female-female interactions.

Variables and data analyses

To answer question (1), on the distribution of female association with nest-owner males, we created a *proximity index* for each female that we recorded visiting at least one nest during the study period ($n = 97$ females). Females that we never observed entering a nest were not included in any of the analyses described below. To calculate the proximity index we divided the number of inspections in which a given female was recorded less than 20 cm from the nest i by the total number of inspections we performed in the nest i . Both numbers were counted before the female visit, in a time window that comprised the fieldtrip in which we observed the female entering the nest and the previous fieldtrip. If the previous fieldtrip occurred more than 2 months before, we considered only the current fieldtrip to calculate the proximity index. For descriptive purposes, we classified as *wanderers* all females with proximity index < 0.3 , and as *residents* all females with proximity index ≥ 0.3 (following Quesada-Hidalgo et al. 2018). To increase the confidence of this classification, we excluded all females for which we had few observations, i.e. less than 5 inspections close to any nest we monitored during the study period.

To answer question (2), on female-female agonistic interactions, we performed two generalized linear models (GLMs) in which the predictor variable was the proximity index

and the response variables (with binomial error distribution) were: if a female attacked (1) or not (0) a conspecific female in the proximity of a nest, and if a female was attacked (1) or not (0) by a conspecific female in the proximity of a nest.

To answer question (3), on the degree of female promiscuity, we counted the number of nests visited by each female in a time window that comprised the fieldtrip in which we observed the female entering a nest and the previous fieldtrip. Once more, if the previous fieldtrip occurred more than 2 months before, we considered only the current fieldtrip to calculate the number of nests visited by each female. We then performed a GLMs in which the response variable (with Poisson error distribution) was the number of nests visited by each female and the predictor variable was the proximity index.

To answer question (4), on female body size, we performed a linear model (LM) in which the predictor variable was the proximity index and the response variable (with Gaussian error distribution) was the dorsal scute width of females.

To answer question (5), on male mating opportunities, we counted the number of different females that visited a nest-owner male and the number of visits he received (regardless of female identity). Both variables were calculated in a time window that comprised the fieldtrip in which we observed the female entering the nest and the previous fieldtrip. If the previous fieldtrip occurred more than 2 months before, we considered only the current fieldtrip to calculate the number of different females that visited a male and the number of visits received by a male. To analyze the data, we performed two generalized linear mixed models (GLMMs) in which the predictor variable was the proximity index and the response variables (with Poisson error distribution) were: (a) the number of different females that visited a nest-owner male and (b) the number of visits received by a nest-owner male. We created a variable called *couple* that contained a code for each pair of female-male and used this variable as a random effect in both GLMMs.

Finally, to answer question (6), on female rejection, we performed a GLMM in which the predictor variables were female size and the proximity index and the response variable (with binomial error distribution) was if a visiting female was rejected (1) or not (0) by the nest-owner male. Again, we used the variable couple as a random effect in the GLMM.

All models (LMs, GLMs, and GLMMs) were performed using the package `glmmADMB` (Fournier et al. 2012) for R version 3.3.1 (R Core Team 2018).

RESULTS

Females association with nest-owner males

The values of the proximity index ranged from 0.04 to 1, with no indication of a bimodal distribution (Figure 2). Most of the females showed low values of proximity index, but some females were recorded close to the same nest for 15 weeks, showing high values of proximity index (Figure 2).

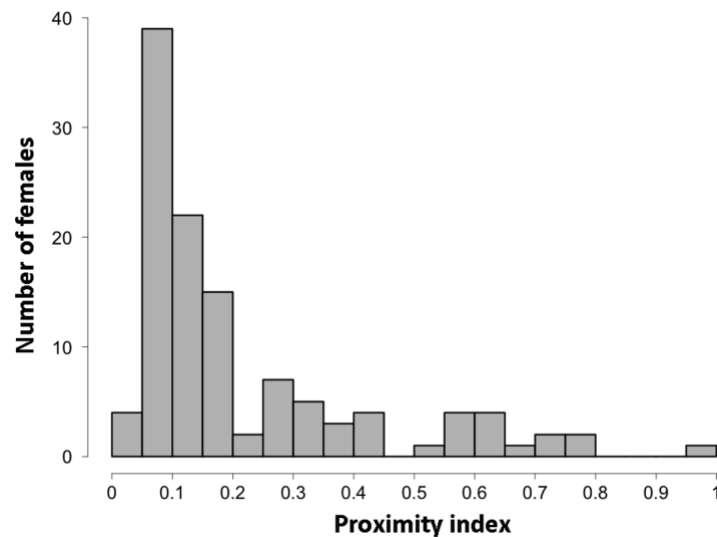


Figure 2. Frequency of inspections each female of the harvestman *Quindina limbata* was recorded close (less than 20 cm) from a given nest divided by the total number of inspections performed in that nest (proximity index). Resident females are those recorded close to a nest in more than 30% of the inspections (proximity index ≥ 0.3), whereas wanderers are those females recorded close to a nest in less than 30% of inspections (proximity index < 0.3).

Female-female agonistic interactions

We observed 15 female-female agonistic interactions close to (< 20 cm) nests. Usually, one female attacked the other by biting the legs of the opponent with her chelicerae. Other type of female-female interactions consisted of one female entering a nest containing a nest-owner male and another female. The female that entered the nest frequently touched the legs of the opponent with her second pair of legs, and the touched females rapidly left the nest. All agonistic interactions ended up with one female (the winner) remaining close to the nest and the other one (the loser) running away from the nest. Based on our classification of female tactics, nine fights occurred between one resident and one wandering female, one fight occurred between two resident females that were associated to nearby nests, four fights occurred between two visitant females, and in one fight the tactic of one the females could not be assessed because we could not determine which nest she was associated with.

The higher the proximity index, the higher the probability of a female to attack a conspecific female that approached the nest she is associated with (coefficient \pm SE = 5.611 ± 1.369 , $z = 4.099$, $p < 0.001$, Figure 3a). However, we did not find any significant relationship between the proximity index and the probability of a female to be attacked by a conspecific female (coefficient \pm SE = -2.446 ± 2.272 , $z = -1.077$, $p = 0.282$, Figure 3a).

Female promiscuity

The number of nests visited by the females before the focal visit ranged from 0 to 4. We found no significant relationship between the proximity index and the number of nests previously visited by the females (coefficient \pm SE = -1.127 ± 0.814 , $z = -1.384$, $p = 0.166$, Figure 3b).

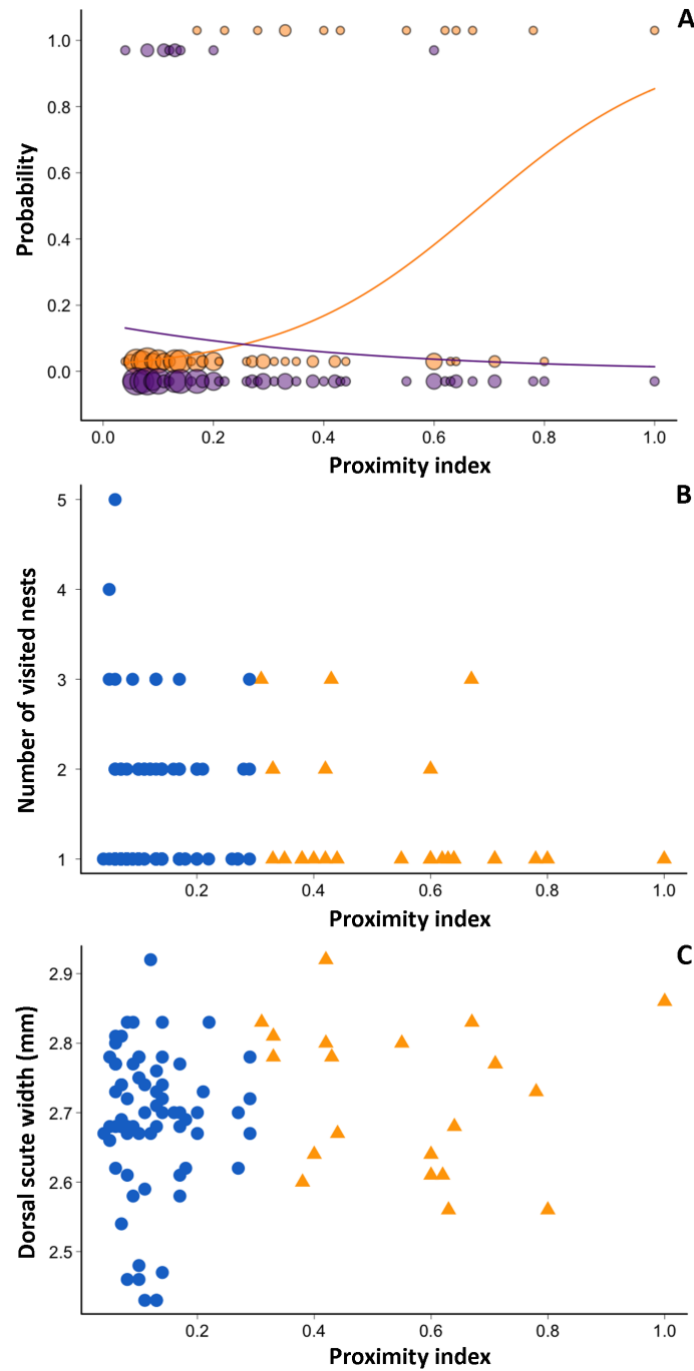


Figure 3. (A) Relationship between the proximity index and the probability of a female of *Quindina limbata* attacking (orange circles) or being attacked (purple circles) by a conspecific female close to (< 20 cm) a nest. The area of the circles represents the number of observations with the same value, the orange line represents the predicted values for attacking females, and purple line represents the predicted values for females that were attacked. (B) Relationship between the proximity index and the number of nests visited by females of *Q. limbata*. (C) Relationship between the proximity index and the size of females of *Q. limbata* and. In (B) and (C), the circles represent wandering females (proximity index < 0.3) and the triangles represent resident females (proximity index \geq 0.3).

Female body size

Female dorsal scute width ranged from 2.43 mm to 2.92 mm (mean \pm SD = 2.69 ± 0.10 mm).

We found no significant relationship between the proximity index and female dorsal scute width (coefficient \pm SE = 0.056 ± 0.053 , $t = 1.057$, $p = 0.294$, Figure 3c).

Male mating opportunities

Each nest-owner male received the visit of 1 to 6 different females during the study period.

The higher the proximity index, the lower was the number of different females that visited a nest-owner male (coefficient \pm SE = -0.806 ± 0.270 , $z = -2.990$, $p = 0.003$, Figure 4a). However, there was no significant relationship between the proximity index and the total number of visits received by a nest-owner male (coefficient \pm SE = -0.169 ± 0.224 , $z = -0.750$, $p = 0.450$, Figure 4b).

Female rejection

We observed a total of 200 nocturnal female visits to the nests. In 64 visits females left the nest by their own after the nest inspection, without mating with the nest-owner male. In 60 visits females mated with the nest-owner males and oviposited in their nest after the inspection. In 27 visits females were clearly attacked by the nest-owner males; in 16 cases the attack occurred before oviposition, in 8 cases the attack occurred after oviposition, and in 3 cases we were not able to define if the attack was before or after oviposition.

We found no significant relationship between female dorsal scute width and her probability of being rejected by a nest-owner male (coefficient \pm SE = 4.640 ± 3.930 , $z = -1.181$, $p = 0.245$, Figure 4c). There was also no significant relationship between the proximity index and the probability of a female being rejected by a nest-owner male (coefficient \pm SE = -0.665

± 1.210 , $z = -0.551$, $p = 0.584$, Figure 4d).

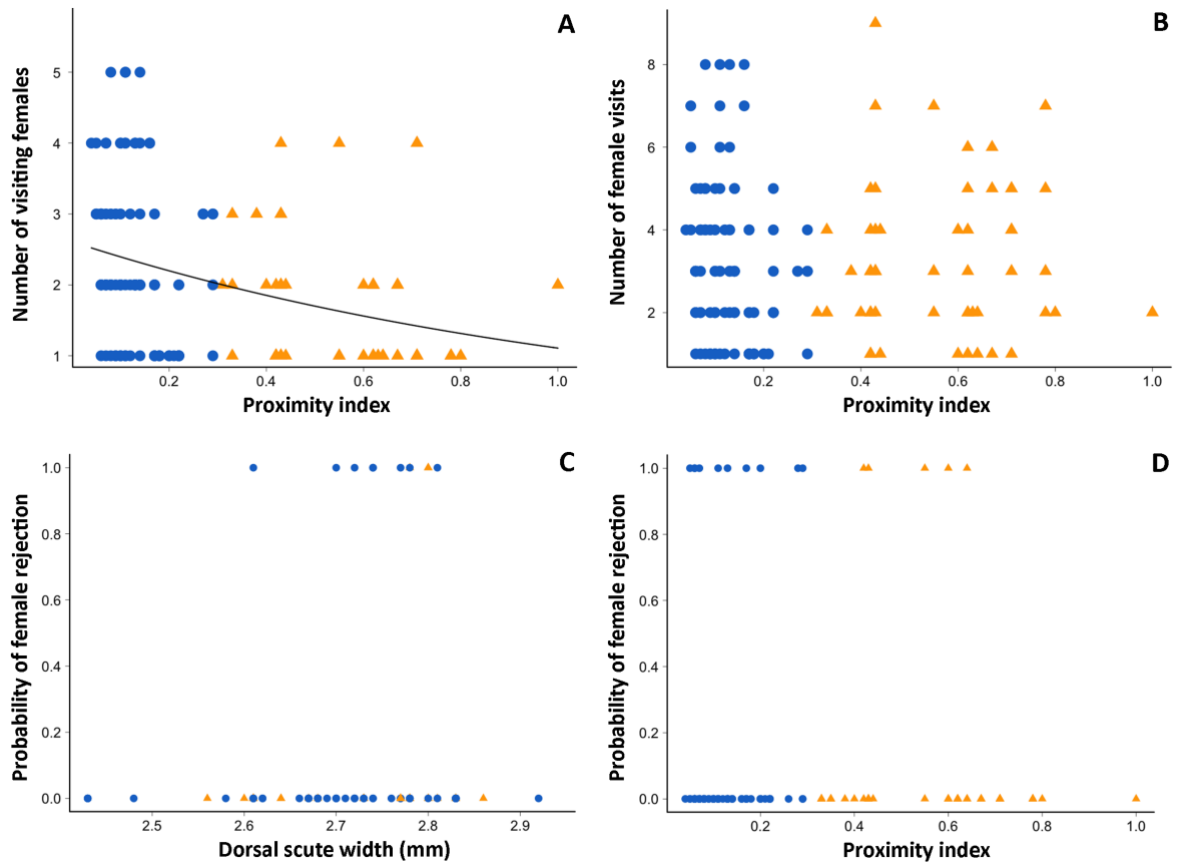


Figure 4. (A-B) Relationship between the proximity index and (A) the number of different females that visited a nest-owner male and (B) the number of female visits received by a nest-owner male of the harvestman *Quindina limbata*. The black line in (A) represents the predicted values estimate by the model. (C) Relationship between female body size (i.e., dorsal scute width) and her probability of being rejected by a nest-owner male of *Q. limbata*. (D) Relationship between the proximity index and the probability of a female being rejected by a nest-owner male of *Q. limbata*. In all graphics, the circles represent wandering females (proximity index < 0.3) and the triangles represent resident females (proximity index ≥ 0.3).

DISCUSSION

Our findings show that the distribution of the proximity index, which refers to the time spent a female in the vicinity of a given nest, shows a unimodal distribution rather than a clear bimodal distribution. However, we found that as the proximity index increases, the probability of females attacking conspecific females that approach the nest with which they

are associated increases. The proximity index did not predict female promiscuity because we found no relationship between the index and the number of nests visited by the females. Moreover, the proximity index also did not related to female size. From the males' perspective, we found that the higher the proximity index of the females associated to a nest, the lower the number of different females that visited this nest. Nonetheless, the total number of visits received by a male (regardless of the females' identity) was not affected by the proximity index of the females associated to his nest. Finally, neither female size nor her proximity index explained the probability of being rejected by a nest-owner male. In following topics, we integrate these results to discuss whether females of the harvestman *Q. limbata* indeed exhibit ARTs and what are the consequences of females' behavior for the males' reproductive success.

Female ARTs

If we define ARTs as alternative ways to obtain fertilizations in both males and females (following Taborsky et al. 2008), a discontinuous distribution of morphological and/or behavioral traits is expected to be found among individuals of the same sex. Among males, in which ARTs are more intensively studied, we can find intrasexual dimorphism in body or weapon size, and discrete variation in behaviors, such as the dichotomy between territorials and sneakers reported for many animal groups (examples in Oliveira et al. 2008). Among females, however, examples of discontinuous distribution of morphological and/or behavioral traits are scarce, and restricted to a few species. In some damselflies, for instance, there is intrasexual female dimorphism in wing pigmentation (e.g., Forbes 1994; Andres et al. 2002). Regarding discrete variation in behavioral traits, the best example occurs among insects, in which some females build nests and provision the offspring, whereas other females behave as social parasites, usurping nests and the food provisioned by conspecifics

(reviewed in Field 1992). In the case of the harvestman *Q. limbata*, the dichotomy between resident and wandering females was not evident and the proximity index did not show a bimodal distribution (Figure 2). Although some females remained close to a nest for several weeks, most of the females remained close to a nest for only a few hours or days. In between these two extremes, we found a great and continuous variation in the values of the proximity index. Therefore, based exclusively on the distribution of this index, we should discard the existence of ARTs in *Q. limbata* females.

The lack of a positive relationship between the proximity index and female body size (Figure 3c) could be an additional evidence that females of *Q. limbata* do not exhibit ARTs. However, although male ARTs are usually coupled with body size differences between individuals exhibiting different tactics, this is not a rule. Among frogs, for instance, some males call to attract females, whereas some satellite males remain silently near vocalizing males to intercept females attracted to the calls. Body size difference between callers and satellites can be found in some species, but these two mating tactics are more frequently associated with body condition: males in good condition behave as callers, whereas males in poor condition behave as satellites. Given that body condition fluctuates over the course of the breeding season, males switch between the two mating tactics in response to how well-fed they are (reviewed in Zamudio and Chan 2008). According to Alonzo (2008), condition-dependent (rather than size-dependent) ARTs are probably common in females, but recognizing discrete variation in female behaviors is more difficult than recognizing the presence or absence of conspicuous courtship displays or fights for territory possession in males. Unfortunately, we did not estimate body condition of *Q. limbata* females, but it is reasonable to suppose that they show variation in how well-fed they are. In the closely related *Q. albomarginalis*, for instance, body condition shows great inter-individual variation in males (Requena and Machado 2015). Moreover, in the harvestman *Iporangaia pustulosa*, the

body condition of egg-tending males decreases over time because they are prevented from foraging while caring (Requena et al. 2012). If the reproductive behavior of *Q. limbata* females is condition-dependent, we can gain some insights on the results reported here.

There is increasing empirical evidence that mate search and assessment are costly to females (Jennions and Petri 1997; Cotton et al. 2006). In the pied flycatcher *Ficedula hypoleuca*, for instance, an increase in mate searching may both reduce the feeding time of females and delay their first breeding event, which ultimately reduces offspring survival (Alatalo et al. 1988; Slagsvold et al. 1988). However, the costs of mate search and assessment should vary in response to female body condition, so that for the same mating tactic, females in poor condition should suffer higher costs than females in good condition (Cotton et al. 2006). Based on this rationale, we argue that *Q. limbata* females with high proximity index are in poor body condition and those with low proximity index are in good body condition. By remaining close to the same nest during long periods, a female may avoid the energy costs related to visiting and inspecting several nests either in the same fallen log or in different fallen logs. Moreover, if the selected nest and/or his owner male provide proper conditions for egg development, this female may gain the benefits of cost-free parental care (Tallamy 2001). Given that most of the eggs of a female with high proximity index are probably laid in a single nest, she should repel conspecific females, which are the most important egg predators in *Quindina* (Mora 1990; Quesada-Hidalgo et al. 2018). Female fights are rare, lasts only a few seconds, and we never observed any kind of injury in the fighting females. Thus, the costs of the fights are probably low and even females in poor body condition should be able to repel conspecific females that approach a nest with which they are associated.

But why should females in good body condition pay the costs of visiting and inspecting several nests? Although nest-owner males are highly effective in preventing egg predation and also fungi infection in their nests (Mora 1990; Quesada-Hidalgo et al. 2018),

they are unable to prevent nest destruction by intense rainfall. In the study population, nearly 50% of the nests are completely destroyed by intense water flow on the fallen log surface (Rojas et al. submitted). Moreover, males may abandon their nests if they are not receiving regular visits from females (Rojas et al. submitted). Therefore, from a female's perspective, to leave their eggs with a great number of different males may be a way of spreading the risk of offspring mortality caused by either nest destruction due to unpredictable storms or male desertion. This bet-hedging strategy has already been reported for several animal groups (reviewed in Seger and Brockmann 1987), including some species with exclusive paternal care. In the pipefish *Syngnathus typhle*, for instance, large females spread their eggs over a larger number of males than small females, and this behavior is interpreted as strategy to avoid the risk of losing all eggs if a slow-moving pregnant male is attacked by a predator (Berglund et al. 1988).

The fact that we did not find a negative relationship between the proximity index and the number of nests visited by females could invalidate the arguments presented above. However, among females with proximity index higher than 0.3 ($N = 27$), only 18.5% visited other nests besides the one with which they were associated (Figure 3b). In turn, 38.6% females with proximity index lower than 0.3 ($N = 89$) visited other nests (Figure 3b). Given that the density of *Q. limbata* nests in some fallen logs may be high, the distance between nests may be less than 20 cm. High nest density provides a possible explanation for the result on the number of visited nests by females. Nest density is an important ecological factor influencing the frequency of extra-pair copulations in birds. For instance, there is empirical evidence that extra-pair copulations are more frequent in colonially-nesting species than among species with dispersed nests, and that nest sites with high nest density have higher rates of extra-pair copulations than sites with low nest density in the same population (reviewed in Griffith et al. 2002). Thus, assuming that all *Q. limbata* females benefit from spreading their

eggs in different nests, some females with high proximity index may still visit nearby nests when nest density is high. In this case, the costs of long displacements are reduced and the risk of losing all eggs laid in a single nest is attenuated. In future studies, therefore, it is important to build a sexual network in which the distance between the nests visited by each female is taken into account. A similar approach has been used to study male ARTs in the harvestman *Serracutisoma proximum*, and the results show that sneakers concentrate their invasions on harems that are spatially aggregated (Muniz et al. 2015).

Sexual conflict

One mechanism that may favor the evolution of female ARTs is the conflict between the sexes over mating (Alonzo 2008). In some damselfly species, for instance, there are two female morphs in the same population: an androchrome morph, with wing pigmentation similar to conspecific males, and the gynochrome morph, with the typical female wing pigmentation. The best hypothesis to explain the evolution of these morphs postulates that some females mimic the wing pigmentation of males to avoid sexual harassment (reviewed in Svensson et al. 2009). However, there is empirical evidence showing that androchrome females mate less often than gynochromes, and that some androchrome females are unable to mate at all (Cordero et al. 1998). Thus, sexual harassment by males and the evolution of wing dimorphism in females may have important consequences for females' reproductive success. The consequences of female ARTs on males' reproductive success, though, are far less studied. The harvestman *Q. limbata* offers a unique opportunity to investigate this question because some females seem to monopolize the access to the nest-owner males, preventing them to receive visits from other females. Thus, the presence of a female close to a nest for a long time may decrease the number of mating partners of nest-owner males.

According to our results, there was a negative relationship between the proximity

index and the number of different females that visited a nest-owner male (Figure 4a). However, there was no relationship between the proximity index and the number of visits (regardless of females' identity) received by a nest-owner male (Figure 4b). Taken together these two findings indicate that the constant presence of a female in the vicinity of a nest repelling conspecific females indeed reduces the promiscuity of the nest-owner males, but the mating rate these males is not reduced. Does it mean that males' reproductive success is not negatively affected by the constant presence of a female in the vicinity of their nests? It is not possible to answer this question because we do not have information on the number of eggs laid by individual females. If females with high proximity index are in fact in poor body condition, we would expect that their oviposition rate would be lower than females with low proximity index, which would be in good body condition (reviewed in Slansky and Scriber 1985). Thus, from a male's perspective, the total number of eggs he receives would be lower if his nest is monopolized by a female. Males in nests that are not monopolized by a female would have a similar mating rate, but would receive more eggs because the visiting females would be more fecund. According to this scenario, nest monopolization by females could reduce males' reproductive success and it would be a source of sexual conflict.

Besides the total number of eggs laid by the females, other important information to estimate males' reproductive success is the paternity of the eggs they receive in their nests. Harvestman females have multiple spermathecae that store sperm from previous copulations, which may increase paternity uncertainty (Macías-Ordóñez et al. 2010). Given that females with high proximity index (> 0.3) seems to be less promiscuous than females with low proximity index (< 0.3), they may represent a lower sperm competition risk for nest-owner males. Thus, the mean number of eggs sired by males in nests monopolized by a female would be higher than in nests not monopolized by a female. Moreover, once females with high proximity index probably lay most of their eggs in the nest with which they are

associated, they should be less prone to cannibalize eggs during nest visits. Females with low proximity index, in turn, probably spread their eggs in more nests and should be more willing to cannibalize eggs. In fact, in a field experiment conducted with *Q. limbata* females never cannibalized eggs in the nests with which they were associated, even after the nest-owner males were removed. In turn, females that were not associated with any nest promptly cannibalized eggs from unattended nests, and were the main source of egg predation when the nest-owner males were removed (Quesada-Hidalgo et al. 2018). According to this scenario, therefore, nest monopolization by females could increase males' reproductive success by decreasing both paternity loss and the risk of egg cannibalism.

Although nest monopolization by a female might have both costs and benefits for the nest-owner males, the proximity index of the females did not influence their probability of being rejected by the males (Figures 4d). Female body size, which is proxy of female fecundity in many arthropods (Bonduriansky, R. 2001), also did not influence the probability of rejection by the males (Figures 4c). In general, female rejection was relatively rare and occurred in only 8% of the visits we recorded during the study period. According to our behavioral observations, it seems that female rejection occurs in two situations: (1) when a female takes a long time to start laying eggs after copulation, and (2) when a female starts scrapping the nest floor after laying an egg (R. Quesada-Hidalgo unpublished data). Because males are constantly touching the venter of the female with their second pair of sensorial legs, they may be able to perceive if the ovipositor is everted and if the female is indeed laying eggs. In the harvestmen *Magnispina neptunus* and *Heteropachylus inexpectabilis*, males also touch the venter of the mates with their second pair of legs and if the female does not lay eggs after copulation or if she tries to cannibalize eggs she is expelled from the nest (Nazareth and Machado 2009, 2010). Thus, female rejection in *Q. limbata* may be a form of egg protection in which males repel females that represent an imminent risk of egg predation

(Quesada-Hidalgo et al. 2018).

Concluding remarks

In conclusion, we suggest that the two previously reported reproductive behaviors of *Q. limbata* females, namely resident and wanderer, are reversible tactics (sensu Brockmann 2001). It means that females can go back and forth between these two tactics and, although females cannot express both tactics simultaneously, they probably adopt both during their lifetime. This flexibility allows females to track changes in intrinsic or extrinsic conditions and switch their reproductive tactics adaptively. As reported for males of some fish species (reviewed in Taborsky 1994), the switch between female tactics may occur in response to body condition. Thus, there should be an individual threshold in body condition that determines whether a female will behave as a resident or as a wanderer. The great variation in the proximity index reported here may be viewed as the result of great inter-individual variation in the threshold determining the body condition that needs to be achieved for the wanderer tactic to be expressed (Tomkins and Hazel 2007). Such inter-individual variation in the threshold suggests that selection on this trait is probably weak, i.e. the fitness costs paid by a female that adopts a reproductive tactic incompatible with her body condition is low. If our interpretation is correct, this is the first case of female ARTs in which one the tactics is based on male monopolization. As we showed here, male monopolization negatively affects the degree of male promiscuity, but not male's mating rate. Although the social monogamy imposed by resident females may have both positive and negative effects for males' reproductive success, this subject deserves further investigation that takes into account the total number of eggs received and sired by the nest-owner males.

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Conclusão geral

— Oi, que bom ver você aqui. Parece que você realmente se interessou pelo meu trabalho.

— Olha, eu achei muito legal mesmo.

— Você estava me contando sobre suas aventuras lá no mato da Costa Rica. Fiquei curiosa para saber o que você descobriu sobre os machos que fazem ninhos e sobre as fêmeas ciumentas.

— Tá bom. Por onde eu começo?

— Antes de você começar, eu tenho uma curiosidade: como você fazia para reconhecer os bichos? Essa galera não é toda igual?

— Eu pintava os opiliões.

— Como assim? Com o quê?

— Com esmalte de unhas ou marcador a base de óleo. Olha só como eles ficam:



— Gente, que fofo! Mas isso não mata eles?

— Então, até onde sabemos afeta muito pouco o comportamento deles. Eles continuam se alimentando, andando, copulando e tal.

— Nossa, legal. Bom, mas vamos lá, me conta o que você achou?

— Vamos começar do começo. No primeiro capítulo, eu tinha três perguntas: (1) Quão eficientes são os machos de *Quindina limbata* em cuidar dos ovos? (2) As fêmeas residentes cuidam dos ovos quando os machos estão ausentes? e (3) Os machos que adotam ninhinhos cuidam tão bem dos ovos quanto os pais originais?

— Isso mesmo.

— Para responder essas perguntas a gente fez um experimento simples: em alguns ninhinhos a gente tirou os machos e em outros ninhinhos a gente deixou os machos. Depois, a gente ficou observando os ninhinhos por alguns dias e contou o número de predadores que visitaram os ninhinhos com e sem machos. A primeira coisa que descobrimos é que a presença dos machos é crucial para a sobrevivência dos ovos. Os ninhinhos sem machos foram três vezes mais visitados por predadores do que os ninhinhos com machos.

— E quem são os predadores dos ovos do seu opilião?

— O principal predador de ovos são as fêmeas da própria espécie, acredita? Elas são canibais

e podem entrar nos ninhos para comer ovos e não para copular com os machos donos.

— Nossa! Mais um comportamento bizarro na novela desse opilião.

— O fato das fêmeas serem canibais pode nos ajudar a entender por que alguns machos atacam as fêmeas e as expulsam do ninho antes mesmo delas colocarem ovos.

— Como assim?

— Os ataques podem estar relacionados à possibilidade das fêmeas quererem só comer ovos do ninho e não colocar novos ovos. Nossa hipótese é que os machos, de alguma forma, percebem a intenção das fêmeas.

— Mas as fêmeas não comeriam os ovos que elas mesmas já colocaram dentro do ninho, né? Por exemplo, as fêmeas residentes não deveriam comer os ovos dos ninhos que elas protegem. Estou viajando?

— Não, claro que não. A gente também esperava que as residentes não canibalizassem ovos dos seus próprios ninhos.

— Falando nisso, as fêmeas residentes cuidaram dos ovos na ausência dos machos?

— Por mais incrível que possa parecer, a resposta é não... Só duas fêmeas adotaram ninhos e elas não eram residentes. De qualquer forma, em não vimos nenhuma dessas duas fêmeas defendendo ativamente os ovos contra predadores. Na verdade, depois que tiramos os machos dos ninhos, a maioria das fêmeas residentes foi embora. Portanto, as fêmeas de *Quindina limbata* não fazem compensação flexível do cuidado parental.

— E vocês têm alguma ideia de por que isso acontece em outros bichos, mas não nessa espécie de opilião?

— Bom, provavelmente porque não compensa para as fêmeas. Talvez os custos de defender um ninho sem macho sejam muito altos ou talvez seja melhor para as fêmeas procurar outro ninho e se associar com um novo macho que vai cuidar dos ovos delas.

— Entendi.

— O legal de fazer pesquisa é que para cada resposta que você consegue surgem várias

outras perguntas. Se alguém quiser, pode ficar estudando a mesma espécie a vida inteira e vai ter sempre coisas novas para descobrir.

— É, mas precisa ser curioso...

— Com certeza!

— E por falar em curiosidade, algum macho adotou um ninho e ficou mais *sexy*?

— Sim! Vários machos adotaram ninhos e a mais da metade deles já estava recebendo visitas de fêmeas no seguinte dia após da adoção.

— Uau! Então essa história de que o cuidado paternal exclusivo é *sexy* funciona mesmo.

— Parece que sim. Na real, essa é uma das descobertas mais importantes do primeiro capítulo da minha tese. O fato dos machos adotarem ninhoss e, logo na sequência, já conseguirem parceiras indica que o cuidado paternal exclusivo em *Quindina limbata* evoluiu tanto por seleção natural quanto por seleção sexual.

— Calma aí! Lembre-se que eu não sou bióloga!

— É simples: a seleção natural tem a ver com aumento das chances de sobrevivência e a seleção sexual tem a ver com o aumento das chances de acasalamento. No caso do opilião que eu estudei, cuidar da prole aumenta tanto a chance de sobrevivência dos ovos quanto a chance de acasalamento dos machos.

— Gente, que legal! Como que isso tudo pode acontecer num bicho tão pequeno?

— Uma das coisas mais legais da biologia é que a gente sempre pode aprender muita coisa com qualquer espécie. Não importa se ela é pequena, como o opilião que eu estudei, ou se é gigantesca, como uma baleia.

— É incrível mesmo. Sabe que eu fico pensando? Será que se os homens soubessem que eles ficam mais *sexy* quando cuidam das crianças eles acabariam cuidando mais?

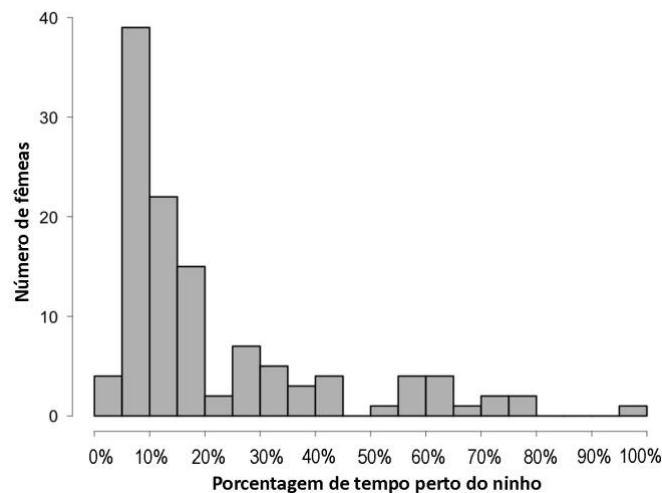
— A gente tem que falar para eles que agora isso está "cientificamente comprovado"!

— Bom, mas tem uma coisa que eu ainda não entendi direito. Por que as fêmeas residentes ficam associadas a alguns ninhoss?

— Então, o segundo capítulo é aquele sobre as táticas alternativas das fêmeas, lembra?

— Lembro, claro. E o primeiro passo era fazer os gráficos sobre o comportamento das fêmeas para ver se existem de fato duas táticas ou se era mais um "dégradé" de comportamentos.

— Exatamente. Fizemos um gráfico do tempo que as fêmeas passam perto dos ninhoss. Vou te mostrar e você me diz o quê você acha? Se a cara dele é mais contínua ou dá para ver duas categorias bem marcadas:



— Bom, para mim parece um contínuo...

— Isso, nós também achamos. Mas as fêmeas que ficam por mais tempo perto dos ninhos têm maior probabilidade de atacar outras fêmeas do que aquelas que ficam pouco tempo perto dos ninhos.

— Bom, para mim isso parece um indicativo de que existem umas fêmeas que atacam mais do que outras. E como são elas que ficam perto dos ninhos, as mais agressivas seriam as residentes, né?

— Foi assim que interpretamos os resultados. Aliás, tem outro detalhe que reforça essa interpretação: a maioria dos ataques que observamos foram de uma fêmea residente contra uma fêmea visitante.

— E as outras características das fêmeas que vocês mediram?

— Bom, primeiro, vou chamar de "proximidade" o tempo que as fêmeas foram observadas perto de um determinado ninho. Isso vai facilitar nossa conversa daqui para frente, tá?

— Tá bom. Esse jargão eu entendo.

— Pois bem, quanto ao tamanho, que é uma característica que geralmente difere entre as táticas alternativas de acasalamento nos machos, não achamos nenhuma relação entre a proximidade e o tamanho das fêmeas. Ou seja, as fêmeas que passam mais tempo perto de um ninho não são nem maiores nem menores do que as fêmeas que visitam muitos ninhos.

— Entendi. Então isso quer dizer que, na real, não existem táticas alternativas de acasalamento nas fêmeas do opilião que você estudou?

— Bom, as diferenças nas táticas não necessariamente envolvem diferenças de tamanho. Existem várias espécies de sapo em que alguns machos não cantam e ficam perto de machos que cantam para interceptar as fêmeas que são atraídas. A gente chama esses machos parasitas de satélites. Aparentemente o que determina se um macho vai se comportar como cantor ou satélite depende do quão bem alimentado ele está. Machos bem alimentados cantam enquanto machos famintos agem como satélites. Se a gente medir o tamanho de cantores e satélites, não vamos encontrar nenhuma diferença. Nesse caso, as táticas dos machos diferem apenas em relação à forma como os machos conseguem parceiras.

— E você acha que acontece alguma coisa parecida com as fêmeas do seu opilião?

— Pode ser. Mas infelizmente não medimos o quão bem alimentadas as fêmeas estavam.

— Nunca imaginei que fosse possível medir isso!

— Possível é, mas não é muito fácil, pois o opilião que estudei é muito pequeno e é muito difícil ter uma balança super-precisa que eu possa levar para o meio de uma floresta tropical.

— Imagino... E em relação às outras características do comportamento das fêmeas, o quê que vocês acharam?

— Bom, lembra que contamos o número de ninhos que as fêmeas tinham visitado? Esperávamos que as fêmeas residentes, ou pelo menos aquelas que ficam muito tempo perto de um ninho, visitassem menos ninhos e, portanto, fossem menos promíscuas do que as visitantes.

— Isso mesmo.

— Por mais estranho que possa parecer, não achamos relação entre a proximidade e promiscuidade das fêmeas. Parece que mesmo as fêmeas residentes saem de vez em quando para copular com outros machos. Mas não fique pensando que esse comportamento é exclusividade de *Quindina limbata*. Tá cheio de aves monogâmicas em que as fêmeas traem

seus parceiros com machos vizinhos!

— Você quer dizer que a traição é regra na natureza?

— Eu não diria isso. Mas definitivamente ela é bem comum. Tanto pelos machos quanto pelas fêmeas.

— Que coisa horrível!

— Amiga, o fato de algum comportamento ser comum entre os animais não quer dizer que ele seja moralmente aceitável na nossa espécie. Na natureza ocorrem comportamentos terríveis, como canibalismo e estupro. Os biólogos têm boas explicações de como esses comportamentos evoluíram, mas isso não quer dizer que a gente deva vê-los como algo justificável nos humanos. É por isso que a gente precisa ter muito cuidado quando nos perguntam que lições podemos tirar da natureza.

— Mmm, entendi. Nunca havia parado para pensar nessas questões...

— Quer saber o que acontece com os machos cujos ninhos têm uma fêmea "residente"?

— Quero! É aquele conflito todo entre machos e fêmeas que você havia me falado antes?

— Sim, e encontramos um resultado muito legal. Existe uma relação entre a proximidade das fêmeas e o número de fêmeas diferentes que visitam o ninho de um macho. Parece, que como as fêmeas residentes repelem outras fêmeas visitantes, os machos copulam com menos parceiras.

— Ou seja, as residentes ciumentas mantêm os machos no cabresto!

— Mas presta atenção nisso: não existe relação entre a proximidade das fêmeas e o número de vezes que um macho copula.

— Peraí, me explica isso com palavras mais simples.

— Ok. Na prática, nossos resultados indicam que em ninhos que têm fêmeas nas proximidades, ou seja, ninhos COM fêmeas residentes, os machos copulam tanto quanto em ninhos que não têm fêmeas nas proximidades, ou seja, ninhos SEM fêmeas residentes.

— Ah, agora entendi. No final das contas, todos os machos copulam a mesma quantidade de

vezes e ficam todos felizes. Então, aquele conflito entre machos e fêmeas acaba não existindo.

— Bom, não é assim tão simples... O conflito pode existir de formas mais sutis. Por exemplo, os machos de *Quindina limbata* certamente não são todos iguais: alguns devem construir ninhos melhores e cuidar melhor dos ovos do que outros. Portanto, as fêmeas que monopolizam esses machos devem ter vantagens sobre outras fêmeas. O mesmo deve acontecer com as fêmeas: elas seguramente diferem em qualidade de forma que algumas colocam mais ovos do que outras. Se uma fêmea residente tiver baixa fecundidade, o macho que é monopolizado por ela pode ganhar menos ovos do que um macho que não é monopolizado por nenhuma fêmea.

— E isso tudo afeta o conflito entre machos e fêmeas. Saquei!

— Mesmo que os machos com e sem residentes tenham uma quantidade similar de cópulas, o número total de ovos que eles recebem deve depender também da qualidade (ou fecundidade) das fêmeas com quem eles copulam.

— E os machos poderiam não copular com as fêmeas de pior qualidade?

— Bom, como eu disse antes, os machos podem atacar algumas fêmeas que visitam seus ninhos. Isso quer dizer que os machos também escolhem suas parceiras. Isso é pouco comum, porque geralmente na natureza são as fêmeas que escolhem os machos.

— E vocês sabem se eles rejeitam as fêmeas de pior qualidade?

— Nós testamos se a rejeição dependia do tamanho das fêmeas ou da proximidade delas aos ninhos, mas não achamos nenhuma relação. As rejeições são raras e precisamos fazer mais observações para entender o que está acontecendo.

— Gente, parece que nesses bichos é tudo ao contrário. Os machos que cuidam dos filhotes e não as fêmeas, as fêmeas que brigam para monopolizar os machos, são elas que parecem ter táticas alternativas de acasalamento, e são os machos que escolhem com quem se acasalar e não as fêmeas.

— Sim. O opilião que eu estudei no doutorado tem o que chamamos de reversão de papéis

sexuais. Porém, a teoria por trás dessa reversão é muito pouco explorada e ainda não sabemos bem definir certinho o que essa reversão significa. Para alguns investigadores só o fato dos machos cuidarem dos filhotes já é uma reversão. Porém, sabemos que existem casos em que não há cuidado paternal, mas mesmo assim são os machos que escolhem suas parceiras.

— E o quê que você acha?

— Eu acho que são comportamentos que, de fato, precisam de mais pesquisa. No opilião que eu estudei, parece que existe uma escolha mútua de parceiros, um conceito que está ficando cada vez mais comum entre os ecólogos comportamentais. Seguramente existem componentes de reversão de papéis sexuais em *Quindina limbata* que precisam ser mais explorados. Espero que pesquisas como a minha ajudem a entender melhor comportamentos como táticas alternativas de acasalamento em fêmeas e rejeição de fêmeas por machos.

— Gente, adorei essa história toda sobre os opiliões. Obrigada por ter me contado. Em geral, as pessoas não têm paciência para explicar de maneira acessível o que elas fazem.

— É que eu gosto muito de divulgação científica. Tenho certeza de que quero tornar o conhecimento científico acessível para todo o público.

— Que legal. Me passa seu contato. Caso eu ache um opilião, posso te mandar a foto.

— Claro! Procura a minha página nas redes sociais, chama *Opilio tracker*. É essa daqui:



— Lá você vai encontrar um montão de informações sobre opiliões e também sobre a minha pesquisa.

— Massa!

