

RESUMO

O cuidado paternal exclusivo à prole é a forma mais rara de investimento parental pós-zigótico na natureza, tendo evoluído independentemente em não mais do que 14 linhagens de artrópodes. Embora muitas dessas espécies sejam facilmente observadas e manipuladas, apenas recentemente os pesquisadores começaram a prestar atenção e a testar hipóteses sobre investimento parental e evolução de papéis sexuais nesses interessantes sistemas biológicos. O principal objetivo desta tese foi investigar características individuais e populacionais que poderiam afetar o sucesso reprodutivo de machos de opiliões (Arachnida: Opiliones) com cuidado paternal. No primeiro capítulo, fazemos uma revisão teórica detalhada, desde trabalhos clássicos de papéis sexuais até os mais recentes avanços, abordando também de maneira sistemática o conhecimento atual acerca de custos e benefícios do cuidado paternal em artrópodes, assim como o papel da seleção sexual sobre a evolução do comportamento parental e dos papéis sexuais. Em seguida, nos capítulos 2 e 3, acessamos os custos energéticos e em termos de sobrevivência do cuidado paternal nos opiliões *Iporangaia pustulosa* e *Zygopachylus albomarginis*. Comparando essas duas espécies, que apresentam diferentes níveis de investimento paternal, testamos previsões direcionadas de que os machos que investem mais na prole deveriam pagar maiores custos do cuidado. No quarto capítulo, por meio de informações da história natural e parâmetros populacionais estimados para *I. pustulosa*, fazemos previsões sobre qual dos sexos deveria ser criterioso ao escolher parceiros e as testamos utilizando observações comportamentais de interações entre machos e fêmeas. Por fim, no capítulo 5, avaliamos a importância relativa do sítio de oviposição, assim como do tamanho e do estado parental dos machos sobre a sua atratividade. Ao final, integramos os resultados obtidos em um corpo teórico sobre a inter-relação entre aparentes demandas conflitantes entre esforço parental e esforço reprodutivo, cuidado paternal e papéis sexuais, abrangendo não apenas as espécies estudadas, mas com um escopo bem mais amplo.

Palavras-chave: Custos do cuidado; Esforço parental; Esforço reprodutivo; *Iporangaia pustulosa*; Seleção sexual; *Zygopachylus albomarginis*.

ABSTRACT

Exclusive paternal care is the rarest form of post-zygotic parental investment in nature, which has independently evolved in no more than 14 arthropod lineages. Although many of those species are easily observed and manipulated, only recently researchers have started to pay attention and test hypotheses on parental investment and evolution of sex roles in such interesting biological systems. The main goal of this thesis was investigating individual and population characteristics that affect the reproductive success of males of harvestmen (Arachnida: Opiliones) with paternal care. In the first chapter, we revise the theory in details, since seminal works on sex roles until the most recent advances in the area, systematically approaching the current knowledge about costs and benefits of paternal care in arthropods, as well as the role of sexual selection on the evolution of parental behavior and sex roles. Then, in chapters 2 and 3, we assess energetic and survival costs of paternal care in the harvestmen *Iporangaia pustulosa* and *Zygopachylus albomarginis*. Comparing these two species, that show different levels of parental investment, we test directional predictions that males which invest more should also pay more intense costs related to care. In the fourth chapter, based on natural history information and population parameters for *I. pustulosa*, we predict which sex should be choosier when selecting sexual partners, and test such predictions using behavioral data on male-female interactions. Finally, in chapter 5, we evaluate the relative importance of oviposition site, males' body size and males' parental state on their attractiveness. At the end, we integrate all the results obtained during the five chapters into a theoretical framework about the interdependence among trade-offs between parental and mating efforts, paternal care and sex roles, not only regarding the pair of species studied, but in a broad way.

Key-words: Costs of care; *Iporangaia pustulosa*; Parental effort, Reproductive effort, Sexual selection; *Zygopachylus albomarginis*.

APRESENTAÇÃO GERAL

O comportamento de cuidado paternal exclusivo à prole é provavelmente a forma mais rara de investimento parental pós-zigótico na natureza. A maioria dos casos ocorre entre anfíbios e peixes (revisões em Wells, 2007; Coleman & Jones, 2011), porém o cuidado paternal está presente também em algumas poucas espécies de poliquetos, artrópodes e aves (revisões em Ridley, 1978; Tallamy, 2001, Owens, 2002). Independentemente do grupo, o que sempre chamou a atenção dos pesquisadores nos casos de cuidado paternal é suposta discrepância em relação a um padrão generalizado na natureza, segundo o qual os machos investiriam principalmente em estratégias competitivas para garantir seu acesso a parceiras e pouco ou quase nada em cuidado à prole. As primeiras explicações teóricas para a evolução do cuidado paternal surgiram no final da década de 60, com um período de efervescência de idéias ao longo da década de 70 (Williams, 1966; Trivers, 1972; Maynard-Smith, 1977). As teorias sobre investimento parental e papéis sexuais lançadas nessa época moldaram nosso conhecimento durante muitos anos (veja, por exemplo, a sétima edição do livro *Animal Behavior, An Evolutionary Approach*, Alcock, 2001). Nas últimas décadas, entretanto, o conhecimento empírico sobre cuidado paternal, tanto no que se refere à descrição de novos casos como ao conhecimento da biologia reprodutiva dessas espécies, vem crescendo exponencialmente (e.g., Bain & Govedich, 2004; Machado & Macías-Ordóñez, 2007; Barreto & Avise, 2008; 2010; Burris, 2010; 2011; Gilbert et al., 2010; Kudo et al., 2011, Requena et al., 2009; 2010). Consequentemente, os paradigmas teóricos têm sido desafiados e vêm sofrendo modificações no sentido de acomodar relações de inter-dependência entre os papéis sexuais e o investimento parental de machos e fêmeas durante a evolução dos diferentes sistemas de acasalamento (e.g. Bonduriansky,

2001; Manica & Johnstone, 2004; Kokko & Jennions, 2008; Barry & Kokko, 2010; McNamara et al., 2010; Alonzo, 2011).

Foi exatamente neste contexto histórico, cheio de questionamentos sobre teorias clássicas, proposições de novas hipóteses, e integração de seleção sexual e cuidado paternal, que, em meados de 2003, iniciei minha carreira acadêmica, ainda durante o meu curso de graduação em biologia. Embora a parte introdutória de teses de doutoramento seja usualmente dedicada à apresentação de revisões teóricas nas quais os trabalhos a seguir estão embasados, optei por um formato menos ortodoxo por duas razões principais. A primeira é que o capítulo 1 desta tese é dedicado exclusivamente a uma revisão teórica detalhada sobre assunto, no qual apresentamos também todo o conhecimento empírico atual com dados de artrópodes com cuidado paternal. Parecia-me um enorme desperdício de tempo (meu e da banca) traduzir esse capítulo para o português a fim de fazer a apresentação da tese. Para aqueles que vêm na introdução geral em português uma síntese acessível a pessoas que não dominam o idioma inglês, ofereço uma alternativa. Em 2009, publiquei em co-autoria uma revisão em português sobre o tema desta tese que, apesar de ser um pouco menos detalhada, está focada exclusivamente nos opiliões, que são os organismos modelo usados aqui e que compreendem mais da metade das linhagens de artrópodes nas quais o cuidado paternal exclusivo evoluiu independentemente. Esse artigo saiu em um volume especial da revista *Oecologia brasiliensis* dedicado à ecologia comportamental no Brasil (Machado et al., 2009). A segunda razão que me motivou a utilizar este espaço de maneira menos formal foi o desejo de expor de maneira clara e didática a meus leitores os desafios enfrentados durante meu doutorado e que moldaram os rumos de toda pesquisa conduzida durante esta etapa da minha formação. Dessa maneira, apresentarei nos dois próximos tópicos quais eram minhas perguntas no começo

do doutorado, quais foram os motivos de não tê-las respondido e como lidei com os desafios encontrados.

O que o meu doutorado poderia ter sido, mas não foi?

O cuidado paternal sempre foi considerado custoso reprodutivamente para os machos por teoricamente limitar seu acesso a fêmeas, comprometendo o investimento em cópulas futuras devido ao investimento atual em cuidar da prole (Sheldon, 2002). Entretanto, hipóteses mais recentes sobre a evolução do cuidado paternal chamam a atenção para o efeito direto da seleção sexual (Alonzo, 2011), propondo que, em determinadas situações, machos poderiam aumentar sua atratividade para fêmeas cuidando, inclusive, de uma prole não relacionada geneticamente a eles (Tallamy, 2001). A avaliação do sucesso reprodutivo de machos em populações cujas fêmeas copulam com um grande número de parceiros diferentes requer a quantificação das relações de parentesco entre os machos e a prole gerada de maneira acurada. Sequências curtas de nucleotídeos (1 - 6 pares de bases) altamente repetitivas, chamadas de microssatélites, são utilizadas como marcadores moleculares individuais e têm sido empregadas com sucesso na determinação de paternidade de diversos organismos (Freeland, 2005). Dessa maneira, o emprego de ferramentas de biologia molecular se fazia necessário para um entendimento mais claro e um teste formal de hipóteses acerca de estratégias reprodutivas utilizadas tanto por machos quanto por fêmeas em espécies com cuidado paternal exclusivo à prole (Figura 1).

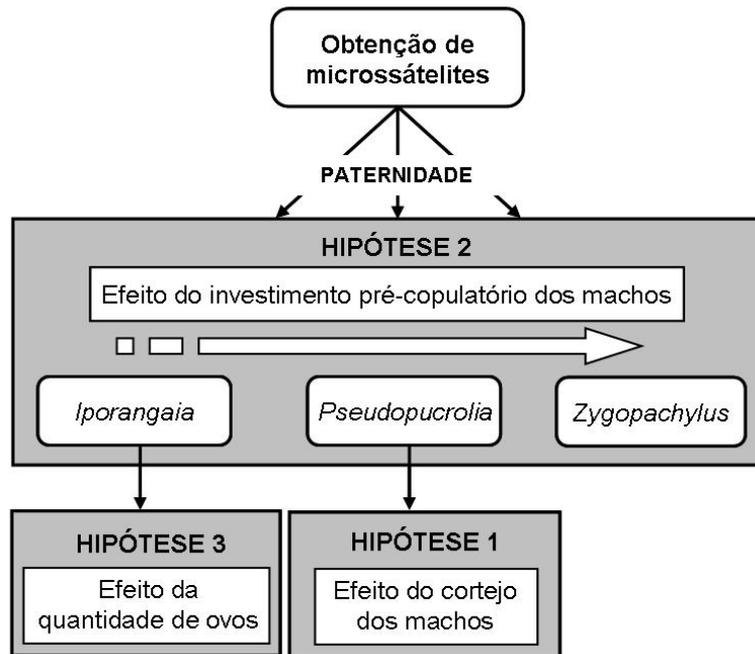


Figura 1. Esquema da estrutura geral do projeto mostrando como todas as hipóteses inicialmente propostas dependiam da obtenção das informações relacionadas à paternidade.

Para um ecólogo comportamental de campo, acostumado a incursões pela Mata Atlântica, às vezes por semanas seguidas sem acesso sequer a um telefone, compreender o dia a dia de coleta de dados em laboratórios de genética parecia o primeiro grande desafio a ser enfrentado. Entretanto, como mencionado anteriormente, apenas com dados robustos sobre a relação de parentesco entre adultos e prole seria possível quantificar o sucesso reprodutivo de machos e associá-los à grande variação nos graus de investimento parental observado entre indivíduos de uma mesma espécie, ou até mesmo em uma comparação inter-específica. Dessa maneira, durante o meu doutorado, investi em me instrumentalizar em técnicas moleculares por meio de disciplinas da pós-graduação e me associando a diferentes grupos de pesquisa especializados em biologia molecular. Neste quesito, estão incluídos os laboratórios do Prof. Dr. Diogo Meyer (Universidade de São Paulo), da Profa. Dr. Anete Pereira de Souza (Universidade Estadual de Campinas), do Dr. Adam G. Jones (Texas A & M University, EUA) e do Dr. Bryan D. Neff (University of Western Ontario, Canadá).

Durante todo esse processo, os conhecimentos e experiências adquiridos nos diversos laboratórios mencionados foram se complementando, pois vivenciei desde procedimentos de construção das bibliotecas genômicas enriquecidas com regiões de microssatélites, passando pela padronização de reações em cadeia de polimerase (PCR) e genotipagem de indivíduos, até a realização de testes de parentesco utilizando programas computacionais específicos para fazê-lo. Apesar da transição de um ecólogo comportamental de campo para um biólogo molecular ter sido absolutamente tranqüila e prazerosa, enfrentei uma série de problemas relacionados à coleta de dados para responder às questões iniciais do meu doutorado. Primeiramente, a quantidade de DNA obtido durante a extração dos ovos dos opiliões era muito baixa, provavelmente devido ao pequeno volume desses ovos e a sua grande quantidade de vitelo. Quando decidi amostrar ovos em avançados estágios de desenvolvimento ou ninfas, obtive uma melhora considerável em relação à quantidade de DNA extraído, porém sua qualidade não parecia razoável. Além disso, as amostras dificilmente eram amplificadas durante a fase de PCR.

Devido à combinação da minha falta de experiência com o fato de ter me associado com laboratórios que tradicionalmente trabalham com amostras de plantas, mamíferos ou peixes, um problema fundamental passou despercebido. Ao amostrar ninfas inteiras, a melanina contida em seus exoesqueletos contaminava as amostras, prejudicando a quantificação do DNA e inibindo as reações de PCR (Giambenardi et al., 1998). Muitas tentativas foram feitas até perceber esse problema e conseguir tratar as amostras com um composto que consegue inativar a melanina, o que aconteceu praticamente no último semestre de meu doutorado. Mesmo assim, para duas das três espécies estudadas eu finalmente consegui amplificar alguns locos de microssatélites. Foi aí então que o último e maior problema apareceu: nos quase 50 adultos amostrados para cada espécie, encontrei

níveis muito baixos de polimorfismo, com apenas dois ou três alelos por loco. A baixa variação genética entre indivíduos, descoberta nos últimos meses do meu doutoramento, impediu por completo que eu conduzisse qualquer análise de parentesco proposta inicialmente e, por esta razão, nenhum dos capítulos aborda questões relacionadas a isso.

O que o meu doutorado realmente foi?

A maioria das espécies de artrópode com cuidado paternal apresenta características comportamentais em comum, como a exibição de comportamentos específicos direcionados à prole que aumentam as chances de sobrevivência dos ovos, assim como o fato dos machos guardiões copularem com diversas fêmeas e cuidarem dos ovos de todas elas simultaneamente (veja revisão no Capítulo 1 desta tese). Entretanto, devido a particularidades da história de vida de cada uma das linhagens nas quais o cuidado paternal evoluiu independentemente, o investimento pré-copulatório dos machos guardiões ocorre de maneira diferenciada entre as diversas espécies. Em algumas espécies os machos constroem e mantêm ninhos (Mora, 1990), em outras os machos defendem sítios específicos de oviposição (Nazareth & Machado, 2010) e, nas formas mais simples de investimento, os machos simplesmente guardam os ovos (Machado et al., 2004; Gilbert et al., 2010, Kudo et al., 2011; Requena et al., 2010). Essa variação observada entre diferentes espécies poderia gerar maior ou menor pressão para os machos guardiões assegurarem a paternidade dos ovos (veja Gross, 2005; Figura 2).

O principal objetivo do plano inicial da minha tese era acessar o grau de parentesco entre machos guardiões e a prole atendida, relacionando esta medida com o nível de investimento parental dos machos (Figura 1). Durante muitos anos foi proposto que a variação na certeza da paternidade deveria explicar as diferenças observadas na

magnitude do cuidado paternal: quanto menores as chances do macho ser o pai de determinada prole, menor seria seu investimento parental (Trivers, 1972; Ridley, 1978; Sheldon, 2002; Figura 2). De fato, essa parece ser a relação que ocorre em espécies cujos machos guardiões são capazes de modular a quantidade de cuidado que oferecem à prole, como é o caso de algumas espécies de peixes (Neff, 2003). Entretanto, em algumas espécies, os machos só conseguem copular se já tiverem investido previamente na construção e/ou manutenção de um sítio reprodutivo apropriado, escolhido pelas fêmeas. Nestes casos, o que eu estava propondo era uma relação inversa entre as variáveis: em espécies cujos machos tivessem um maior investimento reprodutivo, eles deveriam investir mais em mecanismos que assegurassem a paternidade dos ovos (Figura 2) De uma perspectiva teórica mais geral, eu estava propondo uma idéia inovadora, não apenas estabelecendo uma inversão de cause e efeito, mas também um mecanismo para que tal inversão ocorresse (Figura 2).

Para testar minha hipótese, selecionei duas espécies como organismos modelo: (1) *Zygopachylus albomarginis* (Manosbiidae), cujos machos constroem e mantêm ninhos (Mora, 1990) e (2) *Iporangaia pustulosa* (Gonyleptidae: Progonyleptoidellinae), cujos machos não defendem territórios ou recursos e simplesmente guardam ovos postos sob folhas (Machado et al., 2004; Requena et al., 2009). Minha premissa era que essas duas espécies representavam extremos de um gradiente de investimento parental pré-copulatório. Entretanto, durante o desenvolvimento do meu doutorado e simultaneamente às tentativas de obter os dados moleculares de parentesco, fez-se necessário obter quantificações precisas do investimento parental nessas espécies a fim de alicerçar com dados empíricos a premissa central do trabalho. Como fruto do teste dessa premissa, apresento nos Capítulos 2 e 3 os resultados sobre a quantificação dos custos energéticos e

de sobrevivência aos quais os indivíduos parentais das duas espécies estão sujeitos em campo. Vale destacar aqui que dados sobre os benefícios do cuidado paternal já haviam sido avaliados anteriormente para ambas as espécies (Mora, 1990; Requena et al., 2009).

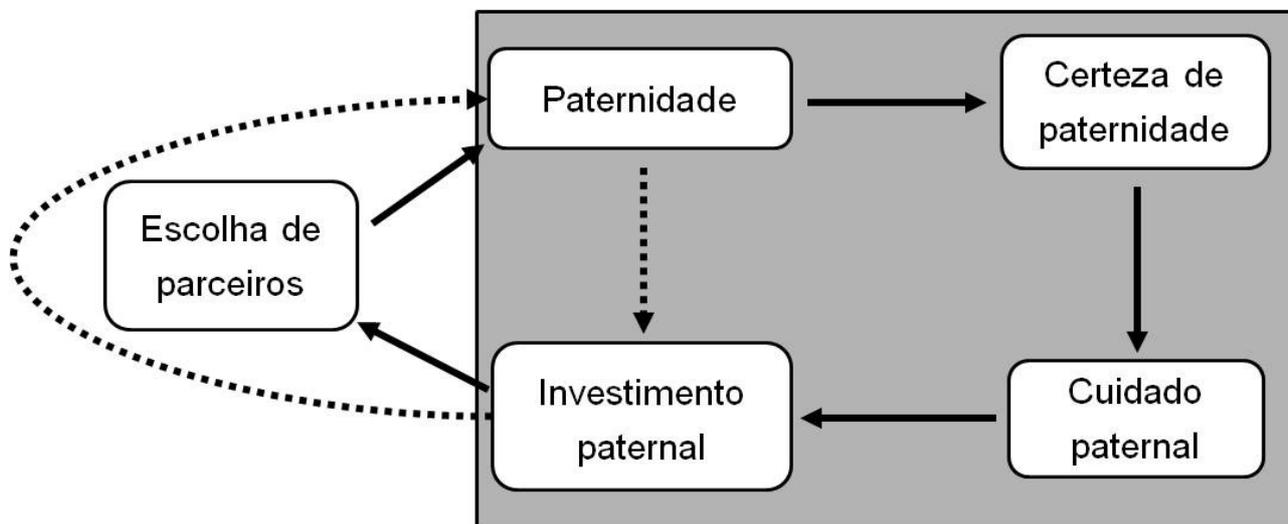


Figura 2. Estrutura teórica relacionando paternidade e investimento paternal. Dentro da caixa cinza está a estrutura clássica (esquema modificado de Sheldon, 2002), no qual paternidade afeta indiretamente (seta tracejada) o investimento parental dos machos por via de mudanças na certeza da paternidade e, conseqüentemente, no esforço parental (setas contínuas indicam efeitos diretos). Dessa maneira, efeitos diretos e indiretos seriam expressos, respectivamente, via respostas comportamentais (em tempo ecológico) ou evolutivas. Minha hipótese inicial estabelecia uma possível rota de retro-alimentação, na qual o investimento dos machos também afetaria a paternidade. Mais do que propor tal rota, eu também lançava mão da via pela qual essa influência ocorreria diretamente: o viés na paternidade seria fruto de processos pós-copulatórios de escolha de parceiros.

Adicionalmente, coletei dados detalhados sobre as interações entre machos e fêmeas em *I. pustulosa* (Capítulo 4). Com essas informações, pude comparar o grau de reversão dos papéis sexuais entre essa espécie e *Z. albomarginis*, para a qual já havia disponível na literatura uma ótima descrição das interações macho-fêmea antes, durante e após a cópula (Mora, 1990). Por fim, avaliei os critérios que determinam o sucesso reprodutivo dos

machos em *I. pustulosa* (Capítulo 5), pois essa informação seria crucial para compreender os resultados obtidos nas análises de paternidade via ligação direta de escolha de parceiros (Figura 2). Apesar dos percalços em laboratório, o trabalho de campo foi extremamente bem sucedido e acabou se transformando em um conjunto coeso de informações comportamentais inéditas que compõem o corpo desta tese. A frustração de não poder responder minha questão inicial foi, sem dúvida, suplantada pelo enorme incremento de conhecimento sobre aspectos centrais da biologia de duas espécies que são organismos modelo para o estudo do investimento parental e dos papéis sexuais. Para além dos resultados apresentados aqui, todo o caminho que percorri durante os mais de 50 meses de doutoramento me deu um sólido ferramental teórico e analítico, ampliou meus horizontes acadêmicos, abrindo portas importantes para colaborações futuras, e consolidou minha formação como pesquisador e futuro orientador.

Apresentação dos capítulos

Minha tese está dividida em cinco capítulos que se conectam por abordarem dois temas inter-relacionados: investimento paternal e papéis sexuais. Como já mencionei anteriormente, duas espécies de opiliões figuram como objeto de estudo ao longo de todo o trabalho: *I. pustulosa* e *Z. albomarginis*. Como para ambas os benefícios do cuidado paternal em termos de proteção da prole já haviam sido investigados, a abordagem empírica sobre o investimento paternal que fiz aqui começa com uma investigação detalhada dos custos do cuidado. Na seqüência, exploro as implicações dos custos e benefícios do cuidado paternal sobre a evolução dos papéis sexuais e sobre os critérios de seleção de parceiros, tanto da perspectiva feminina quanto masculina. A seguir, detalho

especificamente o conteúdo de cada um dos capítulos, que foram sempre redigidos em co-autoria com meu orientador e, eventualmente, com outros colegas.

Capítulo 1: Este capítulo foi aceito como uma contribuição para o livro *Sexual selection: models and perspectives from the Neotropics*, que deve ser lançado no ano que vem pela editora Elsevier. Nele, fazemos uma revisão teórica detalhada dos trabalhos sobre evolução do investimento parental e dos papéis sexuais, desde estudos clássicos da década de 70, nos quais a diferença quanto à produção de gametas entre machos e fêmeas era tida como uma das principais responsáveis pelos padrões de investimento parental atuais, até modelos teóricos mais recentes que incorporam o efeito de parâmetros populacionais e flutuações ao longo do tempo na intensidade de seleção sexual sobre machos e fêmeas. Além disso, apresentamos uma revisão sistemática do conhecimento atual sobre cuidado paternal exclusivo em artrópodes, descrevendo o sistema de acasalamento e a biologia reprodutiva dessas espécies, começando por quantificações dos custos e benefícios do cuidado e chegando no papel da seleção sexual sobre a evolução dos papéis sexuais. Dado que apenas o Capítulo 2 desta tese já foi aceito para publicação, as informações contidas nos outros capítulos não são contempladas nesta revisão.

Capítulo 2: Este capítulo já foi aceito para publicação na revista PLoS One. Nele, acessamos os custos energéticos e de sobrevivência associados ao cuidado paternal no opilião *I. pustulosa*. Primeiramente, quantificamos as atividades de forrageamento de machos guardando desovas, machos não parentais e fêmeas em condições naturais. Além disso, avaliamos como as condições corporais de machos variam de acordo com seu estado parental e também ao longo do período de cuidado. Se o cuidado paternal

impõe restrições alimentares aos machos guardiões, seria esperado que eles comessem menos frequentemente que machos não guardiões. Além disso, seria esperado que a condição física dos machos guardiões decaísse ao longo do período de cuidado. Finalmente, por meio de um estudo populacional de longa duração, estimamos a probabilidade de sobrevivência de machos em diferentes estados parentais e de fêmeas, prevendo que machos parados sobre suas desovas deveriam estar mais expostos a predadores ou parasitóides durante o período de cuidado do que machos não guardiões.

Capítulo 3: Este manuscrito está formatado nos moldes da revista “*Ecological Entomology*”.

Nele, conduzimos um estudo similar ao descrito acima para *I. pustulosa* com o objetivo de acessar os custos do cuidado parental no opilião *Z. albomarginis*. Como a coleta de dados para as duas espécies foi similar, isso nos permitiu fazer uma comparação direta entre elas. Dado que machos de *Z. albomarginis* parecem investir mais na prole, esperávamos que os custos do cuidado paternal nesta espécie fossem maiores do que os custos pagos por machos de *I. pustulosa*.

Capítulo 4: Este manuscrito está formatado nos moldes da revista “*Ethology*”. Baseado em modelos teóricos sobre seletividade frente a parceiros sexuais e utilizando informações da história natural do opilião *I. pustulosa*, assim como parâmetros populacionais estimados no Capítulo 2, fazemos previsões sobre qual dos sexos deveria ser criterioso ao escolher parceiros. Dadas as restrições impostas pelo cuidado paternal aos machos, assim como uma maior liberdade de forrageamento das fêmeas, derivamos uma hipótese específica de que o sistema de acasalamento de *I. pustulosa* gera menores diferenças entre machos e fêmeas e, portanto, indivíduos de ambos os sexos deveriam avaliar seus parceiros em algum nível. Testamos essa hipótese utilizando observações

comportamentais de interações entre machos e fêmeas. Dado que informações equivalentes para *Z. albomarginis* já estão publicadas, fazemos novamente comparações detalhadas entre ambas as espécies.

Capítulo 5: Este manuscrito está formatado nos moldes da revista "*Behavioral Ecology and Sociobiology*". A partir de resultados obtidos nos capítulos prévios, fêmeas de *I. pustulosa* parecem selecionar machos com os quais copular e deixar ovos. Assim, neste último capítulo avaliamos a importância relativa do sítio de oviposição, do tamanho dos machos e do estado parental dos indivíduos tanto na probabilidade de conseguir uma nova desova, quanto no número de ovos que as fêmeas deixam com eles. Além disso, se o estado parental influencia o sucesso reprodutivo dos machos, machos não guardiões poderiam estar dispostos a cuidar de desovas desatendidas como uma estratégia para aumentarem suas chances de cópula. Nesse sentido, conduzimos um experimento em campo no qual removemos machos guardiões de suas desovas e acompanhamos os ovos desatendidos a fim de observar e quantificar o comportamento de machos e fêmeas frente a eles.

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CAPÍTULO 1

Paternal care and sexual selection in arthropods

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(invited chapter in the book "*Sexual Selection: Insights from the Neotropics*" (edited by R.H.F. Macedo and G. Machado))

ABSTRACT

Exclusive paternal care is the rarest form of post-zygotic parental investment in nature. In arthropods, this behavior has independently evolved in 14 lineages, including nearly 1,500 species. Here, we review the theoretical background for the evolution of parental investment and sex roles, contrasting classical views with the most recent theoretical models. Then, we introduce the cases of exclusive paternal care in arthropods, and explore the empirical information focusing on the costs and benefits of male egg-guarding, and the possible role of paternal care for male attractiveness and sex role reversal. Finally, although paternal care is found among arthropod species living worldwide, representatives of nearly 60% of the non-marine species with paternal care occur in tropical regions. Thus, in the last section of this chapter, we analyze the possible effects of temperature and precipitation on the costs and benefits of paternal care and derive macroecological predictions to be tested in future studies.

Key-words: Costs and benefits, Harvestmen, Macroecology, Mating effort, Millipedes, Parental effort, Sea spiders, Sex role reversal, Water bugs.

INTRODUCTION

Exclusive paternal care is probably the rarest form of post-zygotic parental investment in nature. In arthropods, this behavior is known to have independently evolved in no more than 14 lineages, including nearly 1,500 species (Table 1; Figs. 1, 2). This is a tiny fraction of the arthropod diversity, which encompass nearly 1.2 million described species (Hammond et al., 1995). Although many arthropod species exhibiting exclusive paternal care are easily observed and manipulated both in the field and in the laboratory, thus offering unique opportunities to test hypotheses on parental investment and evolution of sex roles, only recently researchers have started to pay attention in such interesting biological systems (Fig. 1B). In fact, there was an exponential increase in the recognition of new and independently evolved cases of paternal in arthropods during the last three decades (Fig. 1A). This accumulation of basic biological information revealed a great diversity of forms that paternal care is expressed, involving specific male morphological traits and behaviors that enhance offspring survival. At the same time, recent advances in the theory of sex roles evolution have incorporated coevolutionary feedbacks between parental investment and sexual selection into the models, challenging classical foundations of behavioral ecology and proposing new hypotheses to be tested (e.g. Manica and Johnstone, 2004; Kokko and Jennions, 2008; McNamara et al., 2010; Alonzo, 2011).

Table 1. Basic biological information and geographic distribution of the arthropod species exhibiting exclusive paternal care. Each line represents a lineage in which male egg-guarding has independently evolved, except for water bugs (Belostomatidae), presented by sub-families separately due to differences in reproductive and parental biology. (?) indicates uncertainty and ? indicates lack of information.

Taxa*	Oviposition site	Multiple clutches	Number of eggs	Brood adoption	Additional investment	Geographic distribution
HEXAPODA: HEMIPTERA						
BELOSTOMATIDAE: Lethocerinae (24 species ^{1,2})	Vegetation ³	Yes ³	ca. 100 ⁴	?	?	Cosmopolitan ²
Belostomatinae (143 species ^{1,2})	Males' dorsum ³	Yes ³	100 – 300 ⁵⁻⁶	Not possible	?	Cosmopolitan ²
PENTATOMIDAE: <i>Edessa nigropunctata</i> ⁷ and <i>Lopadusa augur</i> ⁷	Vegetation ⁷	Yes ⁷	16 – 48 ⁷	?	?	SE Brazil ⁷
REDUVIIDAE: <i>Rhinocoris albopilosus</i> ⁸ , <i>R. albopunctatus</i> ⁹ , and <i>R. tristis</i> ¹⁰	Vegetation ⁸⁻¹⁰	Yes ⁸⁻¹⁰	4 – 40 ⁸⁻¹⁰	Yes ¹¹	Hard eggshell ¹² (?)	Sub-Saharan Africa ⁸⁻¹⁰
DIPLOPODA: PLATYDESMIDA						
ANDROGNATHIDAE: <i>Brachycybe</i> spp. ¹³⁻¹⁶ (8 species ¹⁷) and <i>Yamasinaium noduligerum</i> ¹⁸	Under rotten logs ^{14, 16, 18}	Yes ¹⁶ (?)	10 - 100 ¹⁶	?	?	Japan, Korea, Taiwan, and SE and SW United States ¹⁷
PYCNOGONIDA						
ALL FAMILIES ¹⁹ (ca. 1,100 species ²⁰)	Ovigerous legs ¹⁹	Yes ¹⁹	Dozens to hundreds ¹⁹	Not possible	?	Cosmopolitan (marine) ¹⁹⁻²⁰

ARACHNIDA: OPILIONES						
ASSAMIIDAE: <i>Lepchana spinipalpis</i> ²¹	Under rocks or rotten logs ²¹	Yes ²¹	20 - 130 ²¹	Yes ²¹ (?)	?	Himalayas, Nepal ²¹
COSMETIDAE: <i>Cryptopoecilaema almipater</i> ²²	Vegetation ²²	Yes ²²	20 - 150 ²²	?	Mucus coat ²²	Costa Rica ²²
GONYLEPTIDAE (Gonyleptinae): <i>Gonyleptes saprophilus</i> and <i>Neosadocus</i> sp. ²³	Natural cavities ²³	Yes ²³	8 - 560 ²³	Yes ²³	Eggs covered with debris ²³	SE Brazil ²³
GONYLEPTIDAE (Heteropachylinae): <i>Chavensicola inexpectabilis</i> ²⁴ and <i>Magnispina neptunus</i> ²⁵	Natural cavities ²⁴⁻²⁵	Yes ²⁴⁻²⁵	110 - 160 ²⁴⁻²⁵	Yes ²⁴⁻²⁵	Eggs covered with debris ²⁴⁻²⁵	NE Brazil ²⁴⁻²⁵
GONYLEPTIDAE (Caelopyginae + Progonyleptoidellinae): <i>Ampheres leucopheus</i> ²⁶ , <i>Cadeadoius niger</i> ²⁷ , <i>Iguapeia melanocephala</i> ²³ , <i>Iporangaia pustulosa</i> ²³ , <i>Progonyleptoidellus striatus</i> ²³ , <i>P. orguensis</i> ²⁸	Vegetation ^{23, 26-28}	Yes ^{23, 26}	14 - 420 ^{23, 26, 29}	No ²⁹	Mucus coat ^{23, 26-30}	SE Brazil ^{23, 26-28}
MANAOSBIIDAE: <i>Zygopachylus albomarginis</i> ³¹	Mud-nests ³¹⁻³²	Yes ³¹⁻³²	1 - 100 ³²	No ³²	Eggs covered with debris ³¹⁻³²	Panama ³¹
PODOCTIDAE: <i>Leytpodoctis oviger</i> ²¹ and <i>Ibalonius</i> sp. ²⁸	Males' legs ^{21, 28}	No ^{21, 28} (?)	4 - 17 ^{21, 28}	Not possible	?	Philippines and Melanesia ^{21, 28}
STYGNIDAE: <i>S. flavolimbatus</i> and <i>S. aff. flavolimbatus</i> ³³	Vegetation or under rotten logs ³³	Yes ³³	ca. 100 ³³	?	?	Venezuela ³³
TRIAENONYCHIDAE (Soerensenellinae): <i>Karamea</i> spp. ³⁴⁻³⁵ (6 species ³⁴) and <i>Soerensenella</i> spp. ³⁴⁻³⁵ (10 species ³⁴)	Under rocks or trunks ³⁴⁻³⁵	Yes ³⁴⁻³⁵	ca. 50 - 100 ³⁴⁻³⁵	?	Eggs covered with debris ³⁴⁻³⁵	New Zealand ³⁴⁻³⁵

* We are not considering two arthropod lineages that have been included in the review on paternal care by Tallamy (2001): the leaf-footed bugs of the genera *Scolopocerus* and *Plunentis* (Coreidae), and the thrips of the genera *Hoplothrips*, *Sporothrips*, and *Idolothrips* (Thysanoptera). Although there are records of eggs laid on the body of several species of phytophagous heteropterans, there is no convincing demonstration with decent sample size that only males carry the eggs. On the contrary, there are at least three species in which eggs are laid both on males and females (Panizzi and Santos, 2001), including the well-studied *Phyllomorpha laciniata* (see discussion in Kaitala et al., 2001). In relation to the colonial thrips, the available data indicate that males fiercely defend territories where females lay eggs (Crespi, 1986; 1988). Despite intensive behavioral studies, there is no record of males defending the egg masses against predators. According to the own author of the papers, the mating system is a territory-based mating system with no evidence of parental care (B.J. Crespi, pers. comm.). However, we would like to stress that the inclusion of these two lineages does not change any of our conclusions.

1. Lanzer-de-Souza (1980); 2. Polhemus and Polhemus (2008); 3. Smith (1997); 4. Smith (1979a); 5. Hoffman (1933); 6. Tawfik (1969); 7. Requena et al. (2010); 8. Odhiambo (1959); 9. Nyiira (1970); 10. Thomas (1994); 11. Thomas and Manica (2005); 12. Gilbert et al. (2010); 13. Murakami (1962); 14. Kaestner (1968); 15. Gardner (1974); 16. Kudo et al. (2011); 17. Brewer et al. (2012); 18. Kudo et al. (2009); 19. King (1973); 20. León (1999); 21. Martens (1993); 22. Proud et al. (2011); 23. Machado et al. (2004); 24. Nazareth and Machado (2009); 25. Nazareth and Machado (2010); 26. Hara et al. (2003); 27. Stefanini-Jim (1985); 28. Machado and Macías-Ordóñez (2007); 29. Requena and Machado (unpub. data); 30. Requena et al. (2009); 31. Rodriguez and Guerrero (1976); 32. Mora (1990); 33. Villareal-Manzanilla and Machado (2011); 34. Forster (1954); 35. Machado (2007).

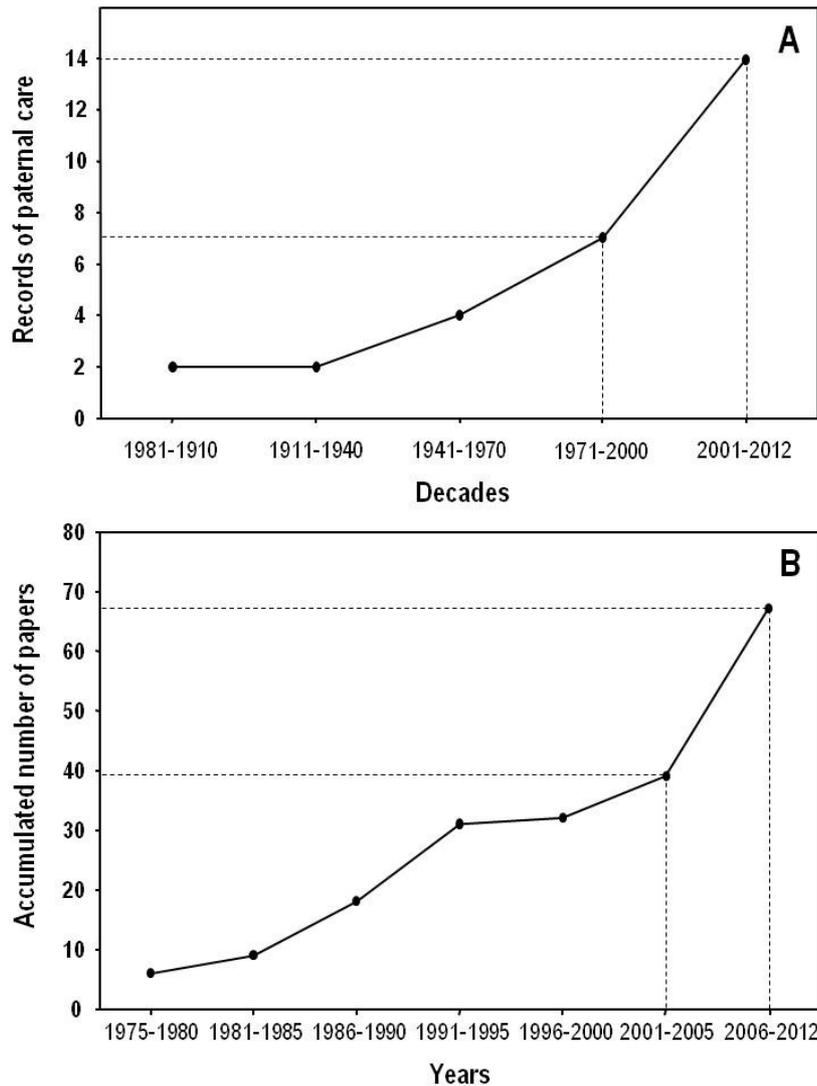


Fig. 1. Increasing in the empirical research on paternal care in arthropods. (A) Records of independently evolved cases of paternal care in arthropods since the first formal description in 1981. Although the most recent time interval is almost three times shorter than the previous ones, we can see that there is an exponential increase in the reports of new records in the last 12 years. Most of these records regard species from the Neotropical region (see Table 1). (B) Accumulated number of empirical papers on paternal care published since 1975. Results were obtained from the Web of Science database using first the key words 'paternal care' or 'male brooding', and then conducting a search on the output for papers containing at least one of the following terms in the title, abstract, or key words: arthropod, insect, Belostomatidae, water bug, Opiliones, harvestman, Diplopoda, millipede, Pycnogonida, or sea spider. Although more than 1,600 papers were found using this procedure, only 67 were selected because the remaining were primarily focused on taxonomy or other aspects not related to behavior. Like the first graphic, there is a clear tendency of exponential increase in the number of empirical papers on paternal care in the last 12 years.

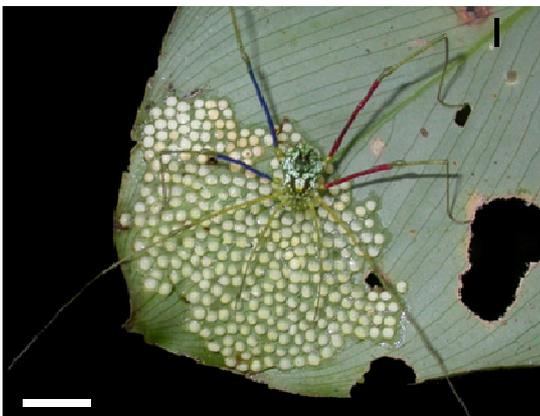
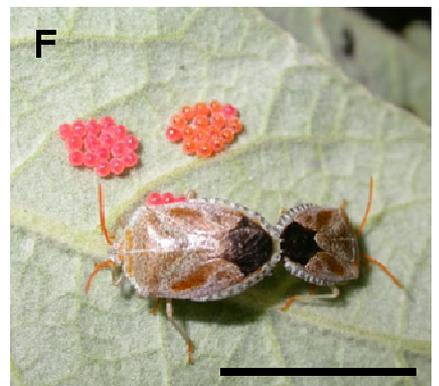
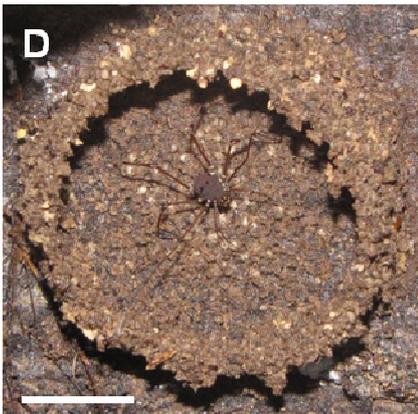
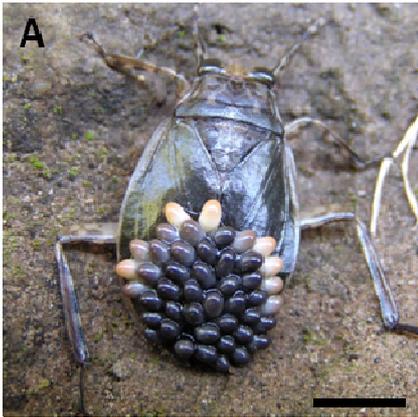


Fig. 2. Arthropod species exhibiting exclusive paternal care. (A) Male of the water bug *Abedus brevices* (Belostomatinae) carrying eggs attached to his back in a small stream in Mexico. Note that eggs differ in color, which indicates that they were laid in two moments either by the same female or by two different females. (B) Male of the harvestman *Leytподoctis oviger* (Podoctidae) carrying large eggs attached on the left fourth leg in a forest from the Philippines (photo: J. Martens). (C) Male of the giant water bug *Lethocerus* sp. (Lethocerinae) guarding a clutch on the emergent vegetation of a lake in the Brazilian Amazon forest (photo: R. Macías-Ordóñez). (D) Male of the harvestman *Zygopachylus albomarginis* (Manaosbiidae) inside his mud nest built on a fallen trunk in Panama. Some white eggs are visible on the nest floor just below the guarding male. (E) Inside view of an artificial nest of the harvestman *Magnispina neptunus* (Gonyleptidae) in the laboratory. The nest is full of eggs, which are also covered by debris by the ovipositing females. (F) Mating pair of the stink bug *Edessa nigropunctata* (Pentatomidae) on the host plant in southeastern Brazil. Under the male (left) there is a recently laid clutch, and close to the mating pair there are two other clutches. (G) Male of the assassin bug *Rhinocoris tristis* (Reduviidae) guarding a multiple clutch on the host plant in Uganda (photo: L.K. Thomas). Note that the clutch is composed of eggs and hatched nymphs, which remain under male protection for a few days. (H) Male of the Japanese millipede *Bachycybe nodulosa* (Andrognathidae) curled around a mass of eggs in the laboratory (photo: S. Kudo). (I) Male of the harvestman *Iporangaia pustulosa* (Gonyleptidae) guarding a multiple clutch on the undersurface of a leaf in southeastern Brazil. Eggs are covered by a mucus coat that confers additional protection to them (see Fig. 3). (J) Male of the harvestman *Cryptopocilaema almipater* (Cosmetidae) guarding eggs in Costa Rica (photo: C. Viquez). Although the oviposition substrate and the presence of mucus around the eggs are very similar to the harvestman *I. pustulosa*, there is no doubt that both features are convergent in these two species. (K) Male of the harvestman *Stenostygnellus* aff. *flavolimbatus* guarding a multiple clutch laid on the petiole of a palm in Venezuela (photo O. Villareal Manzanilla).

In this chapter, we review the theoretical background for the evolution of parental investment and sex roles, contrasting classical views with the most recent criticisms and advances proposed by new theoretical models. In the sequence, we introduce the cases in which males exclusively care for the offspring in arthropods, stressing both particularities and general patterns. We explore the empirical information under the perspective of the modern theoretical models, focusing on the costs and benefits of male egg-guarding, and

the possible role of paternal care for male attractiveness and sex role reversal. Finally, although paternal care is found among arthropod species living worldwide, representatives of nearly 60% of the non-marine species with paternal care occur in tropical regions (Table 1). This pattern may simply reflect the global distribution of arthropod diversity or may indicate that environmental conditions influence the occurrence of paternal care. Therefore, in the last section of this chapter, we analyze the possible effects of temperature and precipitation on the costs and benefits of paternal care and derive specific macroecological predictions to be tested in future studies.

HISTORICAL PERSPECTIVE ON SEX ROLES AND PARENTAL INVESTMENT

Those 70's theories

In 1972, Trivers published his seminal work on the relationship between sexual selection and parental investment, which provided the first theoretical framework for our understanding on sex roles. According to his argument, anisogamy lies at the heart of sexual differences between males and females because gametes are a form of pre-zygotic parental investment. Females, which generally produce large and costly eggs, would make a greater parental investment prior to mating when compared to males, which produce cheap sperm. Indeed, in the great majority of species, male ejaculates represent a smaller total investment per mating than the eggs produced by females. Males, therefore, would be able to replenish their gamete supply and return to the mating pool sooner than females. Consequently, males should intensively compete for the access to females and, assuming that male-male competition prevents any investment in the offspring, males should also not exhibit any sort of parental care. On the other hand, given that females

already invest more than males in gametes, they should be selected to care in order to avoid any breeding failure for lack of additional investment. Therefore, highest past investment in gametes would select females to be choosy in relation to potential partners and to show high future investment in offspring protection. This causal link between anisogamy and parental investment has led to the notion of 'conventional sex roles'. In this sense, sex-role reversal, in which females compete for mates more strongly than males, should only occur when parental investment by males is somehow greater than by females, as observed when males invest in expensive nuptial donations to females or in some cases when they care for the offspring.

Although the association between pre- and post-zygotic parental investment provided a possible explanation for the evolution of sex roles, a central question was still unanswered: why should males care for the offspring at all? Until the late 1970's, the great majority of species exhibiting paternal care occurred among fishes and frogs (see review in Ridley, 1978), leading researchers to propose a causal link between external fertilization, widespread in these two groups, and paternal care. Therefore, the first two explanations for the evolution of exclusive paternal care were based on the mode of fertilization. According to the *certainty of paternity* (Trivers, 1972) and the *order of gamete release* hypotheses (Dawkins and Carlisle, 1976), internal fertilization decreases confidence of paternity in polyandrous species, increasing the costs of parental care for males and promoting a time lag between copulation and oviposition, which allows male desertion and predisposes females towards parental care. In species with external fertilization, on the other hand, females spawn earlier than males, a situation that may increase confidence of paternity, but also puts females in the position to leave males in possession of the zygotes, forcing them into the so called 'cruel bind' (Trivers, 1972). Several empirical

studies, however, have reported the presence of sneaker males close to guarding males in fish, leading to a high proportion of cuckolded broods in nature (review in Coleman and Jones, 2011). Moreover, males from some fish families remain in their nests and take care of the eggs even when they supposedly have the opportunity to desert first, as reported for some teleosts with simultaneous release of gametes (Gross and Shine, 1981) or some catfishes, in which males construct foam nests and release sperm on it before female spawning (Hostache and Mol, 1998).

A third explanation for the evolution of paternal care, known as *association with offspring* or *territoriality* hypothesis, postulates that males would monopolize suitable oviposition sites in an attempt to attract females and acquire mates (Williams, 1975). Males defending a territory would further increase their fitness because they would indirectly defend eggs against conspecific predators. In this case, paternal care does not necessarily decrease the probability of a caring male acquiring additional mating pairs because several females may visit his territory. This hypothesis presupposes that paternal care has evolved under the pressure of natural selection and is ultimately related to males' possession and maintenance of territories or nesting sites. Moreover, this hypothesis predicts that resource defense polygyny mating systems are fundamental to promote the evolution of exclusive paternal care. Even though this hypothesis does not account for the evolution of paternal care in all animal groups, it has been proposed as the primary explanation for two groups in which this behavior is widely distributed, namely fish and anurans (Ridley, 1978; Gross and Shine, 1981; Coleman and Jones, 2011).

In 1977, Maynard-Smith proposed a model to explain sexual differences in post-zygotic parental investment in which individuals of each sex have the option to desert or to care, so that the best tactic adopted for individuals of one sex depends on the tactics

adopted by individuals of the other sex. According to his model, ecological and physiological parameters should determine the costs and benefits of each tactic. The direct benefits of caring were incorporated into the model as the probability of offspring survival when protected by none, one, or two parental individuals. The fecundity costs associated to parental care was also included as females producing fewer eggs when providing care than when deserting. The last parameter is the probability of acquiring an additional mate when males desert, which should depend on the relative proportion of sexually active males to receptive females in the population (OSR). As a result, only a specific combination of parameters is predicted to favor the evolution of exclusive paternal care, i.e., when: (a) offspring survival strongly depends on the presence of at least one parent, but two parents do not significantly increase offspring protection, (b) females have great increase in fecundity if they desert, and (c) males have low probability of acquiring additional mates after desertion. Maynard-Smith's (1977) model was useful to highlight conditions under which each sex is expected to care or to desert, and it was the cornerstone on which subsequent models were built.

Challenging classic theories

During the following decades, Trivers' (1972) theory remained as the most accepted explanation for the evolution of sex roles. However, his original argument that sexual differences in past parental investment condemn females to keep investing in costly post-zygotic parental care hid a logical failure known as the 'Concorde fallacy', i.e., the false impression that past investment in a costly activity makes it more profitable to continue with, rather than abandon it (Dawkins and Carlisle, 1976). Furthermore, in the early 1990's, Clutton-Brock and Vincent (1991) proposed that potential reproductive rates (PRR),

defined as the maximum number of progeny adults of each sex could produce when there is no limitation in the amount of food or sexual partners, should be used as a more general factor determining sex roles (see also Clutton-Brock and Parker, 1992; Parker and Simmons, 1996). The PRR concept leads to the same general pattern predicted by Trivers (1972): due to anisogamy, males generally require fewer nutrients than females to produce their cheap gametes, so that they have higher PRR than females and, consequently, the OSR would be male-biased. This scenario would force males to compete more intensely for access to mates and also to rarely adopt the caring role.

The argument provided by the PRR concept reformulated the causal link between past and future parental investment, prolonging the acceptance of classical verbal models on sex roles until the late 1990's when Queller (1997) stressed the importance of the so called *Fisher consistency*. In any diploid sexually reproducing species, each offspring has a mother and a father, which brings the consequence that the mean reproductive rate of males and females in natural populations with even adult sex ratios should be the same. Fisher consistency challenges the predictions of the classical models pointing out a natural constraint: even with potentially higher PRR, deserting males could not reproduce faster if there are no receptive females to mate with (Queller, 1997). This situation intensifies reproductive competition among males and different outcomes are possible. If there is high mating success variance among males, a subset of males would mate many times, whereas all other males would mate few times or not at all. Therefore, the higher reproductive rate of successful males would make them less prone to care if there is a trade-off between caring and acquiring new mates (Queller, 1997). If there are differences in mortality rates between sexes, it would lead to biases in sex ratio and individual strategies would change dynamically: frequency-dependent selection would favor

parental investment among individuals of the more common sex until OSR reaches 1:1 (Kokko and Jennions, 2008). Finally, if there is not high reproductive variance among males and receptive females are a limiting resource (i.e., the sex ratio is male biased), paternal care could evolve as a strategy to increase males' fitness by improving the viability of their offspring (Kokko and Jennions, 2008).

Once the effect of the Fisher consistency is taken into account, sex role divergence is the expected scenario and, contrary to past theoretical models, male care should be a widespread behavior in nature (Kokko and Jennions, 2008), which is clearly not the case. In an attempt to explain the rarity of male care in nature, it was necessary a new theoretical framework that accounted for additional variables. Recent models for the evolution of sex roles have considered not only fecundity costs and direct benefits for offspring survival associated to parental care, but also population parameters, such as adult density, OSR, and sex differences in mortality and mate searching rates, which ultimately influence mate encounter rate (e.g. Manica and Johnstone, 2004; Kokko and Jennions, 2008; McNamara et al., 2010; Alonzo, 2011). In all these models, parental care in general is favored when the benefits in terms of offspring survival improvement is high. Paternal care, in particular, should evolve when mate encounter rate is high, since it minimizes both the mating cost of caring and the probability of dying without having obtained any mating. In the model proposed by Kokko and Jennions (2008), for instance, another general conclusion is that the reduced relatedness to any given set of offspring due to multiple mating by females (i.e., sperm competition) would select against male care.

Although Kokko and Jennions' (2008) model proposes that the most likely strategy for males with greater reproductive opportunities is deserting the offspring, the authors

recognize that their conclusions would be reversed if paternal care itself is a sexually selected trait. However, their model was built assuming an inherent trade-off between parental and mating efforts, as well as treating the variance in mating success as a fixed characteristic, which was not allowed to evolve in response to changes in other parameters. Subsequent studies, however, have argued that models on sex role evolution should not only demonstrate the interactions between sexual selection and parental investment, but also investigate how sex differences arise from a starting scenario that does not assume any asymmetry between sexes in mating or parental decisions (Stiver and Alonzo, 2009; Alonzo, 2011).

Trade-offs between mating effort and parental effort may arise from different aspects of life-history traits and can be broadly classified into three main categories (Stiver and Alonzo, 2009): (a) resource limitations, since individuals have to invest their limited energy and resources either to attract sexual partners and compete for access to mates or to care for the offspring; (b) temporal limitations, since behaviors related to mating and parenting conflict and cannot be performed simultaneously; (c) mechanistic limitations, since morphological, physiological, or behavioral traits involved mainly in the sexual selection context are at the same time detrimental to effectively perform care. Paternal investment, however, does not necessarily conflict with males' mating effort and recent theoretical studies state that male care could provide an honest signal of male quality subject to female preference (e.g., Tallamy, 2001; Stiver and Alonzo, 2009; Alonzo, 2011). In fact, the transference of material gifts to females or the paternal care of multiple broods at the same site may not only increase offspring fitness, but also enhance male attractiveness and his relatedness to young (e.g. Hoelzel, 1989; Vahed, 1998; Tallamy, 2001; Wagner Jr, 2011; Alonzo, 2011).

PATERNAL CARE IN ARTHROPODS

Overview of cases

The first case of paternal care in arthropods was reported at the end of the 19th century for a sea spider of the family Phoxichilidiidae, in which males carry egg-masses attached to a specialized pair of legs (Sars, 1891; see also Cole, 1901). During the 20th century, a similar behavior was described in seven out of the eight families belonging to the class Pycnogonida (Bain and Govedich, 2004; Table 1). Some years before this first description of paternal care in arthropods, Dimmock (1887) reported water bugs of the subfamily Belostomatinae carrying eggs attached to their backs (Fig. 2A). However, the parental behavior was originally attributed to females, probably because paternal care was totally absent in arthropods until that moment and also because there is no clear sexual dimorphism among the Belostomatinae. The mistake was only corrected twelve years later (Slater, 1899), despite the fact that females are clearly unable to lay eggs on their own dorsum. More recently, two additional cases of males carrying eggs attached to their bodies were described among harvestmen of the family Podoctidae (Fig. 2B, Table 1).

Although water bugs of the subfamily Lethocerinae are the sister group to the Belostomatinae, they show some behavioral differences regarding the form of parental care (Smith, 1997). Lethocerinae males care for the offspring on the emergent vegetation at the margin of lakes and ponds (Fig. 2C), which does not impose a spatial limit to the number of eggs they can care for (see also the topic *Sex role reversal*). Despite this important difference, paternal care has probably evolved only once in the family Belostomatidae and the oviposition on emergent vegetation is regarded as the ancestral state (Smith, 1997). Back brooding has probably evolved as a response to restriction or even absence of appropriate oviposition sites outside the water (Smith, 1997). Moreover,

males in both subfamilies perform specific parental behaviors devoted to protect the eggs from stressful abiotic conditions. In the Belostomatinae, males may either remain in the air-water interface or perform different types of underwater movements that promote egg aeration (Smith, 1997; Munguía-Steyer et al., 2008). In the Lethocerinae, males constantly water the clutch exposed on the vegetation to avoid egg dehydration (Smith, 1997).

Another form of paternal care is found among species in which the mating system and male reproductive success are associated to the possession of a nest. The first example of this type of paternal care was described for males of the Neotropical harvestman *Zygopachylus albomarginis*, which build cup-like mud-nests, used to attract females and where they copulate and guard the eggs (Table 1, Fig. 2D). Males actively invest in repairing the nests and protecting them from invasion of conspecific males, as well as in defending the eggs against fungal attack and predation (Rodriguez and Guerrero, 1976; Mora, 1990). In other two lineages of Neotropical harvestmen, males do not build nests, but rather defend natural cavities (in rocks, trunks, and roadside banks) against conspecific intruders and egg-predators (Table 1, Fig. 2E). Although males of both *Z. albomarginis* and *Magnispina neptunus* (= *Pseudopucroliia* sp.) may attempt to take over nests from other males fighting with the original owners, the behavior of successful intruders are markedly different: individuals from the former species invariably eat all the eggs present inside the mud nest (Mora, 1990), while *M. neptunus* males usually adopt the unrelated eggs, even repelling intruders (Nazareth and Machado, 2010; see also discussion in the topic *Caring males attractiveness, a neglected benefit*).

Finally, male egg-guarding behavior on exposed substrates is found in a couple of species of stink bugs (Fig. 2F), some species of assassin bugs of the genus *Rhinocoris* (Fig. 2G), millipedes of the genera *Brachycybe* and *Yamasinaium* (Fig. 2H), and harvestmen of

five different families (Table 1, Figs. 2 I-K). At least for two of these species, namely *Iporangaia pustulosa* and *R. tristis*, there is experimental evidence that males defend the eggs against predators (Requena et al., 2009; Gilbert et al., 2010). Males of the millipede *B. nodulosa* protect the eggs mainly from fungus infection, and orphaned clutches are promptly covered by hyphae so that no egg hatches (Kudo et al., 2011). Eggs in at least three harvestman families are additionally covered either by mucus or debris coat, both deposited on the eggs by females just after oviposition (Table 1, Figs. 2D, E, I-J). Experimental evidence shows that the mucus coat that surrounds the eggs of the harvestman *I. pustulosa* works as an efficient physical barrier, providing additional protection to the embryos by hampering predator access to them (Requena et al., 2009; see also the topic *Costs and benefits of paternal care*).

Despite the diversity of forms of paternal care found in arthropods, some general patterns seem to occur (Table 1). Given that males are in charge of egg assistance, females are free to forage after oviposition, allocating additional energy in the continuous production of eggs over the course of the breeding season (Maynard Smith, 1977), which would be particularly important in predatory species in which individuals have less regular access to food than in herbivorous species. In fact, females from most of the arthropod species known to exhibit exclusive paternal care are iteroparous, and it has been suggested that female mate preferences towards caring males may have been favored due to the direct, fitness-enhancing gift of cost-free care of the offspring (Tallamy, 2001). Another general pattern observed among almost all arthropods with paternal care is that males are able to sequentially copulate with several females and usually care for all their eggs simultaneously in a single multiple clutch (Table 1, Fig. 2). However, due to the asynchronous development of the eggs laid into their multiple clutches, the total caring

period is inevitably prolonged, imposing additional energetic costs for caring individuals, specially in species in which males are prevented to forage while caring for the offspring (e.g. Gilbert et al., 2010; Nazareth and Machado, 2010, Requena et al., in press). In the following sections, we focus on the trade-offs dealt by arthropod males while caring for the offspring, and discuss how sexual selection affects and is affected by paternal care in well-studied biological systems.

Costs and benefits of paternal care

Virtually all theoretical models proposed to explain the evolution and maintenance of parental care explicitly or implicitly take into account the costs and benefits of this behavior. The benefits of caring are generally related to increasing offspring survival and all experimental studies with arthropods exhibiting exclusive paternal care have obtained the same general result: the absence of caring males condemns the offspring to death (Mora, 1990; Smith, 1997; Munguía-Steyer et al., 2008; Requena et al., 2009; Gilbert et al., 2010; Kudo et al., 2011; Fig. 3). However, males from different lineages exhibit different behaviors that affect offspring survival. In giant water bugs, males actively reduce the risk of dehydration and/or increase egg aeration, as stated before (Smith, 1997; Munguía-Steyer et al., 2008). In other lineages, males protect the eggs against predators (as observed in two harvestman species; Mora, 1990; Requena et al., 2009), parasitoids (as reported to one assassin bug species; Gilbert et al., 2010), and even fungi attack (as described for one harvestman and one millipede; Mora, 1990; Kudo et al., 2011).

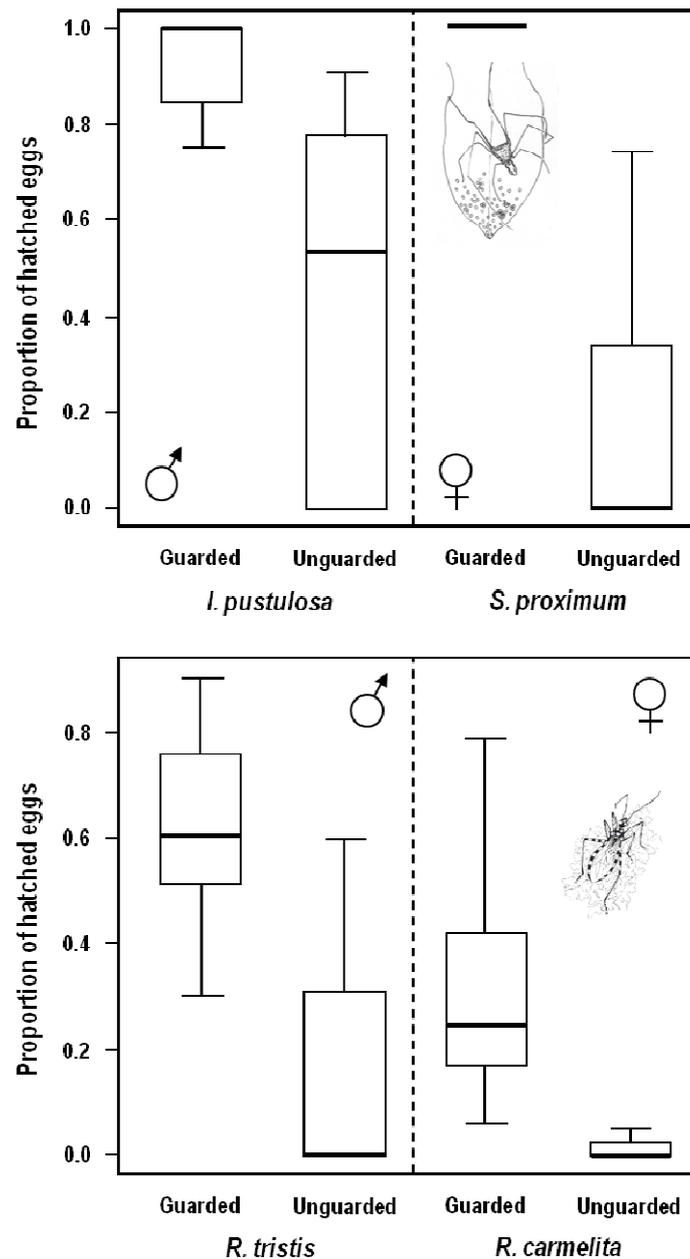


Fig. 3. Efficiency of paternal care when compared to maternal care in two arthropod species. (A) Proportion of hatched eggs in an experiment in which parental individuals of the syntopic harvestmen *Iporangaia pustulosa* (male care) and *Serracutisoma proximum* (female care) were removed from their clutches in the field for two weeks. The median values for guarded clutches in both species are 1.0, indicating that male care is as efficient as female care. However, the median proportion of hatched eggs in unguarded clutches is considerably higher for *I. pustulosa*, in which the eggs are protected by a thick mucus coat that reduces predation even in the absence of the guarding males. (B) Proportion of hatched eggs in a similar experiment in which parental individuals of the syntopic assassin bugs *Rhinocoris tristis* (male care) and *R. carmelita* (female care) were removed from their clutches in the field until all eggs had hatched or disappeared (modified

from Gilbert et al., 2010). In this pair of species, male care seems to be more efficient than female care. Although the median proportion of hatched eggs in unguarded clutches is zero in both species, the number of clutches in which a great proportion of unattended eggs survive is much higher in the species with paternal care. Apparently, the hard eggshell in *R. tristis* may also reduce predation rates even when the guarding males are absent. In both graphics, thick horizontal lines represent the median, boxes represent the interval between the first and third quartiles, and the vertical lines indicate 90% confidence intervals.

Besides the effort of caring males to minimize offspring mortality, females from several species cover the eggs with debris or a thick mucus coat, which may confer additional protection to the eggs and also prevent filial cannibalism (Table 1, Figs. 2D, E, I-J). Eggs experimentally manipulated to remove the mucus coat in the harvestman *Iporangaia pustulosa*, for instance, suffered higher predation than eggs covered by mucus deposited by females after oviposition (Requena et al., 2009). Furthermore, when compared to a syntopic harvestman species with uniparental female care (*Serracutisoma proximum*), in which there is no deposition of mucus on the eggs, egg mortality of unattended clutches of *I. pustulosa* was lower (Requena et al., 2009; Fig. 3A). A comparable pattern has been reported to a pair of syntopic sub-Saharan assassin bug species, *Rhinocoris tristis* with paternal care and *R. carmelita* with maternal care (Gilbert et al., 2010). Although the median proportion of hatched eggs in unattended clutches of both *R. tristis* and *R. carmelita* is zero, the number of clutches in which a great proportion of unattended eggs survive is much higher in the former than in the latter (Fig. 3B). The authors suggested that this pattern is a consequence of an additional investment by *R. tristis* females in eggshell to avoid filial cannibalism, which is commonly observed among caring males (Thomas and Manica, 2003). The protection against filial cannibalism may also provide protection against other natural enemies, which may explain why unattended eggs of *R. tristis* have higher chances of hatching when compared to *R. carmelita*.

Moreover, the additional protection afforded by the hard eggshell may explain why the efficiency of paternal care is higher when compared to maternal assistance in this pair of species (Fig. 3B). These suggestions, however, remain as open and interesting questions to be investigated in *Rhinocoris* and other species exhibiting paternal care.

The costs paid by caring males are generally classified into three main categories (Gross and Sargent, 1985; Clutton-Brock, 1991): (a) increases in mortality risk due to predator, parasite, and parasitoid attacks during the caring period; (b) decreases in body condition as a consequence of either decreasing foraging activity or increasing metabolic expense associated to parental behaviors; and (c) decreases in mating rate due to a trade-off between parental and mating efforts. Empirical data on mortality risks of paternal care are restricted to a few species, with no consensual pattern. In the assassin bug *R. tristis* (Fig. 2G), caring males have higher mortality rates when compared to non-caring males (Gilbert et al., 2010), a result that authors argue to be produced because caring males suppress their escape behavior while guarding the eggs, thus becoming more easily seized by predators. On the other hand, no evidence of survival costs was observed in two species of water bugs. In a laboratory experiment without predators, males of *Belostoma flumineum* that had their egg pads removed showed similar lifespan to either virgin or brooding males (Gilg and Kruse, 2003). The same pattern was also observed in a mark-recapture study conducted under field conditions with *Abedus breviceps* (Fig. 2A), in which parental status of adult males did not influence their survival probabilities (Munguía-Steyer and Macías-Ordóñez, 2007). Even more surprising are the results obtained in the field for the harvestman *I. pustulosa* (Fig. 2I), in which caring males showed higher survival probabilities than non-caring individuals (both males and females). This pattern is interpreted as consequence of differences in movement activities, since non-caring

individuals are constantly moving on the vegetation and are more likely than caring males to be caught by sit-and-wait predators, which are the most important natural enemies of the adults (Requena et al., in press).

As rare and controversial as the data on mortality risks are the results reported for energetic costs of paternal care in arthropods. At the same time that egg carrying allows caring males to move and forage while caring, the extra-weight gained with the eggs may negatively affect movement, foraging efficiency, and food intake of brooding males. Although adults of the sea spider *Achelia simplissima* seek food (sessile worms) in the habitat and non-brooding males cover larger areas and move more frequently than caring males, the extra-weight conferred by the eggs does not affect males' foraging efficiency (Burriss, 2010). On the other hand, empirical results for a Belostomatinae water bug showed that males carrying eggs on their back swim slower than both non-caring males and females (Crowl and Alexander, 1989; Kight et al., 1995), which is likely to affect their foraging efficiency. Additionally, water bug males exhibit expensive parental behaviors that may incur additional energetic costs to parental individuals (see topic *Overview of cases*).

Egg-guarding behavior, on the other hand, would be expected to impose relatively lower energetic costs to caring males because parental activities involve basically staying on the clutch and protecting the eggs against natural enemies. However, the higher potential for multiple and asynchronous mating opportunities in this case may lead to prolonged caring periods with limited foraging opportunities. Indeed, caring males of two harvestmen (Nazareth and Machado, 2010; Requena et al., in press) and one assassin bug species (Thomas and Manica, 2005) feed less frequently than non-caring individuals. The consequences for males' body condition, though, are markedly different: paternal care

erodes caring male body condition over the course of the caring period in the harvestman *I. pustulosa* (Requena et al., in press), while it shows no effect on caring male weight in the assassin bug *R. tristis* (Thomas and Manica, 2005). A plausible explanation for this difference is that males of *R. tristis* usually engage in filial cannibalism during caring (Thomas and Manica, 2003), while males of *I. pustulosa* have never been observed consuming eggs from their own clutches (Requena et al., in press).

Finally, the potential loss of additional mating opportunities while caring, as well as the uncertainty of genetic relatedness with the offspring under protection, have always been pointed out as the strongest selective pressures against the evolution of paternal care (Magrath and Komdeur, 2003). The available data for arthropod species with exclusive paternal care, however, challenge these prohibitive mating cost associated to increased paternal effort. In most of the reported cases, at least some caring males in the population (if not all of them) are able to care for multiple clutches simultaneously (Table 1). This information strongly suggests that males are usually not constrained to care for eggs from just one female at time, but instead they can mate with more than one female during caring period. Therefore, caring for the offspring and acquiring new mates are not mutually exclusive activities for males from most arthropod species, which usually have many mating opportunities while guarding eggs (Table 1). This pattern is similar to what has been described for fishes with exclusive paternal care, in which males also care for multiple clutches (Gross and Shine, 1981; Gross and Sargent, 1985; Stiver and Alonzo, 2009, Coleman and Jones, 2011). In fishes, females preferentially oviposit inside nests whose male owners exhibit high quality care (e.g. Östlund and Ahnesjö, 1998; Pampoulie et al., 2004), and mate choice has probably driven the evolution of extremely specialized male traits, such as egg mimicry, egg thievery, and nest takeovers (Porter et al., 2002).

Could paternal care also play an important role in mating decisions among arthropods? If so, should we keep interpreting male post-zygotic parental investment as a general constraint to future reproductive events? These questions are explored in more details in the next two topics.

Caring males' attractiveness, a neglected benefit

The evidence that males have multiple mates while caring is not strong enough to disprove the existence of a trade-off between parental and mating efforts, which is the underlying assumption of the so-called reproductive costs associated with paternal care. In theory, non-caring males may assess more females and copulate more frequently than caring males, without paying the costs of caring for the offspring. However, for at least two harvestman species there is strong observational evidence indicating that females exclusively mate inside nests and leave their eggs only with territorial males owning such nests (Mora, 1990; Nazareth and Machado, 2010). It is worthy noting, though, that observational data alone cannot disentangle the effect of male individual traits, nest-associated traits, or paternal care quality. Only experimental manipulations are able to isolate these three factors, and they have already been performed using three species with marked differences in their reproductive biology: (a) the harvestman *Magnispina neptunus* (Nazareth and Machado, 2010), (b) the assassin bug *Rhinocoris tristis* (Gilbert et al., 2010), and (c) the sea spider *Achelia simplissima* (Burriss, 2011). In these three species, the presence of eggs indeed influences the probability of males receiving additional eggs, but the specific effect differed among species, and will be discussed in details in the following paragraphs.

Harvestman females were given the opportunity to simultaneously choose between two males with their respective nests in a paired mate choice experiment. First, each female was allowed to select either a caring male (with eggs inside his nest) or a non-caring male (with an empty nest). After female decision and oviposition, individuals were maintained with no contact with each other for some days. The same triplet was allowed to interact again, but the parental state of the males was reversed: offspring of caring males in the first round have hatched and was removed from their nests, while non-caring males in the first round have copulated with other females and received eggs in their nests. The results obtained showed that females consistently preferred to lay eggs inside nests already containing eggs, regardless nest or individual male traits (Nazareth and Machado, 2010).

The experimental design and the results obtained are slightly different for the assassin bug species, in which females sequentially accessed males: females indiscriminately mated with caring and non-caring males, but they preferred to lay eggs in already established broods. Furthermore, caring males were more likely to receive eggs in their broods if female have interacted with a non-caring male first (Gilbert et al., 2010). Observational data of the sea spider *Ammothella biunguiculata* are in accordance with this pattern, since male mate acquisition seems to be random, but once a male encounters a mate, the number of eggs received depends on whether he is already carrying eggs (Barreto and Avise, 2011). Therefore, there are cases in which caring males are clearly more attractive than non-caring males, which are expected to exhibit alternative mating tactics to increase their reproductive success. Examples among arthropods include non-caring males of the harvestman *M. neptunus* taking over nests containing eggs (Nazareth and Machado, 2010) and non-caring males of the assassin bug *R. tristis* adopting eggs from

unattended clutches or from clutches whose owner has been displaced (Thomas and Manica, 2005). These tactics may be profitable to non-caring males because the benefits in terms of increased attractiveness are likely to be higher than the costs of caring for unrelated offspring (Tallamy, 2001).

The last example regards a simultaneous mate choice experiment conducted with the sea spider *Achelia simplissima* to investigate female mate decisions, which revealed a pattern opposite to that described above: females clearly preferred non-caring males. Furthermore, when allowed to select one of two parental males, females preferred to lay their eggs with males carrying fewer egg-masses (Burriss, 2011). The author argued that active aeration of egg masses, the extra weight conferred by the eggs, and the extra resistance to avoid dislodgment by water flow imposed by the egg masses (Burriss, 2010) would deplete caring males' energetic reserves, negatively affecting the quality of parental care they can provide for additional eggs. Different from the cases described above, in which care is non-depreciable (sensu Clutton-Brock, 1991) because the efficiency of male egg-guarding does not depend on offspring number, male care in the sea spider *A. simplissima* is likely to be depreciable since the more the number of eggs males carry, the worse would be the quality of paternal care they provide. Whenever paternal care is depreciable, we predict that caring males will be avoided by ovipositing females because non-caring males are likely to provide a better quality protection to eggs.

Sex role reversal

In the first section of this chapter, we provided a historical perspective on parental investment and the evolution of sex roles. Regardless the model, extreme cases of sex role reversal, characterized in terms of competitive females and choosy males, are expected to

evolve only under particular conditions. The situations in which male mate choice is more likely to be found occur when: (a) males have great parental investment or suffer from sperm depletion, so that they are unable to mate with all available females, (b) females exhibit great variation in quality, and (c) mate searching effort is relatively inexpensive (e.g. Bonduriansky, 2001; Bateman and Fleming, 2006; Barry and Kokko, 2010; Edward and Chapman, 2011). Here we explore the available empirical evidence of sex role reversal among arthropod species exhibiting paternal care.

Sex role reversal in paternally caring arthropods has been described for water bugs, sea spiders, and at least one harvestman species. In the first two groups, males carry eggs attached to their own body, which limits the number of eggs they can receive due to the available space either on their backs (water bugs) or their legs (sea spiders) (Table 1). Belostomatinae males, for instance, can fill up their backs with eggs laid by one to three different females, but rarely accumulate more than 120 eggs (Table 1; Fig. 2A). As an illustrative comparison, the total number of eggs received by Lethocerinae males on emergent vegetation is considerably higher, frequently reaching 150–300 eggs (Table 1; Fig. 2C). Males of both sea spiders and Belostomatinae water bugs are thus likely to have lower PRR when compared to females, and spend proportionally more time in a sexually unreceptive state. At the population level, receptive males will be underrepresented in the OSR, and the mean number of females available as mates is likely to be high relative to the capacity of males to mate with them. If the benefit of mating with specific high-quality females exceeds the cost of assessing them, male choice is expected to evolve (Edward and Chapman, 2011).

Although there is no record of female rejection prior to oviposition among water bugs, male mate choice expressed as premature termination of parental care has been

reported for the Belostomatinae *Belostoma flumineum* (Kight and Kruse, 1992; Kight et al., 2000, 2011). Results obtained under captivity conditions show that males discard small egg pads, condemning the eggs to submerge and die. Extensive fieldwork on *Abedus breviceps* (Belostomatinae), however, show that oviposition of a second egg pad without removing the first one is the rule, with no instance of male abortion ever recorded (R. Munguía-Steyer, unpub. data). Differences between these two species suggest that male abortion may be influenced by the conditions under which data are taken. Captivity experiments maintaining *B. flumineum* adults in a predator free environment greatly reduce the risks of mate searching, increasing the residual reproductive value of parental males in comparison with natural conditions (Kight and Kruse, 1992; Kight et al., 2000, 2011). Under natural conditions, the mortality risk experienced by *A. breviceps* males is very high (Munguía-Steyer and Macías-Ordóñez, 2007). As predicted by the theory (Barry and Kokko, 2010; Edward and Chapman, 2011), males experiencing low mating search costs could afford to selectively discard small clutches as a reproductive tactic to make their backspace available to a new and potentially larger clutch. On the other hand, males living in a risky environment may have their choosiness constrained by the high probability of dieing before encountering a new female. Additional data and experiments specifically designed to test the adaptive role of male abortion are needed, and the influence of mating search costs on the probability of premature termination of parental care remains as an open and interesting line of investigation.

Given that the cost of carrying unrelated eggs is probably very high for males of sea spiders and water bugs, males are also expect to exhibit paternity-assurance strategies. In fact, alternating repeated copulations and ovipositions, and post-copulatory female guarding are common male mating behaviors in water bugs, reported for representatives

of all genera of back-brooders and three species of emergent-brooders (Smith, 1997), suggesting an ancient origin probably related to mate guarding (Alcock, 1994). Although some level of cuckoldry has been detected in a Japanese back-brooder (Inada et al., 2011), extremely high levels of paternity have been reported for a back-brooder from North America (Smith, 1979b), which led the author to interpret the above mentioned mating behaviors as male tactics to diminish the risk of sperm competition (Smith, 1997). Unfortunately, paternity data on Lethocerinae are lacking, thus it is not possible to compare the levels of cuckoldry between back-brooders and emergent-brooders. Among sea spiders, fertilization is external, but the gonopores of males and females are in close contact during mating interactions and the fertilization usually occurs immediately after the transference of eggs onto males' ovigerous legs (Wilhelm et al., 1997; Bain and Govedich, 2004). This tactic is suggested to be responsible for no record of cuckoldry in some species (Barreto and Avise, 2008, 2011).

From the female perspective, some degree of sex role reversal is also observed among water bugs, especially among representatives of Lethocerinae. Given that appropriate oviposition sites on emergent vegetation are usually in short supply, females aggressively compete for the access to them. Competition among gravid females of some species of *Lethocerus* occurs in the form of cannibalism and destruction of non-filial eggs, which is probably a tactic to open space for their own offspring and also to acquire nutritional benefits from the consumed eggs (Ichikawa, 1990; Smith, 1997). Although males attempt to chase attacking females away from the eggs, in most cases they do not succeed and the eggs are eaten and/or dropped into the water. After destruction of the clutch, attacking females always mate with the guarding male following the same general pattern described above, and replace the destroyed clutch with their own eggs. Males, in turn, invariably

care for the new clutch (Ichikawa, 1990). The best explanation why clutch cannibalism and destruction is not widespread among species of *Lethocerus* probably relays on the fact that size dimorphism is relatively rare in the genus. According to this hypothesis, infanticide should occur in species whose females are considerably larger than males, but not in species monomorphic in size, where multiple clutches should be the rule (Smith, 1997).

Among sea spiders, information on either intra or inter-sexual interactions prior to mating is very scarce and no general pattern of sex role reversion is evident. Females of *Propallene saengeri* are the exclusive initiators of courtship, with instances of female-female aggression also described during mate interaction, which strongly suggests sex role reversion (Bain and Govedich, 2004). Curiously, males but not females exhibit courtship behavior in two closely related species, *P. longiceps* and *Parapallene avida*, with no reported instance of competition or aggressive interactions between females (Nakamura and Sekiguchi, 1980; Hooper, 1980). Finally, in another well studied species of sea spider, *Achelia simplissima*, most of the observed mating interactions were initiated by females, which exhibited specific courtship behaviors (Burris, 2011), but they do not seem to compete for mates. Under captivity conditions, however, it has been demonstrated that females reject some mates and, in fact, their mate decisions are based on the number of egg masses that males are already carrying, with a clear preference for non-caring males (Burris, 2011). This finding suggests that males are unlikely to accumulate eggs fast enough to fill up the space on their ovigerous legs, which may explain why nearly all males in the population are carrying much less egg masses than their maximum capacity (Burris, 2010). Therefore, there are likely many more males than females available to mate in the population at any time, implying that *A. simplissima* has conventional sex-roles (Burris, 2011).

Among harvestmen, there is unequivocal observational evidence of sex role reversion for *Zygopachylus albomarginis*, in which males build cup-like mud nests that are visited by females (Mora, 1990; Fig. 2D). Females actively court males when they enter a nest, but males do not copulate with all courting females. Males aggressively reject 14% of the visiting females, biting their legs and chasing them out of the nest. Attacked females never respond to the male's bites, but rather remain quiet on the nest floor or leave the nest without fighting (Mora, 1990). The reasons why some females are accepted while others are rejected remain unknown, but it is likely that males are able to evaluate visiting females through chemical information based on tegumentary hydrocarbons. Given that the great majority (90%) of females in the population visit only one or two nests during the mating season (Mora, 1990), males may recognize their frequent partners and repel newcomer females that visit many nests, and thus represent high risk of sperm competition or egg cannibalism. Field observations also showed that some females spend several days in the vicinity of one or two nests, and sometimes show aggressive behaviors toward newcomer females that approach one of these nests. Given that females are predatory upon eggs, once more the risk of cannibalism may explain female-female aggression (Mora, 1990).

Although aggressive interactions between females and males have never been observed in the harvestman *Magnispina neptunus*, territorial males can reject visiting females of their nests, which suggests some degree of sex role reversion (Nazareth and Machado, 2010; Werneck and Machado, unpub. data). Males of this species do not build a mud nest, but rather occupy natural cavities on roadside banks that are used as oviposition sites (Nazareth and Machado, 2010). Contrary to sea spiders and Belostomatinae water bugs, the number of eggs males of both *Z. albomarginis* and *M.*

neptunus can receive in their nests is not limited and is similar to other harvestman species that do not have nests (Table 1). However, nests are in short supply, either because only good quality males are able to pay the costs of building and maintaining a mud nest or because natural cavities of appropriate dimensions are indeed extremely rare in nature. Given that females of both harvestman species copulate only with males holding a nest (Mora, 1990; Nazareth and Machado, 2010), the OSR may be slightly or strongly female-biased, which may partially explain sex role reversion in these two species.

MACROECOLOGY OF PATERNAL CARE

Considering only non-marine arthropods, i.e., excluding sea spiders from the counts, seven lineages (54% of the total) and 127 species (59% of the total) with paternal care occur in the Neotropical region (Table 1). Among the four lineages that have no representative in the neotropics, one is found in Africa, two in Asia, and one in New Zealand (Table 1). Looking at the geographic distribution of individual species, there is good-quality information for the taxa from the Americas, which includes representatives of all major groups in which paternal care has independently evolved. We used these data to understand species distribution according to climate types. First, we defined a large area in Fig. 4A that encompasses the distribution of 122 arthropod species with paternal, and exclude 28 species of Belostomatidae that are found in northern United States, along southern border of Canada, the southernmost part of Argentina, and all the extension of Chile. Then we calculated the proportion from the total available area under each climate type that is occupied or not by at least one species of arthropod with paternal care. We found that all areas with hot-humid and hot-seasonally dry climates have species with paternal (Fig. 4B). A great proportion of the areas with mild-humid and mild-seasonally

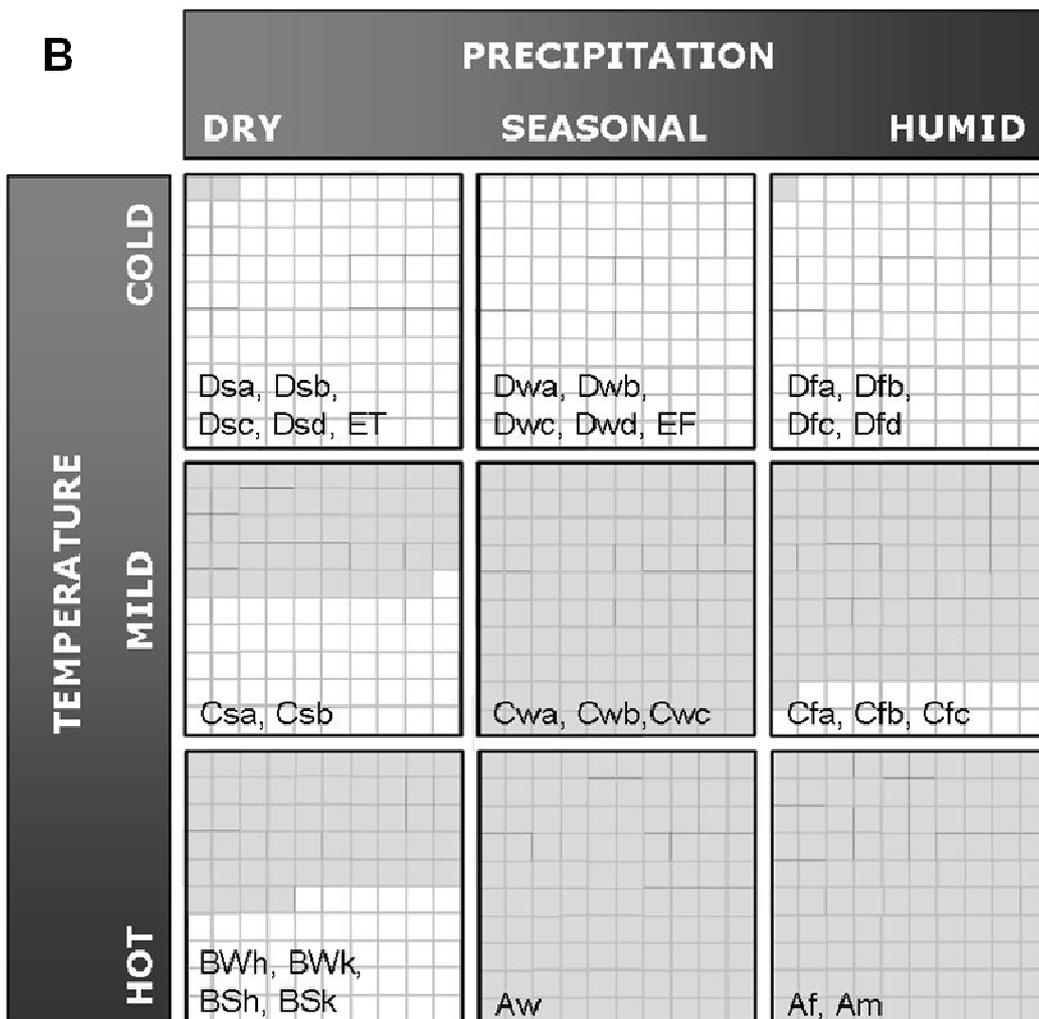
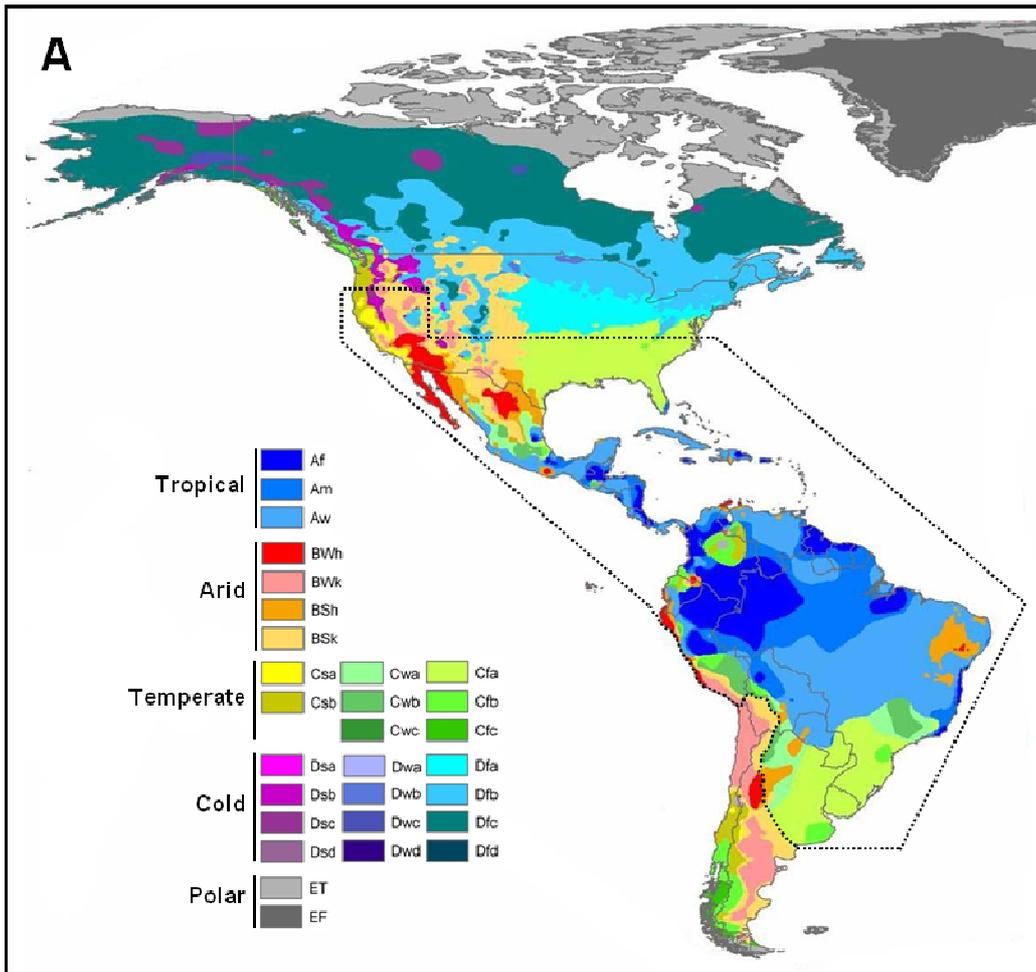


Fig. 4. Distribution of terrestrial arthropod species exhibiting exclusive paternal care in the Americas. (A) Köpen-Geiger climate classification map (modified from Peel et al., 2007) showing the limits of distribution of 122 arthropod species with exclusive paternal care that belong to the following taxa (references in Table 1): Belostomatidae, Lethocerinae (17 species, corresponding to nearly 71% of all species in the subfamily); Belostomatidae, Belostomatinae (83 species, corresponding to nearly 75% of American species and 58% of all species in the subfamily); Pentatomidae (two species); Andrognathidae (all six American species, corresponding to approximately 66% of all species in the family); Cosmetidae (one species); Gonyleptidae, Gonyleptinae (two species); Gonyleptidae, Heteropachylineae (two species); Gonyleptidae, Caelopyginae + Progonyleptoidellinae (six species); Manaosbiidae (one species); and Stygnidae (two species). (B) Diagram representing the proportion of available areas under each climate type in America that is occupied (gray boxes) by arthropod species with exclusive paternal care, considering the limits of distribution of 81% of the American arthropod species with exclusive paternal care. There is a clear bias in the occupancy of species in hot and humid areas, with only a few representatives occurring in extremely cold regions.

dry climates also had (Fig. 4B). On the other hand, nearly 50% of the areas with mild-dry and hot-dry climates, as well as the great majority of the areas with cold climates had no species with paternal care (Fig. 4B). It is worth noticing that species found in the later situations are not specialized in extreme climatic conditions, but rather are species with wide geographic distribution, which also includes regions with low precipitation and temperature.

One simple explanation for this pattern is that it merely reflects the global distribution of arthropod diversity, which is high in hot and humid climates, and low in cold and dry climates (Chown and Nicolson, 2004). An alternative and non-exclusive possibility is that temperature and humidity may affect directly and indirectly the costs and benefits of paternal care. In this section, we explore the potential effects of biotic and abiotic conditions on the occurrence of exclusive paternal care in arthropods. Given that the evolution and maintenance of paternal care are likely to be influenced by both natural

and sexual selection (see references in section *Historical perspective on sex roles and parental investment*), we take into account these two selective pressures when deriving macroecological predictions (Fig. 5). Unfortunately, hard data are very scarce and detailed studies are restricted to a few species, which prevents any formal test of our predictions. We expect, however, that the ideas presented here stimulate researchers to gather basic information on a wider set of arthropod species exhibiting paternal care and also to pay attention on the role of environmental conditions on the costs and benefits of this behavior.

Temperature and precipitation have an important role modulating the intensity and diversity of biotic interactions, including predation and parasitism (Schemske et al., 2009; Moya-Laraño, 2010). Therefore, temperature and precipitation may have indirect effects on the benefits of parental care in terms of offspring protection against natural enemies (Fig. 5). In hot and wet climates, the benefits of egg-guarding, for instance, should be higher than in other cold and dry climates because offspring is expected to be under more intense threat by predators, parasitoids, and fungal attack. In fact, the importance of biotic interactions on the benefits of parental care in general has been recognized a long time ago by Wilson (1971), who stated that intense predation on eggs by conspecifics and ants, as well as the high risk of fungal attack in tropical rain forests may have been the major forces favoring the evolution of parental care in arthropods. Although this hypothesis does not provide an answer to the question of why paternal care has evolved in some species and not in others, it may explain why this behavior is so frequent among tropical arthropods.

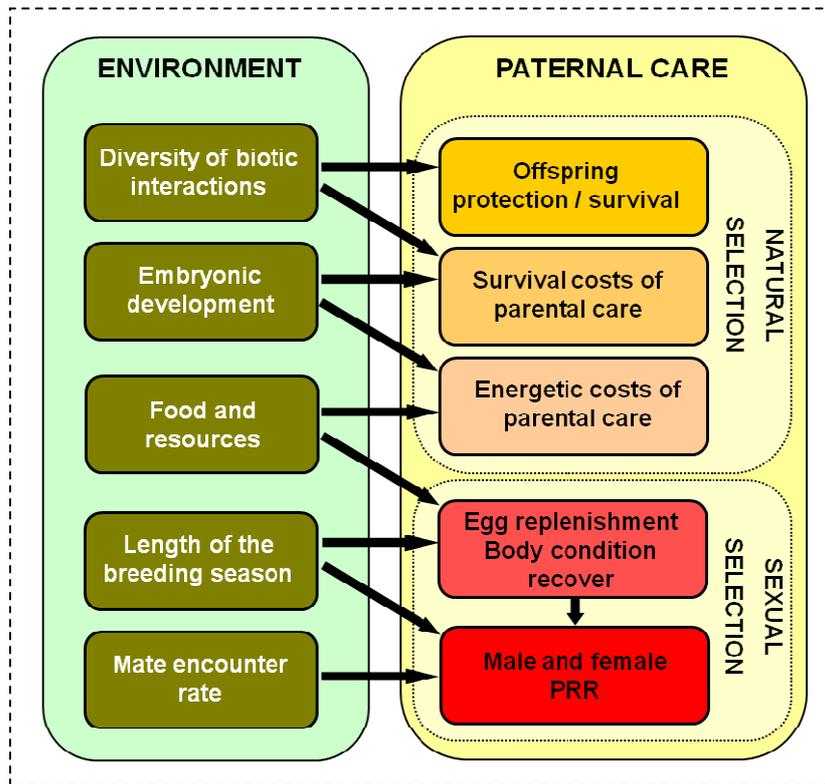


Fig. 5. Macroecology of paternal care. The scheme depicts predictions on the influence of environmental factors on several features of the arthropod ecology and life history. Each one of these features has direct or indirect influence on the costs and benefits of paternal care (natural selection) and also on the intensity and direction of sexual selection. See text for a detailed explanation of the causal link among variables.

For the same reasons hot and wet climates may increase the benefits of paternal care, the costs to parental individuals in terms of mortality risks may also be affected by more frequent encounters with natural enemies (Schemske et al., 2009; Moya-Laraño, 2010; Fig. 5). If egg-guarding makes males more conspicuous, increasing their susceptibility to predator and parasitoid attacks, mortality rates of caring males should be higher in tropical climates. On the other hand, high temperatures accelerate embryonic development of arthropods (Zaslavski, 1988; Fig. 5), thus decreasing the time caring males are exposed to natural enemies. Therefore, the net effect of climatic conditions on the risk

of mortality will depend on how temperature influence the rate of attacks suffered by caring males and the rate of egg development. Variations in these rates among species may partially explain the non-consensual results on mortality costs reported for different arthropod species exhibiting paternal care (see topic *Costs and benefits of paternal care*).

Short caring periods promoted by increases in temperature may also diminish the energetic/foraging costs of caring (Fig. 5). Furthermore, due to the positive effect of temperature and precipitation on ecosystem productivity and resource availability (Hawkins et al., 2003; Moya-Loraño, 2010), individuals are likely to have access to more food in tropical climates. Among species with exclusive paternal care, abundant food in hot and humid climates may accelerate the rate of egg replenishing because females are free to forage just after oviposition and produce additional eggs relatively faster than in cold and dry climates (Fig. 5). Abundant food in hot and humid climates may also reduce the time for male body condition recover after the caring period, during which foraging activities are severely constrained, especially for predatory species (Fig. 5). If the negative effect of temperature and precipitation on female time-out is stronger than on male time-out, the OSR may become female-biased and female PRR may be higher than males (Fig. 5). According to recent models for the evolution of parental care, these conditions would at least in part favor male care (see section *Historical perspective on sex roles and parental investment*).

Temperature and precipitation are also expected to influence the length of breeding season (Fig. 5), so that in hot and humid climates the time of reproductive activity should be longer than in cold and dry climates. Moreover, increases in temperature are also known to positively influence individual activity patterns in arthropods (Chown and Gaston, 2010). The combination of short time-out, high population density, and more

intense activity of individuals in tropical regions may increase encounter rates between receptive males and females (Fig. 5), which is a key parameter for understanding sex role and mate choice (Bonduriansky, 2001; Barry and Kokko, 2010; Edward and Chapman, 2011). Wherever environmental conditions severely decrease encounter rates, the costs of assessing mates is likely to be higher than the benefits of choosing between mates. Therefore, environmental conditions may indirectly influence mutual mate choice and sex role reversal in arthropod species exhibiting paternal care.

CONCLUDING REMARKS

In this chapter, we assembled scattered biological information for a wide set of arthropod species exhibiting paternal care and contextualized the available data on the most recent theoretical frameworks for the evolution of sex roles and parental investment. Several important messages emerge from this synthesis. First, theoretical models for the evolution of parental care have been traditionally based on bird-like systems in which there is a strong trade-off between parental and mating efforts and bi-parental care is widespread. Among arthropods and fish, however, exclusive paternal care is relatively common and the trade-off between parental and mating effort is considerably relaxed or even non-existent because males are able to care for many eggs and simultaneously have access to several mates (Table 1). This finding has profound theoretical implications because these trade-offs should be mathematically incorporated into the models instead of assumed *a priori*. Not surprising, recent attempts of modeling the evolution of sex roles and parental investment using arthropods and fishes to parameterize the models have indeed adopt this approach (Manica and Johnstone, 2004; Alonzo, 2011).

The second important message is that basic information on the costs and benefits of paternal care in arthropods is extremely scarce and the results on the few well-studied species rarely found convergent patterns. Whereas male care always benefits the offspring, protecting the eggs from both natural enemies and stressful abiotic conditions, the costs of egg-guarding markedly differ among species. Divergent results on the costs of male care can be partially explained by methodological differences among studies. Survival costs, in particular, were estimated both in the field and in the laboratory using either an experimental approach or mark-recapture protocols. We strongly suggest that future studies on this subject use a mark-recapture modeling approach because it dissociates survival from recapture through maximum likelihood techniques using the encounter histories of individuals under natural field conditions (Lebreton et al., 1992). Regarding the energetic costs, whenever possible researchers should avoid proxies of body condition correlated with structural body size, such as body mass. To remove the effect of body size and accurately assess body condition, estimates controlling for the effect of structural body size or based on body density should be taken into account because they increase the power to detect condition differences in terms of nutrient storage (Moya-Laraño et al., 2008).

The third message is that high paternal investment does not necessarily lead to sex role reversal in arthropods. In fact, the available empirical data show unequivocal evidence of sex role reversal only in water bugs, a few species sea spiders, and harvestmen. However, given the paucity of detailed behavioral studies of most species known to exhibit exclusive paternal care in the arthropods, it would not be surprising if additional cases of sex role reversion were described as our knowledge increases. Future studies should pay attention on population parameters, such as density and sex ratios,

because recent theoretical models have stressed their importance for the evolution of mate choice, by both males and females (Bonduriansky, 2001; Barry and Kokko, 2010; Edward and Chapman, 2011). Moreover, when focusing on species with exclusive paternal care, researchers should not forget to look closely to female behavior and morphology. Field observations for at least one harvestman species suggest that females may exhibit alternative mating tactics (Mora, 1990), which seems an exciting and promising avenue of investigation. Detailed behavioral data coupled with paternity analyses in this and other species would be extremely welcome to better understand the role of sexual selection in the evolution and maintenance of paternal care and sex roles in arthropods.

Finally, the last message of this chapter is that environmental (climatic) conditions may play an important, yet unexplored role affecting the costs and benefits of paternal care in arthropods. In the future, when more information on paternal care in the group is available, most of the predictions presented here could be tested. There are two equally interesting ways to approach these predictions: (a) inter-specific comparisons that take into account the phylogenetic relationships among the species occurring in different climate types worldwide, and (b) inter-population comparisons using individual species with wide geographic distribution and with populations occurring in different climate types. For both approaches three arthropod groups exhibiting exclusive paternal care seem to be especially adequate as model systems. The first two are the water bugs of the family Belostomatidae and millipedes of the genus *Brachycybe*, whose species are distributed worldwide in markedly different climatic conditions (Table 1), and thus could be used for inter-specific comparisons. Moreover, some species of these groups are distributed over a wide area encompassing a great variety of climate types (Perez-Goodwyn, 2006; Brewer et al., 2012), which makes them particularly suitable for inter-

population comparisons. The third group is the harvestmen, in which paternal care has evolved at least nine times independently (Table 1). There are phylogenies for many groups in the suborder Laniatores, which concentrates all cases of egg-guarding in Opiliones, and basic data on reproduction are accumulating in a fast rate. The group, therefore, may offer unique opportunities to test macroecological predictions using comparative methods.

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CAPÍTULO 2

Paternal care decreases foraging activity and body condition, but does not impose survival costs to caring males in a Neotropical arachnid

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ABSTRACT

Exclusive paternal care is the rarest form of parental investment in nature and theory predicts that the maintenance of this behavior depends on the balance between costs and benefits to males. Our goal was to assess costs of paternal care in the harvestman *Iporangaia pustulosa*, for which the benefits of this behavior in terms of egg survival have already been demonstrated. We evaluated energetic costs and mortality risks associated to paternal egg-guarding in the field. We quantified foraging activity of males and estimated how their body condition is influenced by the duration of the caring period. Additionally, we conducted a one-year capture-mark-recapture study and estimated apparent survival probabilities of caring and non-caring males to assess potential survival costs of paternal care. Our results indicate that caring males forage less frequently than non-caring individuals (males and females) and that their body condition deteriorates over the course of the caring period. Caring males, however, did not show lower survival probabilities when compared to both non-caring males and females. Therefore, given that paternal care significantly decreases egg predation and also improves caring males' attractiveness to females, we conclude that egg-guarding in *Iporangaia* incurs relatively low costs in relation to great benefits for caring males.

Keywords: Energetic costs, mating effort, parental effort, sexual selection, survival costs.

INTRODUCTION

Trivers' [1] classical definition of parental investment postulates that even simple forms of parental care, such as egg-guarding, should include both benefits (enjoyed by the offspring) and costs (directly paid by parental individuals). The benefits of parental care to the offspring include improving micro-climatic conditions, such as reducing risk of dehydration and/or increasing egg aeration, protection against predators, parasitoid or fungal attack, as well as provisioning water or food to juveniles [2]. The costs paid by parental individuals are generally classified into three main categories [2-3]: (a) energetic costs, as a consequence of either reduced feeding opportunities or increased metabolic expense while caring for the offspring; (b) survival costs, as an ultimate consequence of starvation or increased susceptibility of the tending parent(s) to predators, parasites, and parasitoids; and (c) reproductive costs, involving loss of additional mating opportunities.

Parental care generally prevents foraging activities of parental individuals and can also be associated with expensive behaviors, such as providing offspring with food [2]. Therefore, maternal care is energetically costly for females, especially in the case of iteroparous species [4], since it reduces the available energy to produce additional eggs, negatively affecting females' future reproduction and fecundity [e.g., 5-9]. Among species exhibiting exclusive paternal care, the available data do not seem consensual. Studies with giant water bugs (Belostomatinae), whose males carry egg pads attached to their backs, demonstrated that paternal behavior incurs energetic costs to parental individuals by both decreasing their foraging efficiency and food intake [10], and by increasing their muscular activity while promoting water flow and oxygen diffusion through the eggs membrane [11-12]. On the other hand, studies with the sea spider *Achelia simplissima* [13] and the assassin bug *Rhinocoris tristis* [9] showed that, besides differences in movement and activity patterns between caring and non-caring individuals, paternal care does not

negatively affect foraging efficiency or weight loss, respectively.

Conflicting evidence is also the case for the survival costs of exclusive paternal care, for which empirical data is restricted to a few insect species. Observational data on the thrips *Hoplothrips karnyi* showed that caring males remain near communal egg masses, and the protection of these eggs against attacking conspecific males increases their mortality as a consequence of fighting injuries [14]. On the other hand, a laboratory study with the giant water bug *Belostoma flumineum* showed that the mean lifespan of males that had their egg pads removed was not different from either virgin or brooding males [15]. Only two studies using mark-recapture techniques to compare apparent survival probabilities between caring and non-caring males in the field have been conducted so far. For the giant water bug *Abedus breviceps*, males with eggs on their backs paid no survival costs [16], whereas for the assassin bug *R. tristis*, there was evidence of survival costs for caring males [9].

Finally, the reduction of mating opportunities for males during parental care is often pointed out as the most important cost of paternal care in endotherms because a trade-off is expected to exist between parental effort and mating effort [1, 3, 17]. Among many fishes and arthropods, however, eggs laid by different females can be guarded simultaneously, greatly reducing the promiscuity costs for caring males. In fact, observational and experimental evidence for these animal groups clearly show that providing paternal care and acquiring new mates are non-mutually exclusive activities [18-20]. Moreover, theoretical studies have suggested that female preferences for caring males have played an important role in the evolution of paternal care [18, 21-22]. Experimental evidence supporting this suggestion has already been reported for several fishes [e.g., 23-26] and at least two arthropod species [9,27].

Although many theoretical models predict that the maintenance of parental care depends on the balance between costs and benefits of this behavior to the parents [2, 20, 22], empirical information among arthropods is restricted mostly to species exhibiting maternal care [e.g., 6-8, but see 9, 16]. In this paper, we used the Neotropical harvestman *Iporangaia pustulosa* (Arachnida: Opiliones) as a model organism to assess energetic and survival costs of male egg-guarding behavior under field conditions. *Iporangaia* females lay their eggs on the underside surface of leaves, secrete an abundant hygroscopic mucus coat on the clutch, and then abandon the eggs under males' protection [28-29] (Fig. 1A). Paternal care has a crucial protective role, given that unprotected eggs suffer intense predation in a few days [30]. Females show strong preference for caring males [30], which may copulate with several females and care for many eggs simultaneously [28] (Fig. 1B). Given that the clutches are acquired asynchronously by caring males, the total caring period may last up to four consecutive months [28], during which males are likely to experience both higher mortality probabilities and lower food intake when compared to non-caring individuals (males and females).

In this study, we evaluated energetic costs by quantifying foraging activity and estimating how body condition changes over the course of the caring period. Given that *Iporangaia* individuals feed mainly on dead arthropods, actively searching for food on the vegetation (G.S. Requena, unpublished data), we predicted that: (a) caring males would have fewer feeding opportunities when compared to non-caring individuals in the population, and (b) body condition would deteriorate over the course of the caring period. To evaluate survival costs, we conducted a capture-mark-recapture study to estimate apparent survival probabilities and dissociate them from temporary migration and recapture probabilities [31]. Because parental care may increase the susceptibility of caring

males to natural enemies, we predicted that their survival would be lower than that of non-caring males and females. Our study, entirely conducted in the field, provides a comprehensive understanding of the main costs paid by *Iporangaia* caring males. Moreover, our results challenge some widespread ideas on the costs of paternal care and bring insightful implications for the maintenance of paternal care in arthropods as a sexually selected trait.

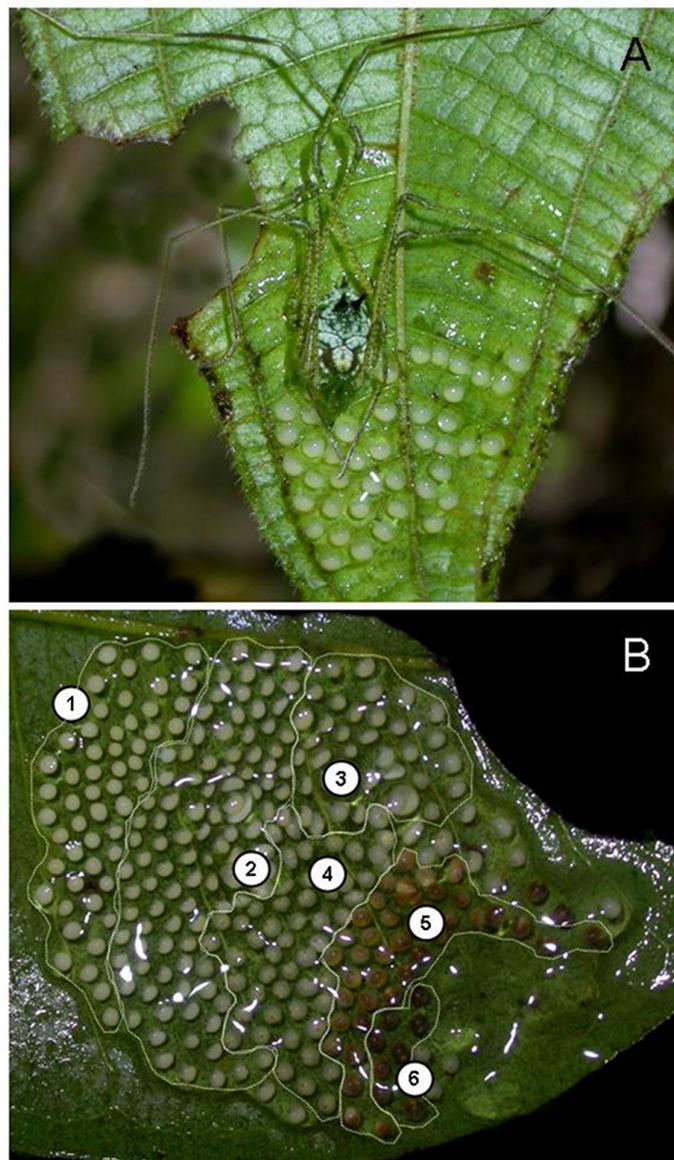


Figure 1. Paternal care in the harvestman *Iporangaia pustulosa*. (A) Male guarding eggs on the underside of a leaf. (B) Egg-batch composed of multiples clutches in different stages of embryonic development (see text for definition of each stage). Note that the eggs are covered by a thick mucus coat.

MATERIAL AND METHODS

Study site

We carried out this study in an Atlantic Forest fragment at Intervales State Park (24°14' S; 48°04' W), in the state of São Paulo, southeastern Brazil. The region has high precipitation levels, with an average annual rainfall of 2000 to 3000 mm/year, and mean annual temperature ranging from 12 to 20 °C. There is a well-marked seasonality in the locality, with a wet and warm period from October to March, and a dry and cold period from April to September, when frosts are common (Fig. 2A). We collected our data along a stream nearly 5 m wide and flanked by abundant vegetation, which sometimes partially covers the stream bed. We established a 200 m transect along this stream and inspected the vegetation at a maximum distance of 1 m from the water in both margins. All procedures presented in the following sections were not conducted with endangered or protected species, and are in accordance with relevant national and international guidelines to ensure ethical appropriateness, for which we obtained all necessary permits from the authority responsible for Intervales State Park (COTEC-IF permit number: 40.625/05).

Capture-mark-recapture

We conducted a one-year capture-mark-recapture study on our 200 m transect between August 2003 and July 2004. We inspected the vegetation flanking the stream three times a day (08.30 - 12.00 h, 14.30 - 18.00 h and 20.30 - 00.00 h) during four consecutive days per month. In each survey, we captured *Iporangaia* adults using an active searching method, recording their sex, their location along the transect (to the nearest 1 m), whether they were feeding and, for individuals captures for the first time, individually marking them with enamel color paint (following protocol described in [32]). After marking, we released the individuals at the same place where we had captured them. We classified males

according to their parental state as caring (i.e., those that were guarding an egg-batch) or non-caring (i.e., those that were not guarding an egg-batch).

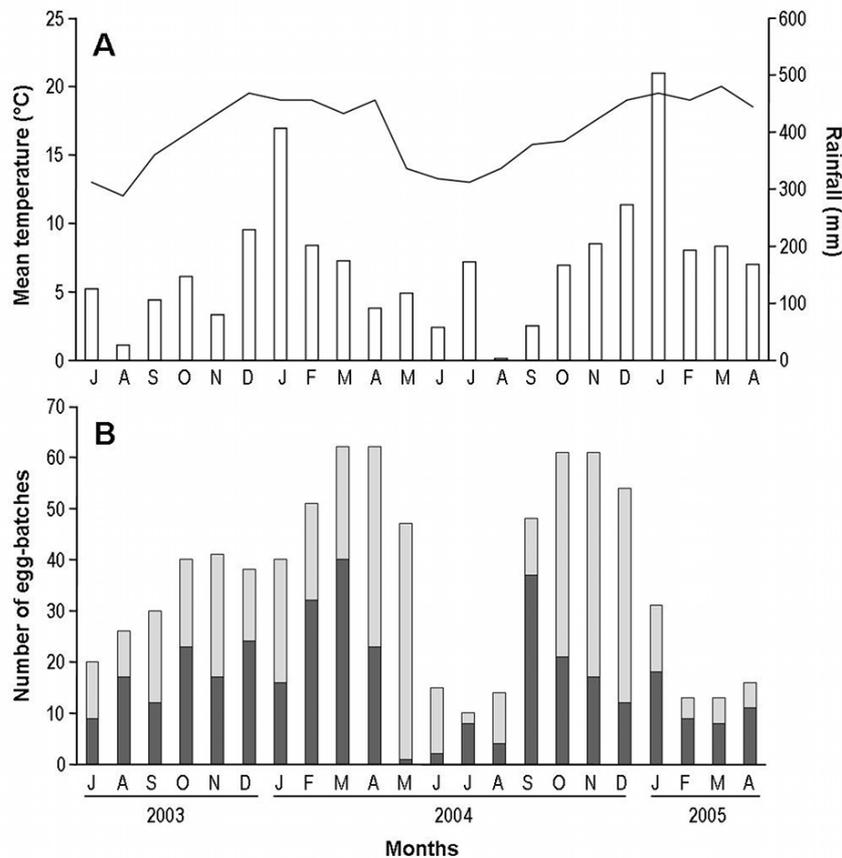


Figure 2. Climatic conditions and number of *Iporangaia* egg-batches observed in the sampled area. (A) The solid line represents temperature; black bars represent the rainfall during the dry-cold season; white bars represent the rainfall during the wet-warm season. (B) Dark-gray bars represent the number of new egg-batches found in each month (i.e., those that were not present in the transect in the previous months); light-gray bars represent old egg-batches (i.e., that were already present in the transect in the previous months).

The capture-mark-recapture procedure also allowed us to estimate how males (caring and non-caring) and females moved along the transect. Considering only individuals that were recaptured at least five times and in at least two different months, we observed that both females (median = 5 m; range = 1 - 31 m) and non-caring males (median = 4 m; range = 1 - 60 m) moved similar distances among different sampling occasions and that it was always longer than the distances moved by caring males (median = 0 m, range = 0 - 10 m)

(analysis of deviance: Δ deviance = 105.5, $df = 1$, $P < 0.001$, Fig. 3A). This information is important to understand the results of the mark-recapture study because movement patterns may influence both recapture and mortality rates (see Discussion). Moreover, given that females and non-caring males have similar movement patterns and do not care for the offspring, we collectively classified them as 'non-caring' individuals in some analyses described below.

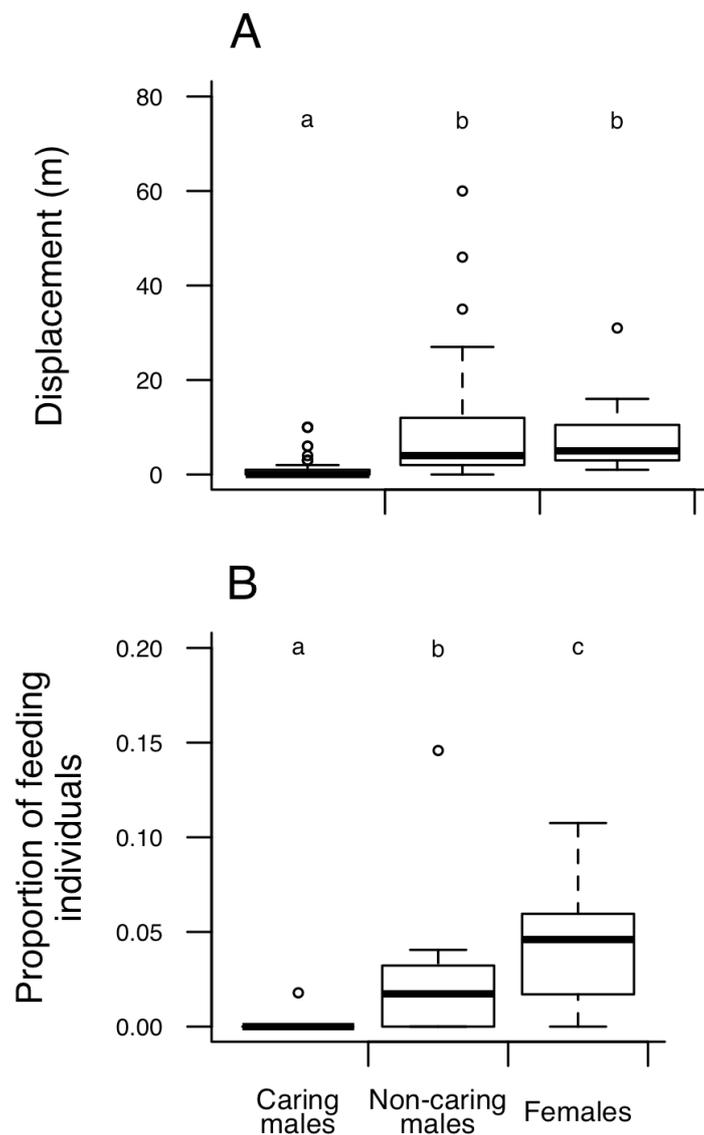


Figure 3. Movement pattern and feeding activity of *Iporangaia* adults. (A) Estimated distances that individuals in different parental states moved in consecutive capture occasions. (B) Observed proportion of individuals (females, caring and non-caring males) found in feeding activities in the field. Different letters represent differences among groups.

Energetic costs

To investigate energetic costs of paternal care in *Iporangaia*, we first quantified if there was any decrease in feeding opportunities to caring males when compared to other individuals in the population. We recorded the monthly number of females and males (caring and non-caring) observed in feeding activities during the capture-mark-recapture period and the total number of individuals recorded monthly in each category during the same period. We used a model selection approach based on the maximum likelihood method [33-34] to compare alternative generalized linear mixed models, representing different biological hypotheses. We built alternative models considering that the monthly proportion of feeding individuals was: (a) not affected by neither sex nor parental state, (b) affected by sex ('males' vs. 'females'), (c) affected by parental state ('caring males' vs. 'non-caring males and females'), and (d) affected both individual categories ('females' vs. 'caring' vs. 'non-caring males'). We built all models considering a binomial distribution of errors and using the sampling month as a random variable.

We used the small sample size bias-corrected version of the Akaike Information Criterion (AIC_c) to rank all models fitted to the data [35]. Then, we selected the model with the lowest AIC_c value as the most parsimonious model describing the data. We also computed the difference in the AIC_c value between the selected model and all other models in the ranking and the relative weight of all models. When this difference is larger than 2, there is strong support to conclude that the model selected is the best one among all candidate models [35]. We built, fit, and selected models using the packages 'bbmle' [36] and 'lme4' [37] in the software R 2.13.1 [38].

Our second approach to evaluate energetic costs of paternal care was to quantify how males' body condition changes over the course of the caring period. Between January and March 2009, we sampled 93 *Iporangaia* males in the field and, using an electronic

caliper (precision of 0.01 mm) and an electronic scale (precision of 0.001 g), we took the following measurements from each individual: (a) dorsal scute length (*DSL*), (b) total body length (*TBL*), (c) body width (*BW*) at the widest portion of the opisthosoma, (d) body height (*BH*) at the highest portion of the opisthosoma, and (e) total body mass (*TBM*). The dorsal scute (or carapace) is a rigid structure that does not change in size with food acquisition and can be classified as a structural body size measure (*sensu* [39]). The last five opisthosomal tergites, on the other hand, are not fused, but rather connected by a highly elastic membrane, allowing body expansion after a meal.

Although body dimensions are presumably correlated with current nutrient storage and have been broadly used to estimate body condition, they actually have the disadvantage of being simultaneously correlated with structural body size [40]. To remove the effect of the latter, we used two different proxies to assess males' body condition (as suggested by [39]): (a) body volume (*V*) controlled by a structural body size measure (i.e. *DSL*), and (b) body mass (*TBM*) controlled by body volume (*V*). In our case, we estimated *V* as an approximation of the ellipsoid according to the following formula:

$$V = \left(\frac{4}{3}\right) \pi \left(\frac{TBL}{2}\right) \left(\frac{BW}{2}\right) \left(\frac{BH}{2}\right).$$

Given that *Iporangaia* eggs change in size and coloration over the course of the embryonic development [28], we estimated the time males had already invested in paternal activities based on features of their egg-batches. During the same summer that we collected data on the body condition of males, we also photographed 20 egg-batches on a daily basis and used the temporal sequence of photos to estimate the duration of each stage of embryonic development, creating an egg development schedule that was used as a proxy for the caring period (Table 1, Fig. 1B). For non-caring males, we attributed the value zero to the duration of their caring period. For caring males, we considered the

oldest eggs in their egg-batches as the time invested in paternal activities (Table 1). The first stage corresponds to recently laid eggs, which are completely white and have not yet absorbed water from the environment. Eggs in the second stage have the same color as those of the first stage, but are larger because of water absorption. In the second stage it is also possible to identify clearly the embryonic formation of legs. Eggs in the third stage are larger than those of the second stage and are generally opaque or milky, with the legs not so clearly identifiable. In the following three categories there is no more difference in egg size. Eggs in the fourth stage are similar in color to those of the third stage, but it is possible to clearly identify two black spots corresponding to the eyes of the embryo. Eggs in the fifth stage are dark yellow or brownish, which corresponds to the beginning of tegumentary pigmentation of the embryo. In the sixth and last stage, immediately before hatching, eggs are almost black (modified from [28]).

Table 1: Duration of each stage of embryonic development in *Iporangaia* eggs during the wet and warm season and the corresponding estimated caring period.

Stage	<i>n</i>	Duration range (days)	Cumulative duration (days)
1 st	20	5 – 10	6.6
2 nd	43	2 – 4	9.7
3 rd	26	6 – 10	18.2
4 th	10	5 – 8	24.5
5 th	10	4 – 5	28.9
6 th	7	2 – 4	32.0

NOTE. - *n* indicates the number of eggs from different oviposition events that were sampled to estimate the duration of each stage. ‘Cumulative duration’ is an estimation of how long it takes for recently laid eggs to complete specific embryonic development stages, calculated as the sum of the median observed duration of all previous stages.

Considering body volume as a proxy for body condition, we conducted the model selection procedure in two steps. First, to control for the effect of body size, we built models in which *DSL*: (a) does not affect *V*, (b) affects only the mean of *V*, (c) affects only the variance of *V*, and (d) affects both the mean and the variance of *V*. We used a linear function to model the influence on the mean parameter of *V* and a power function to model the variance parameter of *V* (as recommended by [34]). Using the best model selected in this first step, we then incorporated the effect of paternal care as: (a) the effect of caring period on the mean of *V* (controlled by body size), (b) caring period on the variance, (c) caring period on both parameters, (d) parental state on the mean, (e) parental state on the variance, and (f) parental state on both parameters. Using total body mass (*TBM*) as another proxy for body condition, we used the same two analytical steps described above to control for the effect of *V* and to evaluate the effect of paternal care on the mean and variance parameters of the *TBM* distribution. We conducted all these analyses and the model selection using the packages 'bbmle' [36] and 'lme4' [37] in the software R 2.11.1 [38], using AIC_c to rank the models fitted to the data [35], as described above.

Survival costs

To quantify the potential survival costs of male egg-guarding behavior, we estimated apparent survival ($\hat{\phi}$) and recapture (p) probabilities of females and males (caring and non-caring), using a statistical modeling approach [31] implemented in the software MARK [41]. We analyzed the data of all individuals together to be able to explicitly address models that consider the same $\hat{\phi}$ or p among individuals of the same sex or individuals performing similar behaviors (parental state). Moreover, we pooled the capture-recapture data obtained during the three periods of the same day to generate a single sampling occasion per day. Therefore, our capture-recapture data set comprises 12 primary occasions (sampling months) and 46 secondary occasions (due to heavy rains, two months had only three days sampled). This so-called robust design model assumes that the population is open during the intervals between primary occasions, during which

individuals may migrate, die or molt to the adult stage in the sampling area, but it is considered closed within each primary occasion because secondary occasions are so close together in time [42]. Therefore, it combines the advantages of closed capture models to estimate p within primary occasions, at the i^{th} month, with the advantages of the Cormack-Jolly-Seber live recapture model to estimate ϕ between consecutive primary occasions, at the interval between the i^{th} and the $(i+1)^{th}$ months (the model is described in details by [43]).

Male parental state is a varying condition in *Iporangaia* because caring males become non-caring males when nymphs hatch and disperse, while non-caring males may copulate and obtain a first clutch, thus becoming caring males. Therefore, we used multi-state models to estimate transition probabilities (ψ) between caring and non-caring states, at the interval between the i^{th} and the $(i+1)^{th}$ months [44-46]. We used a 'Huggins closed robust design multi-state model', which does not include the abundance of individuals as a parameter of the model [42]. Furthermore, we also assumed the same probability to capture individuals for the first time and to recapture them within each primary occasion, and fixed ψ between males and females as zero.

We first assessed the fit of the global model to the capture-recapture history data. This global model considered that ϕ and p were a function of time and individual categories ('caring males' vs. 'non-caring males' vs. 'females'). Furthermore, the global model also considered that ψ between male states ('caring' vs. 'non-caring') was a function of time and parental state of males at the i^{th} month. Our global model did not include interactions between time and individual categories (or parental state) because such a model did not reach convergence. We used the ratio of the model deviance by the model degrees of freedom, obtained by the goodness-of fit test for multi-state models performed

in the software U-CARE [47], to estimate the overdispersion parameter of the global model (\hat{c}). The global model was considered to fit the data adequately if the estimated value of \hat{c} fell between 1 and 3, though the closer the value of \hat{c} is to 1, the better the fit of the model [31].

We compared the global model to three other general models incorporating different surrogates for time-dependent parameters. In the first model, we divided the study period into two seasons, corresponding to the wet-warm season (between October and March) and the dry-cold season (between April and September) (Fig. 2A). In the second and third models, we incorporated either temperature or rainfall as covariates representing time variation over the course of the study period, since both variables are strongly correlated with the reproductive activity of *Iporangaia* (Fig. 2B). For ϕ and ψ estimates, we used the mean values of temperature and rainfall recorded for the days between two consecutive sampling occasions. For p estimates, we used the mean values of temperature and rainfall recorded during the four sampling days of each month. All additional models included interactions between time surrogates and individual categories (or parental state) We compared these four general models and selected the most parsimonious one using the small-sample size Akaike information criterion in the same way described in the *Energetic Costs* above, but corrected for overdispersion (QAIC_c).

After the selection of the general model, we built models in the following way. First, we fixed the global structure for ϕ and p as dependent of the additive effect between the time-related variable and individual categories, and built alternative models that considered ψ between caring and non-caring males as being: (a) constant and not affected by male state at the i^{th} month, (b) affected by the selected time variable, (c) affected by male state at the i^{th} month ('caring' vs. 'non-caring'), and (d) affected by the additive effect

of the selected time-related variable and the male state at the i^{th} month. We also built two additional models in which (e) ψ from caring to non-caring state was constant, but ψ from non-caring to caring state was dependent of the selected time variable, and (f) ψ from non-caring to caring state was constant, but ψ from caring to non-caring state dependent of the selected time variable. We compared all alternative models and selected the best one using the QAIC_c.

With the best selected structure for ψ , and with the structure for \square still fixed as the additive effect between the time-related variable and individual categories, we built a new set of alternative models that considered p as being: (a) constant and not affected by individual categories, (b) affected by the selected time-related variable, (c) affected by individual categories, (d) affected by the additive effect of the time-related variable and individual categories, (e) constant for caring and non-caring males, but affected by the time-related variable for females, (f) constant for caring males and females, but affected by the time-related variable for non-caring males, (g) constant for non-caring males and females, but affected by the time-related variable for caring males, (h) affected by sex ('all males together' vs. 'females'); (i) affected by the additive effect of the time-related variable and sex, (j) constant for males, but affected by the time-related variable for females, (k) constant for females, but affected by the time-related variable for males, (l) affected by parental state ('non-caring males and females together' vs. 'caring males'), (m) affected by the additive effect of the time-related variable and parental state, (n) constant for caring individuals, but affected by the time-related variable for non-caring individuals, and (o) constant for non-caring individuals, but affected by the time-related variable for caring individuals. Finally, we built the same last 15 alternative models for \square and repeated the model selection procedure.

We used the 'step-down' approach described above (and first presented by [31]) to avoid the comparison of all possible models in a single analysis, i.e., (4 structures for the general model) \times (6 structures for ψ) \times (15 structures for p) \times (15 structures for \square) = 5,400 models, which would be a prohibitive, time consuming procedure and would greatly increase the possibility of spurious results [35, 48]. However, it is still not clear if the order in which the structure of parameters is fixed or modeled affects the convergence of different approaches to the same best selected model [31, 48]. In an attempt to avoid biased results due to our specific analytical implementation, we also performed the model selection procedure starting with a general model in which all parameters were considered constant. Both procedures converged to the same best supported model given the capture history data observed. Therefore, for the sake of simplicity, we will focus our results on the first step-down model selection procedure, in which we started with all parameters as dependent of the additive effect between the time-related variable and individual categories. Furthermore, since the estimates of p and ψ are not the main goal of our study, we will focus here on the results on estimates of \square . The results of the second model selection procedure are presented in the Appendix A1 and the values of all additional probabilities included in the best supported model can be found in the Appendix A2.

RESULTS

Energetic costs

We found 501 males and 349 females of *Iporangaia*, recording a total of 3,503 captures and recaptures between August 2003 and July 2004. Of all males captured in the study area, 66.4% were recorded only in the non-parental state ($n = 333$), 12.4% were recorded only in the parental state ($n = 62$), and 21.2% were recorded in both parental states ($n = 106$).

During this period, we observed 35 females, 24 non-caring males, and only one caring male feeding on the vegetation. Therefore, there was a clear effect of individual categories on feeding activity: the frequency of caring males feeding in the field was significantly lower than the frequency of non-caring males, and females were more often found in feeding activities than males in general (Table 2; Fig. 3B).

Table 2: Summary of the model selection statistics for the analysis that evaluated the monthly feeding activity of *Iporangaia* individuals between August 2003 and July 2004.

Predictor variables	AIC _c	K	ΔAIC _c	Weight
Individual category (♂_C vs. ♂_{NC} vs. ♀)	53.4	4	0.0	0.926
Parental state (♂ _C vs. ♂ _{NC} + ♀)	58.7	3	5.4	0.063
Sex (♂ vs. ♀)	62.3	3	9.0	0.011
No effects	76.5	2	23.2	< 0.001

NOTE. Models are ranked by increasing order of their bias-corrected modified Akaike Information Criterion (AIC_c). The best model is indicated in bold. ΔAIC_c is the difference between the AIC_c value of model *i* and the AIC_c value of the most parsimonious model; *K* is the number of estimable parameters in the model *i*; Weight is the Akaike weight of model *i*. The symbols ♂_C, ♂_{NC} and ♀ represent caring and non-caring males, and females, respectively.

The best model to describe the relationship between males' structural body size (*DSL*) and males' body volume (*V*) was the one considering that *DSL* affects only the mean parameter of *V* distribution (Table 3). Then, using this model to control for the effect of body size, the most supported model taking into account the effect of paternal care considers that caring period negatively affects both the mean and the variance of *V* (Table 3). This means that males caring eggs for longer periods have more homogenous and smaller body volumes than non-caring males or males that have just started to care (Fig. 4A-B). Therefore, we showed that our first proxy of body condition (body volume

controlled by structural body size) decreases and is more homogeneous among *Iporangaia* males as caring period increases.

Table 3: Summary of the model selection statistics for the analysis that evaluated the relationship between paternal care and the mean and the variance of body condition proxies (controlled by structural body size) of *Iporangaia* males.

Models		AIC _c	K	ΔAIC _c	Weight
Mean	Variance				
Males' body volume - <i>V</i> (Step 1)					
<i>DSL</i>	-	651.6	3	0.0	0.752
<i>DSL</i>	<i>DSL</i>	653.8	4	2.2	0.248
-	<i>DSL</i>	685.8	3	34.3	< 0.001
-	-	686.5	2	34.9	< 0.001
Males' body volume - <i>V</i> (Step 2)					
<i>DSL + caring period</i>	Caring period	630.6	5	0.0	0.955
<i>DSL + caring period</i>	-	636.8	4	6.2	0.044
<i>DSL + parental state</i>	Parental state	644.7	5	14.0	< 0.001
<i>DSL + parental state</i>	-	646.2	4	15.5	< 0.001
<i>DSL</i>	Caring period	647.4	4	16.8	< 0.001
<i>DSL</i>	Parental state	648.5	4	17.8	< 0.001
<i>DSL</i>	-	651.6	3	20.9	< 0.001
Males' body mass - <i>TBM</i> (Step 1)					
<i>V</i>	<i>V</i>	604.7	4	0.0	0.525
<i>V</i>	-	604.9	3	0.2	0.475
-	<i>V</i>	692.2	3	87.5	< 0.001
-	-	706.6	2	101.9	< 0.001

Cont. Table 3

Models		AIC _c	K	ΔAIC _c	Weight
Mean	Variance				
Males' body mass - TBM (Step 2)					
V + parental state	V + parental state	581.4	6	0.0	0.677
V + parental state	Parental state	583.6	5	2.1	0.231
V + parental state	-	586.4	4	4.9	0.057
V + parental state	V	587.5	5	6.0	0.033
V + caring period	V + caring period	594.5	6	13.0	< 0.001
V + caring period	Caring period	597.6	5	16.2	< 0.001
V + caring period	-	599.3	4	17.9	< 0.001
V + caring period	V	599.7	5	18.3	< 0.001
V	V + parental state	603.9	5	22.5	< 0.001
V	V + caring period	604.5	5	23.1	< 0.001
V	V	604.7	4	23.3	< 0.001
V	-	604.9	3	23.5	< 0.001
V	Parental state	606.1	4	24.7	< 0.001
V	Caring period	606.4	4	25.0	< 0.001

NOTE. - Models are ranked by increasing order of their bias-corrected modified Akaike Information Criterion (AIC_c). The best models in each stage are indicated in bold. ΔAIC_c is the difference between the AIC_c value of model *i* and the AIC_c value of the most parsimonious model; *K* is the number of estimable parameters in the model *i*; Weight is the Akaike weight of model *i*; *DSL* is the males' dorsal scute length (mm); *V* is the males' body volume (mm³); caring period is the estimated time males have already invested in parental activities (see text for methodological details); and parental state is the classification of males into the caring or non-caring categories.

Using total body mass (*TBM*) as another proxy for males' body condition, we found two equally plausible models to describe its relationship with the body size of males: considering the effect of males' body volume on the mean and variance of *TBM* distribution, and considering the effect of *V* only on the mean parameter of *TBM* (Table 3). Therefore, we used both model structures in the second analytical step, which revealed that the best supported model fitted to the observed data takes also into account the influence of parental state on the mean and variance of *TBM* (Table 3). For males of the same size, caring individuals were always lighter than non-caring males (Fig. 4C), although the variance in their body mass was higher (Fig. 4D). Therefore, our second proxy for body condition (body mass controlled by body volume) also negatively responded to variation in the caring period.

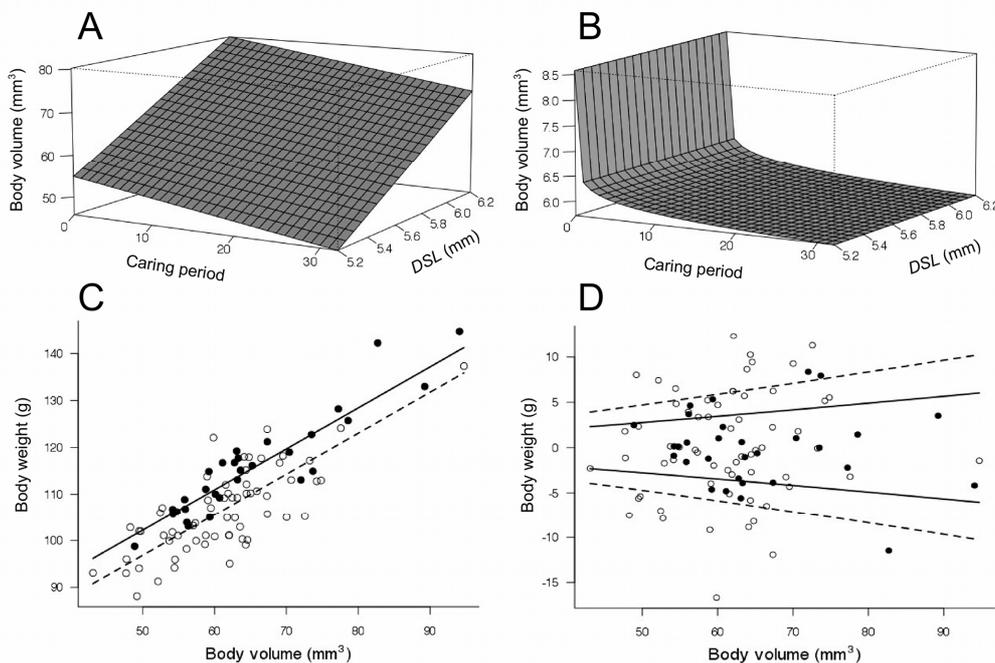


Figure 4. Energetic costs paid by *Iporangaia* caring males. Effect of caring period on the mean (A) and the variance (B) of males' body volume controlled by structural body size, i.e., dorsal scute length (*DSL*). Effect of parental state on the mean (C) and the variance (D) of males' weight controlled by body size, i.e., body volume (*V*). Filled circles and solid lines represent the predicted values for non-caring males, and open circles and dashed lines represent predicted values for caring males.

Survival costs

The global model considering time dependence of all parameters fitted satisfactorily to the capture-recapture history data (goodness-of fit test for the JMV model: $\chi^2 = 535.266$, $df = 485$, $P = 0.057$), resulting in a \hat{c} -value of 1.1036. The global model including the additive effect of time and individual categories was by far the best supported by the data when compared to the models considering rainfall, temperature or seasons as time-related covariates (all had $\Delta \text{QAIC}_c > 50$). The summary of the step-down model selection starting from the global model is shown in Table 4. At the end, the most supported model to explain the observed capture-recapture history data considered: (a) ψ as being influenced by the additive effect between time and the state of males; (b) p as being influenced by the additive effect between time and the individual categories in each sampled month; and (c) ϕ as being influenced by parental state of individuals, with estimates for females and non-caring males depending on time, and estimates for caring males constant over the sampling period (Table 4).

In this sense, non-caring males and females, which share similar behaviors, also showed similar apparent survival probabilities throughout the year. However, the best supported model revealed a general pattern that does not corroborate our initial hypothesis: caring males did not show lower survival than non-caring individuals. In fact, in almost all sampling months the survival estimates for parental males were higher than or at least similar to those of non-caring individuals (Fig. 5).

Table 4. Summary of the step-down model selection procedure for the capture-recapture analysis that investigated the influence of time, sex, parental state, and individual category on the transition probabilities between male parental states, as well as their influence on apparent survival and recapture probabilities of *Iporangaia* individuals between August 2003 and July 2004.

Parameter structure	QAIC _c	K	ΔQAIC _c	Weight
First step – transition probability (ψ)				
Time + initial state	14,802.6	39	0.0	0.919
Time (δ_{NC} to δ_C) & Constant (δ_C to δ_{NC})	14,807.8	39	5.2	0.067
Time (δ_C to δ_{NC}) & Constant (δ_{NC} to δ_C)	14,811.0	39	8.4	0.013
Initial state	14,829.5	29	26.9	< 0.001
Time	14,890.2	38	87.6	< 0.001
Constant and not affected by initial state	14,897.4	28	94.8	< 0.001
Second step – recapture probability (p)				
Time + individual category	14,802.6	39	0.0	0.692
Time (δ_{NC} and ♀) & Constant (δ_C)	14,805.1	38	2.5	0.191
Time + parental state	14,806.1	38	3.5	0.117
Time (♀) & Constant (δ_C) & Constant (δ_{NC})	14,857.5	39	54.9	< 0.001
Time (δ_{NC}) & Constant (δ_C) & Constant (♀)	14,910.0	39	107.4	< 0.001
Time (δ_C) & Constant (δ_{NC}) & Constant (♀)	14,925.9	39	123.3	< 0.001
Time (δ_C) & Constant (δ_{NC} and ♀)	14,928.1	38	125.5	< 0.001
Individual category	14,966.7	28	164.1	< 0.001
Parental state	14,969.0	27	166.4	< 0.001
Time (♀) & Constant (δ)	16,143.1	38	1,340.5	< 0.001
Time + Sex	16,194.4	38	1,391.8	< 0.001
Time (δ) & Constant (♀)	16,238.1	38	1,435.5	< 0.001
Sex	16,254.3	27	1,451.7	< 0.001
Time	16,459.3	37	1,656.7	< 0.001
Constant and not affected by individual category	16,505.9	26	1,703.3	< 0.001

Cont. Table 4

Parameter structure	QAIC _c	K	ΔQAIC _c	Weight
Third step – apparent survival probability ($\hat{\phi}$)				
Time (δ_{NC} and φ) & Constant (δ_C)	14,799.6	38	0.0	0.781
Time + individual category	14,802.6	39	3.0	0.181
Time + Sex	14,806.9	38	7.3	0.021
Time (δ_{NC}) & Constant (δ_C) & Constant (φ)	14,808.5	38	8.9	0.009
Time + parental state	14,809.4	38	9.8	0.006
Time (δ) & Constant (φ)	14,815.3	38	15.7	< 0.001
Time (φ) & Constant (δ_C) & Constant (δ_{NC})	14,815.5	39	15.9	< 0.001
Individual category	14,815.6	29	16.0	< 0.001
Sex	14,816.7	28	17.1	< 0.001
Time (φ) & Constant (δ)	14,816.7	38	17.1	< 0.001
Time	14,820.5	37	20.9	< 0.001
Parental state	14,823.1	28	23.5	< 0.001
Time (δ_C) & Constant (δ_{NC}) & Constant (φ)	14,824.9	39	25.3	< 0.001
Constant and not affected by individual category	14,829.4	27	29.8	< 0.001
Time (δ_C) & Constant (δ_{NC} and φ)	14,832.7	38	33.1	< 0.001

NOTE. - Models are ranked by increasing order of their small-sample size and \hat{c} adjusted Akaike Information Criterion (QAIC_c) for $\hat{c} = 1.1036$. The best models in each stage are indicated in bold. 'ΔQAIC_c' is the difference between the QAIC_c value of model i and the QAIC_c value of the most parsimonious model; 'K' is the number of estimable parameters in the model i ; 'Weight' is the Akaike weight of model i ; 'initial state' represents the status individuals were classified at the i^{th} month. 'Individual category' is a three-level classification factor for females (φ), caring (δ_C) and non-caring males (δ_{NC}); 'parental state' is a two-level a classification factor for caring and males and non-caring individuals (φ and δ_{NC}); 'sex' is a two-level classification factor for females and males (φ and δ); '+' represents the additive effects.

DISCUSSION

Our results indicate that parental behavior imposes energetic costs to *Iporangaia* caring males given that they feed less frequently than other individuals in the population and

that their body condition deteriorates over the course of the caring period. However, the deterioration of body condition while guarding eggs does not seem to negatively affect the survival of caring males. Contrary to our initial hypothesis, survival estimates of males during the caring period were consistently higher than (or at least similar to) during the period in which they were not caring for the offspring. In the following sections, we will discuss these results in details and integrate them to explore the implications of our main findings for sexual selection and parental care theory.

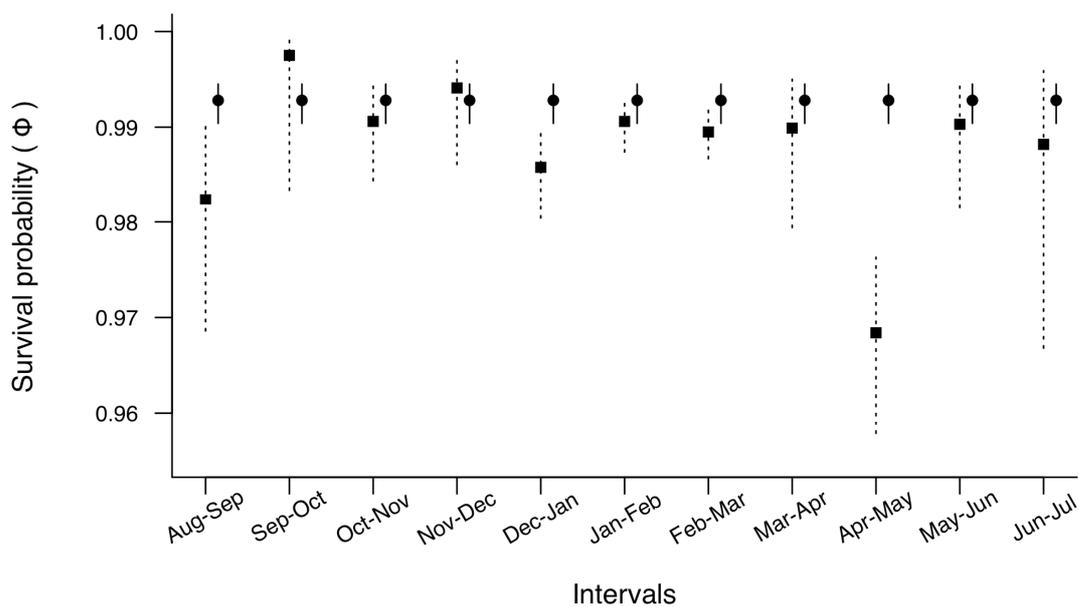


Figure 5. Apparent survival probability estimates for *Iporangaia* individuals according to their parental state. Vertical lines represent 95%CI of the monthly estimates, solid squares and dotted lines represent estimates for non-caring individuals (males and females), while solid circles and solid lines represent estimates for caring males.

Energetic costs

The longer the parental effort of *Iporangaia* males, the worse was their body condition, measured and analyzed using two different proxies. Both body volume (controlled by structural body size) and body mass (controlled by body volume) of caring males decreased as the time invested in egg-guarding increased. Given that the only parental

activity exhibited by *Iporangaia* males is egg-guarding [29], the deterioration of body condition over the course of the caring period is probably the result of reduced food intake, rather than increased metabolic expense while caring for the offspring. Indeed, our field data indicate that caring males feed much less frequently, if at all, than caring males, a result similar to that obtained for another harvestman species with exclusive paternal care, *Magnispina neptunus* (= *Pseudopucroliia* sp.) under laboratory conditions [27].

Most arthropod species exhibiting exclusive paternal care are predators or detritivorous [18], and males have developed strategies to maintain their body condition during the caring period. For example, sea spider males can carry egg-masses and seek food during brooding [13], while caring males of the assassin bug *R. tristis* usually perform filial cannibalism [49]. Instead of cannibalizing eggs, a rare behavior that we have observed only twice during more than 3,000 h of field observations, *Iporangaia* caring males may temporarily abandon the offspring to search for food [28]. However, given that body volume and mass of caring males clearly decreases over the course of the caring period, it seems that *Iporangaia*'s foraging behavior is not as efficient in maintaining caring males' body condition as the strategies reported for sea spiders [13] and assassin bugs [9] — probably because foraging is limited to a small area on the vegetation around the egg-batch where dead arthropods, an unpredictable food source, are likely to be scarce. Our results suggest, therefore, that the energetic costs of male care in this harvestman species are probably higher than the ones paid by the other two arthropod species in which the costs of paternal care have been measured.

Paternal care not only erodes *Iporangaia* males' body condition, but also results in a homogeneous body condition in caring individuals after a month of parental activities (Fig. 4B). Due to the increased attractiveness of males caring for recently laid egg-batches

[30], they may copulate with several females at different moments, potentially prolonging the total caring period to more than three months [28] and, consequently, intensifying the cumulative energetic costs of caring. These costs associated with the extended period of care could explain why only a small fraction of males in the population (33.6%) were found caring for the offspring during one year of intensive sampling and why females avoid mating with males guarding egg-batches containing old eggs [30]. Poorly-fed males or males infested by larvae of phorid parasitoids [50] probably have limited endogenous energy reserves and are unable to pay the energetic costs of paternal care. Furthermore, depleted energy reserves may negatively affect the expected future quality of paternal care, measured as both the ability of males to protect the eggs against potential predators and the frequency and/or the duration of their temporary desertions to seek for food. These males, therefore, should be avoided by ovigerous females, just like has been reported for some fishes with paternal care (e.g. [51-52], but see [53]).

Survival costs

Although parental activities negatively affect body condition of *Iporangaia* males, egg-guarding per se does not seem to impose survival costs upon caring males. Here, we considered that the observed differences in apparent survival probability estimates among adults do represent real mortality, although the effects of permanent emigration and mortality are still confounded, even using a robust design model approach [31, 45]. Two other field studies using a mark-recapture approach showed completely distinct patterns for arthropods. For the giant water bug *Abedus breviceps*, there was no difference in the apparent survival probability between males in caring and non-caring states [16]. The authors argued that males in both parental states are equally exposed to predators (mainly birds) during similar time periods, such as when water bugs go to the surface to take air.

For the assassin bug *R. tristis*, on the other hand, the apparent survival probability of caring males was lower than that of non-guarding males [9]. Apparently, suppression of escape behavior in caring males, rather than their conspicuousness on the host plant, accounts for their lower survival. Due to the paucity of empirical evidence and the controversial results found by the available studies, the effect of paternal care on the survival of caring males certainly deserves further investigation in other arthropod groups. It seems clear, however, that the so-called effect of increased visibility of parental individuals to natural enemies [54] cannot account for all the empirical results reported so far.

Studies with Namib Desert beetles [55] and milkweed beetles [56] have reported that individuals that were more active during the reproductive period (males) were more frequently captured by ambush predators than sedentary individuals (females). In *Iporangaia*, females and non-caring males are constantly searching for mates and/or food inside home-range areas bigger than those of caring individuals, as it seems evident by measurements of individual movements (Fig. 3A). Like the above mentioned beetles, it is plausible that females and non-caring males should be under stronger predation pressure than caring males, which remain close to their egg-batches for long periods. The natural predators recorded for Neotropical harvestmen in southeastern Brazil are frogs, mammals, insects, and spiders [50]. Half of the 18 known predatory species are active hunters (all vertebrate species, one assassin bug, and one ant species), and 66.6% of them forage primarily at the ground level. Thus, they are unlikely to prey on *Iporangaia*, which lives exclusively on the vegetation, mostly between 50 and 250 cm from the ground (G. S. Requena unpub. data). Conversely, the predators that adopt an ambush hunting strategy are spiders (nine different species) that catch their prey on the vegetation. Therefore, it is

reasonable to assume that individuals of *Iporangaia* are more likely to be at risk of predation by ambush predators than by active hunters. Interestingly, the only predation event we witnessed in the field was by a corinnid spider that ambushed a female on the foliage (see Fig. 9.3 in [50]).

Implications for sexual selection

Post-ovipositional maternal care in arthropods is a costly behavior because it reduces foraging opportunities for guarding females during long periods of care and, consequently, their lifetime fecundity [e.g. 7, 9, 57]. Given that the production of sperm and other seminal products generally requires fewer nutrients than does the production of eggs [58], care-related reductions in feeding activities are predicted to be less costly for male reproductive potential than they are for female reproductive potential [1]. In species in which post-zygotic care is crucial for offspring survival, and such care can be provided effectively by either one or two parents, paternal care may minimize the physiological costs related to egg-guarding by females, allowing them to forage immediately after oviposition without sacrificing offspring survivorship [18]. Laboratory data on the harvestman *M. neptunus* demonstrated that foraging rates of females were indeed much higher than those of caring males [27]. Here we demonstrated that, under field conditions, the foraging rate of *Iporangaia* females is also much higher than that of caring males, and similar to that of non-caring males. Allied to the fact that females are iteroparous [28], the maintenance of exclusive post-zygotic paternal care in *Iporangaia* may be favored through sexual selection because it increases male mating opportunities by offering females the direct, fitness-enhancing gift of cost-free care of their offspring [18].

Additionally, paternal care may provide an honest signal of the male's quality as offspring defender, and thus caring males should be selected by females [18]. Indeed, results from another field study provide support for the hypothesis that paternal care in *Iporangaia* is a sexually selected trait, and that caring males are preferred by females [30]. Female choice, however, seems to be influenced not only by the presence of eggs, but also by the age of the guarded offspring: caring males are preferred when they are guarding recently laid egg-batches and avoided when they are guarding old egg-batches in which nymphs have already hatched [30]. As we showed here, the longer the caring period, the worse is the male body condition, which may negatively affect the quality of paternal care (see *Energetic Costs* above). Therefore, female rejection, mediated by poor male body condition and/or his low frequency of egg attendance, may prevent an indefinite increase in the number of eggs in an egg-batch. It is worth noticing, however, that the mucus coat secreted by *Iporangaia* females after oviposition may be viewed as a naturally-selected trait that confers protection to the eggs when starving males temporarily abandon their clutches to forage [28-29]. As a result, if the frequency of egg attendance decreases over the course of the caring period in response to the energetic costs imposed by prolonged male care, females are expected to invest more in the mucus coat when ovipositing in old egg-batches, whose males are probably food deprived. This is a testable hypothesis and *Iporangaia* offers the opportunity to investigate this putative conflict between sexes over the relative parental investment.

Concluding remarks

Most models about life-history theory predict that parental care could evolve only when the benefits in terms of offspring survival outweigh the costs to the parents [2]. Furthermore, classic models usually assume that a trade-off does exist between parental

and matting effort [20]. However, recent theoretical studies propose new benefits for males resulting from egg-guarding (as increased attractiveness and paternity for caring males), and point out that paternal care does not necessarily conflict with males' mating effort [18-20, 22, 59]. Previous results from our research group indicate that paternal care in *Iporangaia* has an important protective role for the offspring, significantly decreasing egg predation [29], at the same time as it increases the attractiveness of caring males [30]. The findings we report here clearly show that food intake and body condition decline during the caring period, but this energetic cost does not reduce the survival of caring males. We conclude, therefore, that paternal care in this arthropod species incurs relatively low costs in relation to great benefits for caring males. Since the male egg-guarding behavior observed in *Iporangaia* is a simple form of parental assistance, further investigations in arthropod species in which males heavily invest in nest defense [e.g. 27, 60] or carry large masses of eggs attached to their own body [11-12], would contribute to a more general understanding of the relationship among the intensity of paternal investment, the costs of caring, and the strength of sexual selection.

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APPENDIX

A.1 Results of the second model selection procedure for the capture-recapture data

Table A1 shows the results of the second model selection procedure for the capture-recapture data. This model selection procedure converged to the same best supported model found in the first step-down procedure, in which we started with all parameters as dependent of the additive effect between the time-related variable and individual categories. The best supported model in both procedures consider: (a) ψ being influenced by the additive effect between time and the state of males; (b) p being influenced by the additive effect between time and the individual category in each sampled month; and (c) ϕ being influenced by parental state of individuals, with estimates for non-caring males and females depending on time and estimates for caring males constant over the sampled period.

Table A.1 Summary of the first step-down model selection procedure for the capture-recapture analysis

Parameter structure	QAIC _c	K	Δ QAIC _c	Weight
First step - transition probability (ψ)				
Time + initial state	16,622.5	14	0.0	0.617
Time (δ_C to δ_{NC}) & Constant (δ_{NC} to δ_C)	16,623.4	14	0.9	0.381
Initial state	16,634.2	4	11.7	0.002
Time	16,648.8	13	26.3	< 0.001
Constant and not affected by initial state	16,650.8	3	28.3	< 0.001
Second step - recapture probability (p)				
Time + individual category [M1]	14,829.4	27	0.0	0.835
Time (δ_{NC} and ϕ) & Constant (δ_C) [M1]	14,832.7	26	3.3	0.160
Time + individual category [M2]	14,840.8	27	11.4	0.003
Time + parental state [M1]	14,841.6	26	12.2	0.002
Time (δ_{NC} and ϕ) & Constant (δ_C) [M2]	14,845.0	26	15.6	< 0.001
Time + parental state [M2]	14,851.8	26	22.4	< 0.001
Time (ϕ) & Constant (δ_C) & Constant (δ_{NC}) [M1]	14,883.7	27	54.3	< 0.001
Time (ϕ) & Constant (δ_C) & Constant (δ_{NC}) [M2]	14,892.6	27	63.2	< 0.001

Cont. Table A.1

Parameter structure	QAIC _c	K	ΔQAIC _c	Weight
Time (δ_{NC}) & Constant (δ_C) & Constant (φ) [M1]	14,965.7	27	136.3	< 0.001
Time (δ_{NC}) & Constant (δ_C) & Constant (φ) [M2]	14,977.5	27	148.1	< 0.001
Time (δ_C) & Constant (δ_{NC}) & Constant (φ) [M1]	15,004.7	27	175.3	< 0.001
Time (δ_C) & Constant (δ_{NC} and φ) [M1]	15,012.9	26	183.5	< 0.001
Time (δ_C) & Constant (δ_{NC}) & Constant (φ) [M2]	15,013.6	27	184.2	< 0.001
Time (δ_{NC}) & Constant (δ_{NC} and φ) [M2]	15,022.0	26	192.6	< 0.001
Individual category [M1]	15,045.8	16	216.4	< 0.001
Parental state [M1]	15,054.1	15	224.7	< 0.001
Individual category [M2]	15,055.0	16	225.6	< 0.001
Parental state [M2]	15,063.3	15	233.9	< 0.001
Time (φ) & Constant (δ) [M1]	16,188.4	26	1,359.0	< 0.001
Time (φ) & Constant (δ) [M2]	16,189.4	26	1,360.0	< 0.001
Time + Sex [M1]	16,254.0	26	1,424.6	< 0.001
Time + Sex [M2]	16,255.0	26	1,425.6	< 0.001
Time (δ) & Constant (φ) [M1]	16,322.1	26	1,492.7	< 0.001
Time (δ) & Constant (φ) [M2]	16,323.1	26	1,493.7	< 0.001
Sex [M1]	16,345.3	15	1,515.9	< 0.001
Sex [M2]	16,346.3	15	1,516.9	< 0.001
Time [M1]	16,558.4	25	1,729.0	< 0.001
Time [M2]	16,559.3	25	1,729.9	< 0.001
Constant and not affected by individual category [M1]	16,622.5	14	1,793.1	< 0.001
Constant and not affected by individual category [M2]	16,623.4	14	1,794.0	< 0.001

Cont. Table A.1

Parameter structure	QAIC _c	K	ΔQAIC _c	Weight
Third step - apparent survival probability ($\hat{\psi}$)				
Time (δ_{NC} and $\delta_{\text{♀}}$) & Constant ($\delta_{\text{♂}}$)	14,799.6	38	0.0	0.781
Time + individual category	14,802.6	39	3.0	0.181
Time + Sex	14,806.9	38	7.3	0.021
Time (δ_{NC}) & Constant ($\delta_{\text{♂}}$) & Constant ($\delta_{\text{♀}}$)	14,808.5	38	8.9	0.009
Time + parental state	14,809.4	38	9.8	0.006
Time ($\delta_{\text{♂}}$) & Constant ($\delta_{\text{♀}}$)	14,815.3	38	15.7	< 0.001
Time ($\delta_{\text{♀}}$) & Constant ($\delta_{\text{♂}}$) & Constant (δ_{NC})	14,815.5	39	15.9	< 0.001
Individual category	14,815.6	29	16.0	< 0.001
Sex	14,816.7	28	17.1	< 0.001
Time ($\delta_{\text{♀}}$) & Constant ($\delta_{\text{♂}}$)	14,816.7	38	17.1	< 0.001
Time	14,820.5	37	20.9	< 0.001
Parental state	14,823.1	28	23.5	< 0.001
Time ($\delta_{\text{♂}}$) & Constant (δ_{NC}) & Constant ($\delta_{\text{♀}}$)	14,824.9	39	25.3	< 0.001
Constant and not affected by individual category	14,829.4	27	29.8	< 0.001
Time ($\delta_{\text{♂}}$) & Constant (δ_{NC} and $\delta_{\text{♀}}$)	14,832.7	38	33.1	< 0.001

NOTE. - Models are ranked by increasing order of their small-sample size and \hat{c} adjusted Akaike Information Criterion (QAIC_c) for $\hat{c} = 1.105$. The best models in each stage are indicated in bold. 'ΔQAIC_c' is the difference between the QAIC_c value of model i and the QAIC_c value of the most parsimonious model; 'K' is the number of estimable parameters in the model i ; 'Weight' is the Akaike weight of model i ; 'initial state' represents the status individuals were classified at the i^{th} month. 'Individual category' is a three-level classification factor for females ($\delta_{\text{♀}}$), caring ($\delta_{\text{♂}}$) and non-caring males (δ_{NC}); 'parental state' is a two-level a classification factor for caring and males and non-caring individuals ($\delta_{\text{♀}}$ and δ_{NC}); 'sex' is a two-level classification factor for females and males ($\delta_{\text{♀}}$ and $\delta_{\text{♂}}$); '+' represents the additive effects. Since two models were equally plausible during the first step, 'M1' and 'M2' represent the specific parameter structure of ψ between parental states during the second step of the model selection procedure, given by the best and the second best models, respectively.

A.2 Supplemental parameter estimates of the best supported model for the capture-recapture data

Although the estimates of the transition probabilities between male parental states fluctuated over the sampling period, the transition estimates from non-caring to caring states were always lower than the corresponding transition estimates from caring to non-caring states (Fig. A1-A). Therefore, at any time, it is less likely than a non-caring male acquire a first clutch and become a caring male than the converse, probably because females prefer to lay eggs with caring males [28]. Regarding the estimates of recapture probabilities, they showed an overall increase between January and March 2004, which coincides with the increase in the reproductive activity registered for that year (Fig. 2B). Furthermore, recapture estimates for caring males were between three and six times higher than estimates for non-caring males during the whole sampling period. Recapture estimates for non-caring males, on the other hand, were just a little higher than estimates for females. This pattern was probably generated due to behavioral differences related to movement patterns and parental care. While caring males usually stay close to their egg-batches, non-caring individuals (males and females) are actively searching for mates, food or shelters on the vegetation (Fig. 3A).

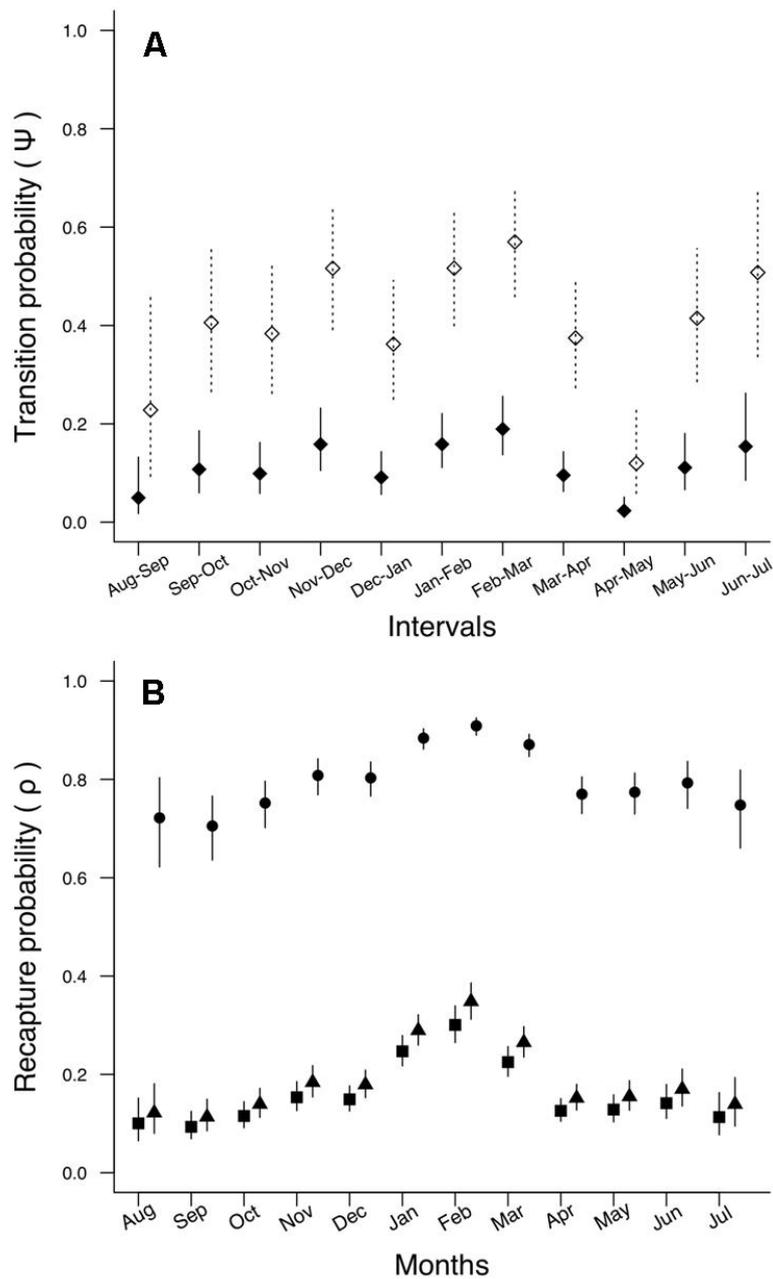


Figure A1: Transition and recapture probabilities estimates for the harvestman *Iporangaia pustulosa*. (A) Transition probability estimates between male parental states. Solid diamonds represent estimates for the transition from non-caring to caring states, while filled diamonds represent estimates for the transition from caring to non-caring states. (B) Recapture probability estimates. Solid squares represent estimates for females; solid triangles represent estimates for non-caring males; and solid circles represent estimates for caring males. Vertical lines in all graphs represent 95%CI of the estimates in corresponding periods.

CAPÍTULO 3

Comparing survival and energetic costs between two Neotropical harvestmen with exclusive paternal care: does higher parental effort impose higher costs to males?

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ABSTRACT

1. Meaningful information on the costs of paternal care should be obtained comparing species with similar biology, but that differ in their parental effort. In these cases, the higher the paternal effort, the higher should be the costs paid by caring males.
2. We tested this hypothesis using two Neotropical harvestman species with exclusive paternal care as model organisms: *Zygopachylus albomarginis* and *Iporangaia pustulosa*. Although males of both species perform parental activities that protect offspring against natural enemies, *Z. albomarginis* males also heavily invest in the construction, maintenance, and cleaning of mud nests. On the other hand, *I. pustulosa* males do not build or defend nests and eggs are laid on leaves, an abundant substrate.
3. We assessed male body condition over the course of the caring period and compared survival rates of caring and non-caring males of these two species using a mark-recapture procedure.
4. Our results do not indicate any difference in body conditions among *Z. albomarginis* territorial and non-territorial males. Moreover, there is no evidence that body condition deteriorates over the course of the caring period, contrary to what happens with *I. pustulosa* caring males. Survival estimates depend on territorial behavior, but the observed pattern is in the opposite direction of our initial hypothesis, with territorial males showing higher survival probabilities than non-territorial individuals (males and females).
5. We discuss possible explanations for discrepancies between theoretical expectations and empirical results, and also point out some useful directions for field studies on the costs of paternal care in arthropods.

Keywords: Body condition, Foraging activity, *Iporangaia pustulosa*, Mark-recapture, *Zygopachylus albomarginis*.

INTRODUCTION

Virtually all models proposed to explain the evolution and maintenance of paternal care explicitly or implicitly take into account the costs and benefits of this behavior (e.g., Trivers, 1972; Maynard-Smith, 1977; Manica & Johnstone, 2004; Kokko & Jennions, 2008; Alonzo, 2011). The benefits of paternal care are generally related to increasing offspring survival, and the great majority of the experimental studies with different taxa exhibiting exclusive paternal care have obtained similar results: the absence of caring males condemns the offspring to death, either because males actively prevent the attack of natural enemies or because males attenuate stressful abiotic conditions (e.g. fishes: Wiegmann & Baylis, 1995; Mackereth et al., 1999; amphibians: review in Wells, 2007; arthropods: review in Requena et al., in press). The costs of caring affect different aspects of the male fitness, increasing mortality risks due to higher exposure to natural enemies during the caring period, decreasing body condition as a consequence of either decreasing foraging activity or increasing metabolic expense associated to parental activities, and decreasing the mating rate due to a trade-off between parental and mating efforts (Gross & Sargent, 1985; Clutton-Brock, 1991).

Among fishes, amphibians, and arthropods with exclusive paternal care, caring for the offspring and acquiring new mates are generally not mutually exclusive activities for males, which usually have many mating opportunities while guarding eggs (fishes: Pampoulie et al., 2004; Lindström et al., 2006; amphibians: review in Wells, 2007; arthropods: review in Tallamy, 2001). Therefore, most studies on the costs of paternal care in these three groups focus on survival and energetic costs. Looking to arthropods, in particular, there are detailed information for representatives of at least four lineages in which paternal care has independently evolved: (a) the water bugs *Belostoma flumineun*

and *Abedus breviceps*, whose males carry egg pads attached to their backs (Crowl & Alexander, 1989; Kight et al., 1995; Munguía-Steyer & Macías-Ordóñez, 2007); (b) the sea spider *Achelia simplissima*, whose males carry egg masses attached to a modified pair of legs (Burriss, 2010); (c) the assassin bug *Rhinocoris tristis*, whose males guard eggs laid on the low vegetation (Gilbert et al., 2010); and (d) the harvestman *Iporangaia pustulosa*, whose males guard multiple clutches on the high vegetation (Requena et al., 2012).

Given that the form and duration of parental activities show great variation among species of the four arthropod lineages mentioned above, the relative importance of survival and energetic costs is likely to show some variation as well. In fact, contrary to the general patterns obtained for the benefits of paternal care, empirical studies on survival costs, for instance, show no consensual results. Whereas caring males have higher mortality rates when compared to non-caring males in the assassin bug *R. tristis* (Gilbert et al., 2010), paternal care does not influence mortality rates of the water bug *A. breviceps* (Munguía-Steyer & Macías-Ordóñez, 2007). In the harvestman *I. pustulosa*, on the other hand, caring males have lower mortality rates than non-caring individuals, including both males and females (Requena et al., 2012). These three studies were conducted in the field, using similar mark-recapture modeling approaches to estimate mortality rates, so that discrepant results cannot be ascribed to methodological differences. Inter-specific comparisons on the costs of paternal care in representatives of such distantly related taxa may be hampered by the fact that many aspects of their ecology and behavior are markedly different. Water bugs, for instance, are aquatic whereas assassin bugs and harvestmen are terrestrial. Moreover, assassin bugs regularly feed on eggs of their own clutches (Thomas & Manica, 2003) whereas harvestmen never practice filial cannibalism (Requena et al., 2012). Meaningful information on the costs of paternal care should be

obtained comparing species with similar biology, but differ in their parental effort. In these cases, we predict that the higher the paternal effort, the higher should be the costs paid by caring males.

In this study, we selected a pair of Neotropical harvestman species exhibiting exclusive paternal care, *Zygopachylus albomarginis* and *I. pustulosa*, to test the prediction mentioned above. Although these species belong to distantly related families in which paternal care has independently evolved (Machado & Macías-Ordóñez, 2007), both live in forested habitats, have similar defense mechanisms and food requirements, and males do not practice filial cannibalism. Parental effort, on the other hand, shows fundamental differences between them. Males of *Z. albomarginis* build cup-like mud nests that are visited by ovipositing females. Males actively invest in repairing these nests and protecting them from invasion of conspecific males, and also in defending the eggs against fungal attack and intense predation by conspecifics and ants (Rodríguez & Guerrero, 1976; Mora, 1990). On the other hand, *I. pustulosa* males do not build or defend nests, and eggs are laid on an abundant and predictable substrate, i.e., leaves on the vegetation flanking streams (Machado et al., 2004). Males effectively defend the eggs against predators, but are unable to prevent fungal infection on the eggs (Requena et al., 2009). Due to asynchronous oviposition events, paternal care in both species can be prolonged, lasting up to five months for *Z. albomarginis* males (range= 20 to 150 days; Mora, 1991) and up to four months for *I. pustulosa* males (range = 30 to 120 days). Besides the difference between species regarding the total caring period, *Z. albomarginis* males seem to periodically leave their nests to forage (Mora, 1991), although there is no precise information on their caring frequency and foraging success, whereas *I. pustulosa* males just occasionally abandon their clutches to forage, but rarely succeed in finding food (Requena et al., 2012).

Costs of paternal care in *I. pustulosa* have already been studied and it is known that egg guarding imposes severe energetic costs to caring males since they feed less frequently than non-caring individuals and their body condition deteriorates over the course of the caring period. However, as stated before, the deterioration of body condition does not negatively affect the survival of caring males since their survival probabilities were consistently higher than non-caring individuals (Requena et al., 2012). Given that parental effort in *Z. albomarginis* is considerably higher than in *I. pustulosa*, we tested two alternative and mutually exclusive hypotheses related to the energetic costs of paternal care: (a) *Z. albomarginis* males should leave their nests with low frequency as a tactic to avoid nest takeovers or egg predation, showing a higher deterioration of their body condition when compared to *I. pustulosa*; or (b) *Z. albomarginis* males could not afford the energetic costs associated to costly parental activities exhibited for long periods, and thus should leave their nests to forage more frequently than *I. pustulosa* males, sustaining good body condition over the course of the caring period. Furthermore, we hypothesize that survival costs should be also higher for *Z. albomarginis* males because they remain for long periods inside a nest that may make them more conspicuous or more easily detectable by potential predators, when compared to *I. pustulosa* caring males which care for the eggs almost hidden under leaves. Additionally, if high metabolic expense associated to parental activities in *Z. albomarginis* imposes severe energetic costs to caring males, it may increase their mortality rates when compared to *I. pustulosa* males.

MATERIAL AND METHODS

Study site

We conducted this study on Barro Colorado Island (09°09' N; 79°51' W), Panama, a biological reserve administered by the Smithsonian Institution. This island has a total area of 1,500 ha and was isolated from the surrounding mainland in 1914, after the Rio Chagres was dammed to form the Gatún Lake, in the middle of the Panama Channel. The island is almost completely forested, with the northeast half portion composed by secondary forest, recovering from widespread cutting dating from the beginning of the 19th century. The other half is composed of old forest that has been maintained undisturbed for the last 400 or 500 years (Windsor, 1990). Given that the average monthly temperature is relatively constant around 27 °C, varying a little throughout the year, the most important seasonal change is associated to fluctuations in rainfall, responsible for two well-marked seasons: (a) the dry season, between late December and early May, when the cumulative rainfall is usually less than 120 mm; and (b) the rainy season, between mid-May and December, when nearly 90% of yearly rainfall occur totaling 1,100 mm (Windsor, 1990).

The reproductive season of *Z. albomarginis* usually lasts six months, between June and December (Mora, 1990, 1991). We carried out our observations between September and November 2009, when most of the males have already constructed their mud nests. We sampled populations in two main sites at the plateau area of the island, where the abundance of individuals is high (Mora, 1991; pers. obs.). The first area (site 1) was a plot approximately 80 m long and 20 m wide, located between Wheeler and Drayton trails. Inside this plot, we conducted both a capture-mark-recapture study to assess survival costs and naturalistic observations to better describe paternal behavior. The second area

(site 2) was a plot approximately 500 m long and 5 m wide along the final portion of Donato trail, where we collected data on caring frequency and conducted additional naturalistic observations.

Capture-mark-recapture study

We conducted a capture-mark-recapture study on site 1 between September and November 2009. Prior to the start of the study, we individually identified three fallen logs and 81 trees in the area, ignoring small trees (with diameter at breast height < 10 cm) where the occupancy by *Z. albomarginis* individuals is very low (Mora, 1991). During this preliminary search, we carefully scanned logs and trees to flag all occupied nests. After that, we inspected each identified log and tree for 2 min, from the ground to the height of 2 m, twice a day for two consecutive days. Since we conducted the study during the rainy season, when intense storms are common (Windsor, 1990), the sampling periods varied, but always included the night (20:00 – 01:00 h). This two-day period was our primary sampling occasions, which we repeated six times, with regular intervals of 10 days between them. In each survey, we actively searched for *Z. albomarginis* adults, recording their sex and the identity of the tree/log where they were found, and individually marking all individuals captured for the first time with enamel color paint (as previously used by Mora, 1990). We additionally classified males as *territorials* (i.e., those owning a nest) or *non-territorials* (i.e., those without nest). After manipulation, we released the individuals at the same place where we captured them.

To derive a direct comparison with the results previously obtained for the harvestman *I. pustulosa* (Requena et al., 2012), we used a similar modeling approach to estimate apparent survival (ϕ) and recapture (p) probabilities of females, territorial males,

and non-territorial males between consecutive primary sampling occasions (Lebreton et al., 1992). Furthermore, given that territorial males may abandon their nests or may be displaced by intruders, and non-territorial males may build a nest, occupy an empty nest or displace territorial males from their nests, we also estimated transition probabilities (ψ) between territorial states in the interval between consecutive primary sampling occasions (Brownie et al., 1993; Lebreton & Pradel, 2002). Therefore, we implemented a 'Huggins closed robust design multi-state model' in MARK program (White & Burnham, 1999), assuming the same probability to capture individuals for the first time and to recapture them within each primary sampling occasion, and fixing ψ between males and females as zero.

We assessed the fit of the global model, fully parameterized as ϕ and p depending on the interaction between time (intervals between consecutive primary sampling occasions) and individual categories (females vs. territorial males vs. non-territorial males) and ψ depending on the interaction between time and territorial state in the previous sampling occasion, by the goodness-of fit test for multi-state models performed in the software U-CARE (Choquet et al., 2009). Since this global model fitted well to the capture-recapture data (goodness-of fit test for the JMV model: $\chi^2 = 54.5$, $df = 69$, $P = 0.899$), we proceeded to the model selection approach. To avoid the comparison of all 605 possible models in a single analysis, we used the step-down approach, first presented by Lebreton et al. (1992) and already used to assess the survival costs of paternal care (Requena et al., 2012). First, we fixed the structure of ϕ and p as in the global model, and built alternative models that considered ψ between territorial and non-territorial males as: (a) constant and not dependent on male state, (b) dependent on time, (c) dependent on male territorial state, (d) dependent on the additive effect of time and male territorial state, and (e) dependent on

the interaction between time and male territorial state.

We ranked the alternative models using the small sample size bias-corrected version of the Akaike Information Criterion (AIC_c) and computed the difference in the AIC_c value between the best ranked model and all other models in the ranking (Burnham & Anderson, 2002). We selected all models in which the difference in the AIC_c was smaller than 2 as the models containing the best structure for ψ . In the second step, we fixed the structure of ψ as observed in the selected models, kept the structure of ϕ still fixed as in the global model, and built a new set of alternative models that considered p as: (a) constant and not dependent on individual categories, (b) dependent on time, (c) dependent on individual categories, (d) dependent on the additive effect of time and individual categories, (e) dependent on the interaction between time and individual categories, (f) dependent on sex (all males together vs. females), (g) dependent on the additive effect of time and sex, (h) dependent on the interaction between time and sex, (i) dependent on territorial state (non-territorial males and females together vs. territorial males), (j) dependent on the additive effect of time and territorial state, and (k) dependent on the interaction between time and territorial state. In the third and last step, we fixed the structure of ψ and p obtained in the best models selected in the second step, and then we built the same 11 alternative models for ϕ , repeating the model selection procedure.

Male body condition

During the capture-mark-recapture study, we also evaluated how male body condition varied between consecutive sampling occasions. With an electronic caliper (precision of 0.01 mm), we measured the following structures of 17 *Z. albomarginis* territorial males and six non-territorial males: (a) dorsal scute length (*DSL*), (b) total body

length (*TBL*), (c) body width (*BW*), and (d) body height (*BH*). Then, we calculated individual body volume as an approximation of the ellipsoid, a procedure previously used for the harvestman *I. pustulosa* (Requena et al., 2012). Moreover, to remove the effect of structural body size (*DSL*) on our body condition proxy (volume), we built alternative generalized linear mixed models (GLMM) in which body volume of *Z. albomarginis* males depended on: (a) *DSL*, (b) the additive effect of *DSL* and male territorial state, (c) the additive effect of *DSL* and the interval between sampling occasions, (d) *DSL* and the additive effect of male territorial state and the interval between sampling occasions, and (e) *DSL* and the interaction between male territorial state and the interval between sampling occasions. All models considered male identity as a random variable because we were dealing with repeated measures.

Additionally, at the last sampling occasion, we collected eight territorial males and nine non-territorial males in the field and brought them to the laboratory where we accessed their mass using an electronic scale (precision of 0.001 g). To directly compare the effect of male attendance both in *Z. albomarginis* and *I. pustulosa*, we included in our data set information on body dimensions of the later species collected in 2009 and already published elsewhere (Requena et al., 2012). We built alternative general linear models that controlled for individual body size and for differences between harvestman species in the absolute values of body structures. Therefore, all models included male body volume and species identity as co-variables, differing in the sense that they also considered male body mass as dependent on: (a) male state (territorial vs. non-territorial for *Z. albomarginis*, and caring vs. non-caring for *I. pustulosa*), (b) the interaction between co-variables, (c) male state and the interaction between co-variables, (d) male state and its interaction with male body volume, (e) male state, its interaction with male body volume, and the interaction

between co-variables, (f) no other predictor variable. Although we built models including the interaction between species identity and male state, all of them were worse than their corresponding models without this term (data not shown) and, for the sake of simplicity, we do not report them in the results.

Besides male body condition, we also recorded whether territorial males were found inside their nests. Given that we followed males in several surveys during the capture-mark-recapture study, we were able to quantify how many times they were or they were not inside their nests. However, as stated before, we surveyed individuals at site 1 mostly at night (18:00 – 01:00 h). Thus we used this information as an estimate for caring frequency at night, using additional data at site 2 (surveys at 08:00 - 12:00 h and 14:00 – 18:00 h) to gather equivalent estimate during daytime. We assessed the same information for *I. pustulosa* males between December 2003 and April 2004, which allowed us to directly compare caring frequency between the two harvestman species since data were collected in similar moments of their reproductive seasons (Mora, 1990; Requena et al., 2012). For both species, we only considered individuals sampled more than six times. We built different GLMMs establishing that male caring frequency depends on: (a) harvestman species, (b) period (day vs. night), (c) the additive effect of species and period, (d) the interaction between species and period, and (e) neither of these two variables. All models considered the binomial distribution of the errors and included the sampling month as a random variable.

In all the analyses described above, we used AIC_c to rank competitive models fitted to the data and applied a model selection approach to simultaneously compared alternative biological hypothesis (Burnham & Anderson, 2002). We built, fit, and selected models using the packages 'bbmle' (Bolker, 2011) and 'lme4' (Bates et al., 2011) in the

software R 2.13.1 (R Development Core Team, 2011). Our two mutually excluding hypotheses lead to two different predictions. If the risk of nest takeover and/or egg predation is high, we expected that *Z. albomarginis* males abandoned their eggs less frequently than *I. pustulosa* males, what should impose stronger energetic costs to the former due to the higher caring frequency. On the other hand, if *Z. albomarginis* males cannot afford the energetic costs associated to costly parental activities exhibited for long periods, we expected they abandoned their eggs more frequently than *I. pustulosa* males, sustaining good body condition over the course of the caring period. Therefore, it is expected a congruence between the results of body condition and caring frequency.

RESULTS

Survival costs

After the step-down procedure to find the best structures of ψ and p , the two selected models considered that ϕ estimates depend only on territorial state (Table 1). The estimates obtained from these selected models show that transition probability from territorial to non-territorial state (1st model: 95% CI = 0.019 - 0.166; 2nd model: 95% CI = 0.020 - 0.172) is lower than the transition probability from non-territorial to territorial state (1st: 95% CI = 0.125 - 0.375; 2nd: 95% CI = 0.121 - 0.367). Territorial males have always higher recapture probabilities than both non-territorial males and females (Fig. 1). Finally, both best ranked models estimate the same apparent survival probabilities, but the results do not corroborate our hypothesis of high survival costs to territorial males. On the contrary, territorial males showed higher survival probabilities ($\phi = 0.984$; 95% CI = 0.970 - 0.991) than non-territorial males and females ($\phi = 0.957$; 95% CI = 0.943 - 0.968).

Table 1. Summary of the step-down model selection procedure for the capture-recapture analysis that investigated the influence of time, sex, male state (territorial vs. non-territorial), and individual category (female vs. territorial vs. non-territorial males) on the transition probabilities (ψ) between territorial states, as well as their influence on recapture probabilities and apparent survival of *Zygopachylus albomarginis* individuals between September and November 2009.

Parameter structure	AIC _c	K	ΔAIC _c	Weight
First step – transition probability (ψ)				
Initial territorial state	1382.9	35	0.0	0.836
Constant and not dependent on initial territorial state	1386.6	34	3.7	0.132
Temporal variation + initial territorial state	1389.8	39	6.9	0.027
Temporal variation	1393.7	38	10.8	0.004
Temporal variation * initial territorial state	1396.6	43	13.7	< 0.001
Second step – recapture probability (p)				
Temporal variation + individual category	1373.1	25	0.0	0.455
Temporal variation + initial territorial state	1374.4	24	1.3	0.245
Individual category	1375.8	20	2.7	0.119
Temporal variation * initial territorial state	1376.2	29	3.1	0.100
Initial territorial state	1376.6	19	3.5	0.079
Temporal variation * individual category	1382.9	35	9.8	0.003
Temporal variation + sex	1475.7	24	102.6	< 0.001
Sex	1478.5	19	105.4	< 0.001
Temporal variation * sex	1480.8	29	107.7	< 0.001
Temporal variation	1559.5	23	186.4	< 0.001
Constant and not dependent on any category	1567.5	18	194.4	< 0.001

Cont. Table 1

Parameter structure	AIC _c	K	ΔAIC _c	Weight
Third step – apparent survival probability ($\hat{\phi}$)				
Initial territorial state^A	1351.6	12	0.0	0.446
Initial territorial state^B	1352.8	11	1.2	0.244
Individual category ^A	1353.7	13	2.1	0.0155
Individual category ^B	1354.8	12	3.2	0.089
Sex ^A	1358.2	12	6.6	0.016
Sex ^B	1358.7	11	7.1	0.013
Constant and not dependent on any category ^A	1358.9	11	7.3	0.012
Temporal variation + initial territorial state ^A	1359.3	16	7.7	0.009
Temporal variation + initial territorial state ^B	1360.5	15	8.9	0.005
Constant and not dependent on any category ^B	1360.7	10	9.1	0.005
Temporal variation + individual category ^A	1361.5	17	9.9	0.003
Temporal variation + individual category ^B	1362.6	16	11.0	0.002
Temporal variation + sex ^A	1366.1	16	14.5	< 0.001
Temporal variation * initial territorial state ^A	1366.3	20	14.7	< 0.001
Temporal variation + sex ^B	1366.5	15	14.9	< 0.001
Temporal variation ^A	1366.6	15	15.0	< 0.001
Temporal variation * initial territorial state ^B	1367.4	19	15.8	< 0.001
Temporal variation ^B	1368.1	14	16.5	< 0.001
Temporal variation * sex ^A	1370.7	20	19.1	< 0.001
Temporal variation * sex ^B	1371.2	19	19.6	< 0.001
Temporal variation * individual category ^A	1373.1	25	21.5	< 0.001
Temporal variation * individual category ^B	1374.4	24	22.8	< 0.001

Models are ranked by increasing order of their small-sample size Akaike Information Criterion (AIC_c). The best model is indicated in bold. K is the number of estimable parameters in the model i ; ΔAIC_c is the difference between the AIC_c value of model i and the AIC_c value of the most parsimonious model; weight is the AIC_c weight of model i ; the symbols + and * represent additive and interaction effects, respectively. In the third step, the letter A represents models in which p depends on the additive effect of temporal variation and individual category, and the letter B represents models in which p depends on the additive effect of temporal variation and initial territorial state.

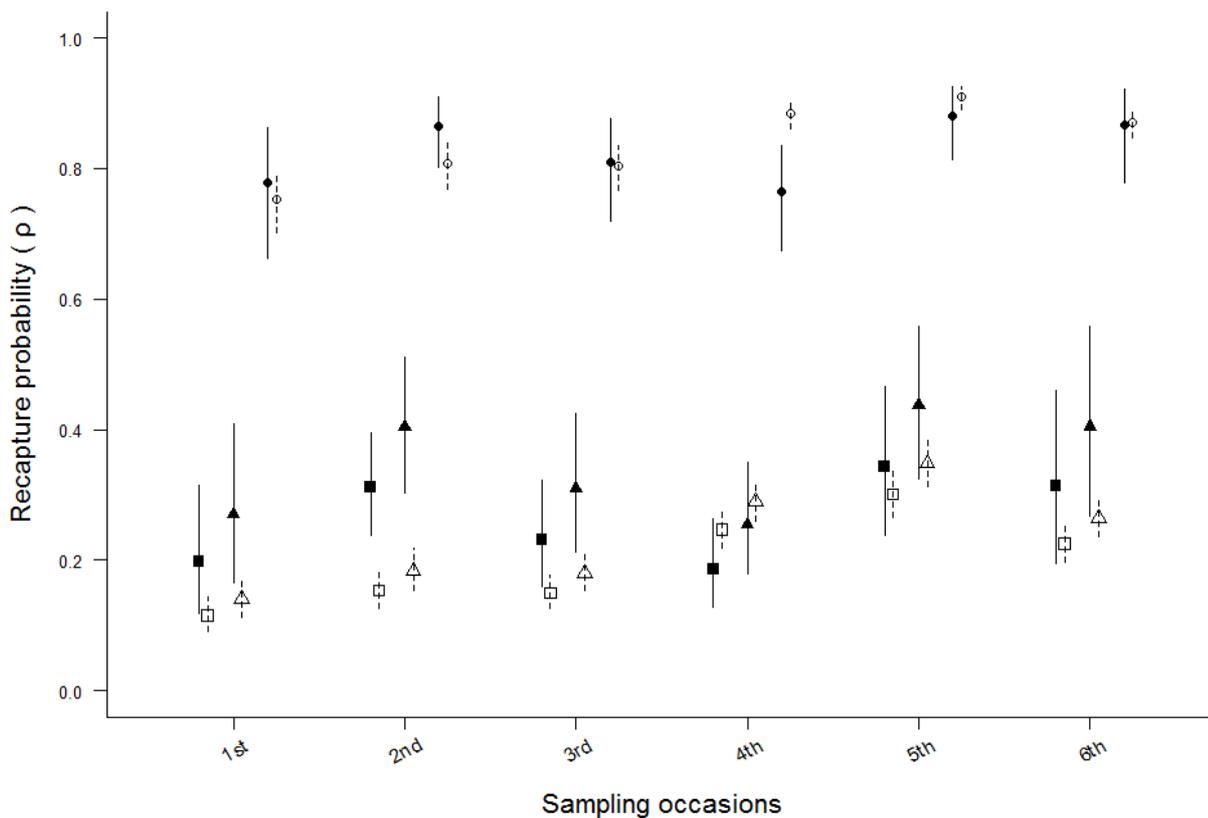


Figure 1. Recapture probability estimates for *Zygodactylus albomarginis* and *Iporangaia pustulosa* individuals during the peak of their reproductive season. Squares represent estimates for females, triangles represent estimates for non-territorial or non-caring males, and circles represent estimates for territorial or caring males. Solid symbols are estimates for *Z. albomarginis* and filled symbols, for *I. pustulosa* individuals. Vertical lines represent 95%CI of the estimates in the corresponding sampling occasions. Values for *Z. albomarginis* were obtained from the best ranked model (Table 1).

Energetic costs

Body volume controlled by structural body size measure is not affected by male territorial state or the interval between two sampling occasions (Table 2). It means that there is no difference in body condition between males in different territorial states, and that their body condition does not decay over time (Table 2). Regarding male body mass controlled by body volume, there is an effect of harvestman species identity and also of the interaction between male parental state and body volume (Table 3). As already reported in a previous study (Requena et al., 2012), *I. pustulosa* non-caring males are in better body condition than caring individuals (Fig. 2A). In *Z. albomarginis*, however, the interaction term of the best fitted model reveals that the difference in body condition between territorial and non-territorial males is very subtle (Fig. 2B).

The best supported model for male caring frequency takes into account the interaction between harvestman species and period of the day (Table 4). Territorial males of *Z. albomarginis* show a more constant caring frequency throughout the day (Fig. 4), since they are equally present inside their nests both during daytime and at night (estimated caring frequency: daytime = 0.766, night = 0.788). On the other hand, *I. pustulosa* caring males show lower caring frequency during daytime (estimated caring frequency: 0.608) than at night (estimated caring frequency: 0.822, Fig. 3).

Table 2. Summary of model selection approach to investigate the effect of male state (territorial vs. non-territorial) and parental investment on male body volume in the harvestman *Zygopachylus albomarginis*.

Predictor variables	AIC _c	K	ΔAIC _c	Weight
Dorsal scute length	109.0	4	0.0	0.767
Dorsal scute length + male state	111.6	5	2.6	0.210
Dorsal scute length + parental investment	116.4	5	7.4	0.019
Dorsal scute length + male state + parental investment	119.2	6	10.2	0.005
Dorsal scute length+ male state * parental investment	128.3	7	19.2	< 0.001

Models are ranked by increasing order of their small-sample size Akaike Information Criterion (AIC_c). The best model is indicated in bold. *K* is the number of estimable parameters in the model *i*; ΔAIC_c is the difference between the AIC_c value of model *i* and the AIC_c value of the most parsimonious model; weight is the AIC_c weight of model *i*; the symbols + and * represent additive and interaction effects, respectively.

Table 3. Summary of model selection approach to compare the effect of harvestman species (*Zygopachylus albomarginis*, n = 17 males; *Iporangaia pustulosa*, n = 93 males), body volume, and male state (territorial vs. non-territorial) on male body mass.

Predictor variables	AIC _c	K	ΔAIC _c	Weight
Harvestman species + body volume * male state	677.2	6	0.0	0.584
Harvestman species * body volume + male state * body volume	679.4	7	2.2	0.201
Harvestman species + body volume + male state	679.9	5	2.7	0.153
Harvestman species * body volume + male state	681.7	6	4.5	0.062
Harvestman species + body volume	698.4	4	21.2	< 0.001
Harvestman species * body volume	700.5	5	23.3	< 0.001

Models are ranked by increasing order of their small-sample size Akaike Information Criterion (AIC_c). The best model is indicated in bold. *K* is the number of estimable parameters in the model *i*; ΔAIC_c is the difference between the AIC_c value of model *i* and the AIC_c value of the most parsimonious model; weight is the AIC_c weight of model *i*; the symbols + and * represent additive and interaction effects, respectively.

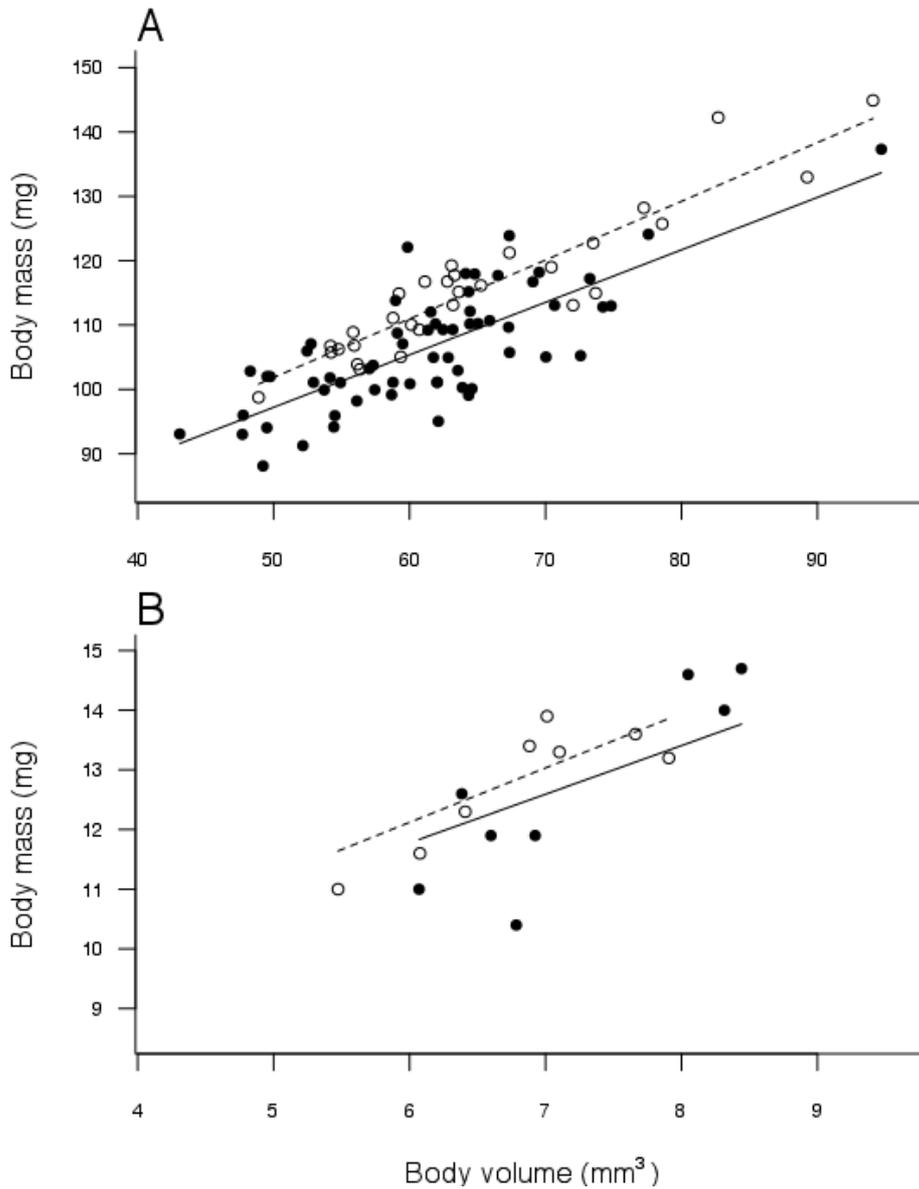


Figure 2. Energetic costs paid by caring males in two harvestman species with exclusive paternal care. Comparison between (A) *Iporangaia pustulosa* and (B) *Zygopachylus albomarginis* regarding the effect of parental state on the relationship between male body mass and male body volume. Solid circles represent caring males and open circles represent non-caring males. Continuous lines represent values for caring males, predicted by the best fitted model to the data (Table 3) and the dashed lines represent values for non-caring males.

Table 4. Comparison of the caring frequency between *Zygopachylus albomarginis* (n = 60 males) and *Iporangaia pustulosa* (n = 361 males) during the peak of the reproductive season. Period of the day refers to data collected during daytime (from 08:00 18:00 h) or at night (from 18:00 to 01:00 h).

Predictor variables	AIC _c	K	ΔAIC _c	Weight
Harvestman species * period of the day	1370.4	5	0.0	0.999
Period of the day	1385.1	3	14.7	< 0.001
Harvestman species + period of the day	1387.2	4	16.7	< 0.001
No effect	1572.9	2	202.5	< 0.001
Harvestman species	1573.1	3	202.7	< 0.001

Models are ranked by increasing order of their small-sample size Akaike Information Criterion (AIC_c). The best model is indicated in bold. *K* is the number of estimable parameters in the model *i*; ΔAIC_c is the difference between the AIC_c value of model *i* and the AIC_c value of the most parsimonious model; weight is the AIC_c weight of model *i*; the symbols + and * represent additive and interaction effects, respectively.

DISCUSSION

There are fundamental differences in the mating system and male parental effort between the harvestmen *Z. albomarginis* and *I. pustulosa*. Although males of both species perform parental activities that protect offspring against natural enemies, *Z. albomarginis* males also heavily invest in the construction, maintenance, and cleaning of mud nests (Mora, 1990). On the other hand, *I. pustulosa* caring males abandon the offspring for short periods, when the protection of eggs is still guaranteed by a mucus coat deposited by females after oviposition (Requena et al., 2009). Due to these differences, we predicted that the energetic and survival costs of paternal care should be higher in *Z. albomarginis* than in *I. pustulosa*. Our results, however, do not indicate any difference in body conditions among *Z. albomarginis* territorial and non-territorial males, with no evidence that body condition deteriorates over the course of the caring period. Moreover, although survival estimates do depend on territorial behavior, the observed pattern is in the opposite direction of our

initial hypothesis, with territorial males showing higher survival probabilities than non-territorial individuals (including males and females). In the following sections, we will first provide a detailed comparison between the energetic and survival costs paid by egg-guarding males of these two harvestman species. Then, we will discuss possible explanations for discrepancies between theoretical expectations and empirical results, and finish pointing out some useful directions for field studies on the costs of paternal care.

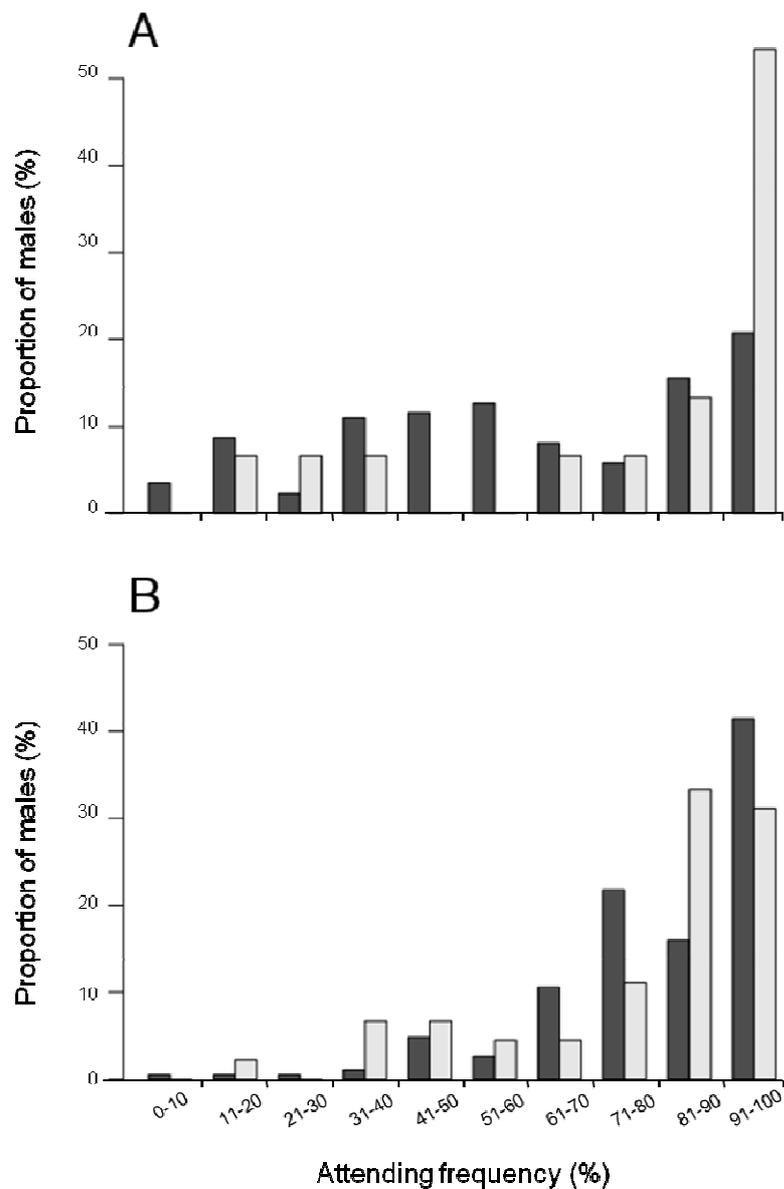


Figure 3. Comparison of the caring frequency between *Zygodachylus albomarginis* (light gray bars) and *Iporangaia pustulosa* (dark gray bars) during daytime (A) and at night (B).

Energetic costs

Territorial males of *Z. albomarginis* may be found in their nests for up to 150 days, but the mean (\pm SE) residency observed in the field during a previous study was 53.9 (\pm 5.05) days (Mora, 1991), which is almost twice longer than the total interval between our first and last sampling occasions when we took body condition measurements. In this interval, however, there was no difference in body condition between territorial and non-territorial males. In a similar time window, *I. pustulosa* caring males showed a marked deterioration of body condition when compared to non-caring males (Requena et al., 2012). It seems, therefore, that *Z. albomarginis* caring and non-caring males have access to food at the same frequency, contrary to what happens with *I. pustulosa* (Requena et al., 2012). In fact, one of our proposed hypotheses considered such possibility, stating that the energetic costs of long lasting parental activities would be so expensive that territorial males would frequently abandon their nests to forage. Caring frequency, however, is very similar between harvestman species at night, while *Z. albomarginis* males were found, in fact, more frequently inside their nests during daytime than *I. pustulosa* males, suggesting that even during the caring period males of the former are not food deprived and probably have access to food while attending their nests.

The lack of influence of parental effort on male body condition has already been described for other two arthropods with exclusive paternal care: the sea spider *Achelia simplissima* (Burris, 2010) and the assassin bug *Rhinocoris tristis* (Gilbert et al., 2010). Authors argued that males of these two species are able to maintain their body condition because they may either forage while carrying the eggs attached to their bodies (sea spiders) or engage in filial cannibalism (assassin bugs). These two explanations, however, seem not to apply to *Z. albomarginis*, since males show high caring frequency and they

have never been observed eating eggs inside their own nests (Mora, 1990). The list of food items of *Z. albomarginis* territorial males includes carcasses of dead insects, fruits, insect larvae, and termites (Mora, 1991), which are frequently seen close to the nests on logs (pers. obs.). Furthermore, nests are frequently infected with fungi and males may spend several days eating fungal hypha to avoid egg infection (Mora, 1990). On the other hand, during more than 3,000 h of field observations, we found only one *I. pustulosa* caring male feeding on the vegetation and only two engaging in filial cannibalism (Requena et al., 2012). Thus, frequent access to food while caring for the eggs in a tropical rainforest may significantly reduce energetic costs to *Z. albomarginis* males and explain why they are able to sustain parental activities for up five consecutive months.

Survival costs

Territorial males of *Z. albomarginis* were not more vulnerable to be attacked by predators due to their long permanence inside a conspicuous structure, which also make caring males a predictable prey both in space and time (Fig. 1). Contrary to our initial prediction, territorial males showed higher survival probability than non-territorial males and females. Naturalistic observations obtained during our fieldwork indicate that non-territorial males and females move more than territorial males. Although we have not accessed vertical movements on trees, we have data on individuals recaptured on different trees inside the site 1 where we conducted the mark-recapture study. All individuals (n = 8) that moved from one tree to another were in the non-territorial state, including four females and four non-territorial males. Moreover, females and non-territorial males showed lower recapture rates when compared to territorial males, which is probably a consequence of differences in movement patterns among them (see also Buzatto et al.,

2011, Requena et al., 2012). The causal link between movement patterns and survival probability seems to be more general than previous thought. The same relationship was obtained for *I. pustulosa*, with caring males consistently showing higher survival probabilities than non-caring males and females. In this species, precise quantification of individual movements showed that non-caring individuals (both males and females) move longer distances on the vegetation than caring males, which are stationary most of the time (Requena et al., 2012). We discussed that lower survival probabilities of non-caring individuals may be related to stronger predation by ambush spiders, which direct their attacks almost exclusively to preys moving on the vegetation. In several arthropods with no parental care, males move more than females during the breeding season and, at least for two beetle species, moving individuals are more frequently captured by ambush predators than sedentary individuals (McCauley & Lawson, 1986; Polis et al., 1998).

Concluding remarks

In the last 20 years, we watched dramatic changes and fast advances in our theoretical comprehension of parental investment (e.g., Manica & Johnstone, 2004; Kokko & Jennions, 2008; McNamara et al., 2009; Alonzo, 2011). These advances, however, do not seem to have increased our predictive power. We began this study arguing that meaningful information on the costs of paternal care should be obtained comparing species with similar biology, but that differ in their parental effort. We then presented two harvestman species for which we have information on several aspects of their reproductive biology. Based on the available information, we formulated the hypothesis that higher paternal effort should impose higher costs to caring males. Even though this hypothesis directly derives from widely accepted theories of parental investment and life-history trade-offs (Clutton-Brock,

1991), we found surprising discrepancies between theoretical expectations and empirical results. Rather than invalidating the theory, these discrepancies call our attention to the need of basic data on the behavior and ecology of the studied species. For *Z. albomarginis*, for instance, we lacked crucial a priori information on the caring frequency and the foraging opportunities of caring males. For both *Z. albomarginis* and *I. pustulosa*, we still do not have any information on the main natural enemies of the adults.

One of the great powers of behavioral ecology is its strong mechanistic basis and the possibility to formulate clear a priori hypotheses based on a solid theoretical framework. More than looking for patterns, behavioral ecologists are interested in the understanding processes (Alcock, 2009). Therefore, investigations on the costs of paternal care should be preceded by quantification on relevant aspects of the natural history of the study organism. Moreover, we should also pay attention on methodological issues because divergent results on the costs of paternal care in arthropods can be partially explained by differences on how data were gathered and analyzed. Survival costs, in particular, have been estimated both in the field and in the laboratory using either an experimental approach or mark-recapture protocols. At the same, different authors have been using different proxies of body condition to access energetic costs.

As a recommendation to increase our scientific rigor and to make results from different studies more comparable, we strongly suggest that future studies on the survival costs of paternal care use a mark-recapture modeling approach because it dissociates survival from recapture through maximum likelihood techniques using the encounter histories of individuals under natural field conditions (Lebreton et al., 1992). Regarding the energetic costs, whenever possible researchers should avoid proxies of body condition correlated with structural body size, such as linear size measures or body mass. To remove

the effect of body size and accurately assess body condition, estimates controlling for the effect of structural body size or based on body density should be taken into account because they increase the power to detect condition differences in terms of nutrient storage (Moya-Laraño et al., 2008). Finally, whenever possible, we should feed our models and hypotheses with fine naturalistic information otherwise we will be condemned to post hoc explanations.

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CAPÍTULO 4

Sex roles in the Neotropical harvestman *Iporangaia pustulosa*: evidence of mutual mate choice in an arachnid with exclusive paternal care

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ABSTRACT

Paternal care has independently evolved in at least 14 arthropods lineages, but male mate choice has been described for only three species belonging to distantly related taxa. Here we first describe the mating behavior of the Neotropical harvestman *Iporangaia pustulosa*, in which males care for eggs laid on the vegetation. Then, based on information of the reproductive biology and population ecology of this species, we tested a theoretical prediction that both males and females would be expected to exhibit some degree of mate choice. Our data were entirely obtained under natural field conditions and are based on 46 mating interactions. Although pre-copulatory and copulatory courtship is performed exclusively by males, mate search is performed exclusively by females, which always approached stationary caring males on the vegetation. Upon arrival, more than 30% of the visiting females were repelled by caring males before copulation. After copulation, on the other hand, nearly 50% of the females abandoned the male without ovipositing. Given that harvestmen are non-visual organisms, chemical and tactile cues probably play a decisive role in mutual evaluation during and after mating. Chemical cues, in particular, may come from the mating partner, signaling body condition or fecundity, and also from the clutch, honestly indicating the presence or the quantity of eggs. Based on chemical information, males may reject females with low fecundity, and both copulated and non-copulated females may abandon males without laying eggs.

Keywords Copulation, Courtship, Mate choice, Mate search, Oviposition, Sexual selection, Sperm depletion

INTRODUCTION

Most of the empirical and theoretical studies on mate choice have focused on female preferences (Clutton-Brock 2009), although both males and females can selectively choose their sexual partners (Kraaijeveld et al. 2007). This bias is probably a side effect of the recognition of an apparently widespread pattern in nature, according to which males are more competitive, less discriminating in relation to sexual partners, and less willing to care for the offspring than females (Clutton-Brock 2009). However, the list of species in which males heavily invest in nuptial gifts or in parental care has exponentially increased in the last decades, revealing cases of partial or complete sex-role reversal, with female-female competition for access to males and even exclusive male mate choice (e.g. Vahed 1998; Wilson et al. 2003; Wells 2007; Gwynne 2008; Aisenberg & González 2011; Requena et al. *in press*). In fact, several authors have suggested that rather than an exception to the rule, male mate choice has been underestimated in the past and it is probably more common than previously thought (Bonduriansky 2001; Bateman & Fleming 2006; Kraaijeveld et al. 2007; Edward & Chapman 2011).

The traditional framework first put forth by Trivers (1972) postulates that biases on the ratio of sexually active individuals in a population at a given time (OSR) leads to intense intra-sexual competition among individuals of the over-represented sex, and choosiness among individuals of the limiting sex. However, recent mathematical models on the evolution of sex roles have challenged classic foundations of behavioral ecology, highlighting conditions that promote either conventional or reversed sex roles (Kokko & Johnstone 2002; Manica & Johnstone 2004; Kokko & Jennions 2008; Barry & Kokko 2010; Alonzo 2011). Early models, for instance, did not take into account the so-called *Fisher condition*, which states that under an even adult sex ratio population, males cannot, on

average, produce offspring faster than females (Queller 1997). This constraint has profound implications for the evolution of choosiness, so that new theoretical models suggest that male mate choice may occur under a broader combination of conditions than previously realized, even under conventional sex roles and male-biased OSR (e.g. Houston & McNamara 2005; Kokko & Jennions 2008). Mate choice is expected to be affected not only by the level of parental investment or potential reproductive rates (which ultimately changes the OSR), but also by mate encounter rates, the costs of breeding and searching for mates, sex-specific mortalities, and variations in mate quality (e.g. Bonduriansky 2001; Bateman & Fleming 2006; Barry & Kokko 2010; Edward & Chapman 2011; Table 1).

Among arthropods, paternal care has independently evolved in at least 14 lineages (Requena et al., *in press*), but male mate choice has been described for only three species belonging to distantly related taxa: the harvestman *Zygopachylus albomarginis* (Mora 1990), the giant water bug *Belostoma flumineum* (Kight & Kruse 1992; Kight et al. 2000; Kight et al. 2011), and the sea spider *Propallene saengeri* (Bain & Govedich 2004). These species show different degrees of sex role reversal, with evidences of female exclusive initiation of courtship behavior in *P. saengeri* and *Z. albomarginis*. Moreover, females of these two species fight each other for the access to potential mates (Mora 1990; Bain & Govedich 2004). The paucity of cases of sex role reversal in arthropods should not be taken as synonym of rarity because only a few species have been detailed investigated. It would not be surprising, therefore, if additional cases of sex role reversal were described as our knowledge increases. A good candidate to start with is the Neotropical

Table 1. Factors predicted to influence mate choosiness and their respective proxies. Values are presented for males and females of the Neotropical harvestman *Iporangaia pustulosa*, and were collected between October 2003 and March 2004 (peak of the reproductive season).

Factor	Proxy	Sex	
		Male	Female
Encounter rate ^a	Population density (individuals/m ²)	0.912 (\pm 0.172)	
	Sex ratio (males:females)	1.426 (\pm 0.245)	
	Mate encounter rate ^b	0.764	1.089
Breeding cost	Gamete replenishment	No data available	No data available
	Daily mortality rate when caring ^a	0.0072 (0.0055 – 0.0096)	Not applied
Mate searching cost ^a	Daily mortality rate when not caring	0.0098	0.0098
		(0.0025 – 0.0196)	(0.0025 – 0.0196)
Quality variation	Carapace length (mm) ^c	Non-caring males: 3.9% (3.2 - 4.7%)	
		Caring males: 3.4% (2.8 – 4.0%)	3.8%
		All males: 3.8% (3.1 - 4.6%)	
	Body volume (mm ³) ^d	Non caring males:19.5%	
		Caring males: 14.1%	15.2%
		All males: 16.7%	

Population density and sex ratio are expressed as mean \pm SE. Given that daily mortality rate estimates vary over the course of the reproductive season, values are expressed as mean (95%CI in parenthesis). (a) Population estimates obtained from a previous work (Requena et al. 2012). (b) The rate at which an individual encounter receptive mates is equal to the population density when sex ratio is 1:1. In case of biased sex ratio, males encounter females at a rate of the ratio between population density and the square root of sex ratio, whereas females encounter males in a rate of the product of both quantities (Kokko & Monaghan 2001). (c) The carapace is a rigid structure in harvestmen that does not change in size with food acquisition and is frequently used as a proxy for fixed, genetic quality of individuals. Due to differences in sample size for males (caring males: n = 254 individuals; non-caring males: n = 973 individuals) and females (n = 44 individuals), we generated estimates for males by resampling 44 individuals without replacement 1,000 times and the values are expressed as mean (95%CI in parenthesis) of the distribution of coefficient of variation. (d) The last five opisthosomal tergites in harvestmen are not fused, but rather connected by a highly elastic membrane, allowing body expansion after a meal. For this reason, we used body volume as a proxy for condition-dependent and fecundity quality of individuals, and we calculated it as an approximation of the ellipsoid from adults collected between January and March 2009 (31 non-caring males, 63 caring males and 44 females).

harvestman *Iporangaia pustulosa*, for which we have accumulated information for several aspects of its reproductive behavior and population ecology, which allow us to test specific predictions from recent models about sex role evolution.

Females of this harvestman species lay their eggs exclusively on the underside surface of leaves of plants growing at the margin of forest streams, and abandon their eggs under the males' guard (Machado et al. 2004). Although paternal care has a crucial protective role in this species, since unprotected eggs suffer intense predation in a few days, *I. pustulosa* females also invest in offspring protection depositing a thick mucus coat on the eggs just after oviposition. This mucus coat has already been demonstrated to provide physical protection to the egg, decreasing predation rates by conspecifics (Requena et al. 2009). Despite the great benefit in terms of offspring protection, paternal care reduces feeding opportunities during the caring period and deteriorates body condition of caring males. However, the energetic cost paid by caring males does not negatively affect their survival, since mortality rates of caring males is consistently higher than the mortality of non-caring individuals, including males and females (Requena et al. 2012). Additionally, paternal care does not constrain mate acquisition, so that caring males can accumulate eggs from several females in multiple clutches that may have up to 400 eggs (Requena et al. *in press*).

Like females of other harvestman species with paternal care, *I. pustulosa* females undergo continuous gonadotrophic cycles (Machado et al. 2004), suggesting that they are able to mature eggs relatively faster. Although we have no information on the costs of sperm production in the species, foraging activities of caring males are severely limited (Requena et al. 2012) and it may negatively affect gamete replenishing. Moreover, whereas males and females seem to have a similar degree of variation in carapace length (a proxy

of structural body size that does not change through the time with food acquisition), females show a little more variation than males in body volume (a proxy of body condition or fecundity that may change through the time; Table 1 and Fig. 1). At the population level, the combination of a high abundance of individuals and a male biased adult sex ratio observed in the field during the peak of reproductive season (Requena et al. 2012) leads to females potentially encountering receptive mates more frequently than males (Table 1). Finally, as stated before, females are slightly more prone to die while looking for mates than caring males (Table 1).

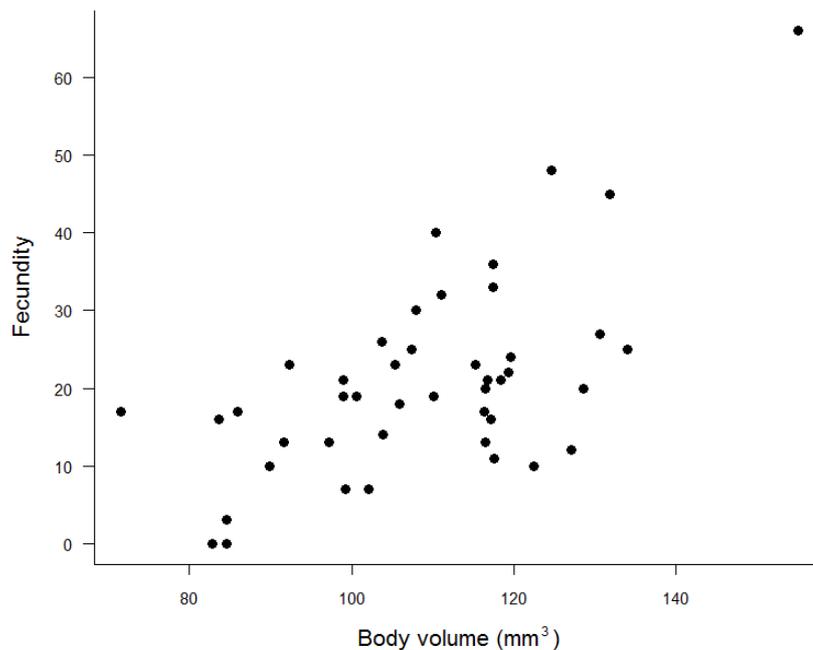


Fig. 1. Correlation between female body volume and fecundity in the harvestman *Iporangaia pustulosa*. Body volume was estimated as an approximation to ellipsoid and the fecundity was accessed dissecting females collected in February 2009 (in the middle of the peak of the reproductive season), and counting the number of mature eggs inside their reproductive tract. Given that *I. pustulosa* females undergo continuous gonadotrophic cycles, we found oocytes in several stages of the gametogenesis in the reproductive tract, but we included only mature eggs (sensu Machado et al. 2004) as a measure of fecundity.

Under the conditions specified above, the differences between sexes seem to be reduced and recent theoretical models predict that both males and females would be expected to exhibit some degree of mate choice (e.g. Bonduriansky 2001; Bateman & Fleming 2006; Barry & Kokko 2010; Edward & Chapman 2011). Here we tested this prediction using behavioral data on mating interactions between caring males and females. Our data were entirely obtained under natural field conditions, in the same population where previous behavioral and ecological data have been gathered. The results obtained here add more information on the *I. pustulosa* reproductive biology and provide empirical evidence of mutual mate choice in an arthropod species with paternal care.

MATERIAL AND METHODS

We conducted the study in an Atlantic Forest fragment at the Intervales State Park (24°14' S; 48°04' W), in the state of São Paulo, southeastern Brazil. The region has a markedly seasonal weather, with a wet period lasting from October to March, and a dry period from April to September. Temperature, on the other hand, is relatively stable throughout the year, ranging from 12 °C to 20 °C. Between February and March 2009, which corresponds to the peak of the reproductive season (Requena et al. 2012), we inspected the vegetation up to 1 m from both margins of a forest stream (approximately 5 m wide) along a transect of 200 m.

We located 42 clutches of *I. pustulosa* and individually marked all males observed guarding them using enamel color paint applied to their dorsum and forelegs (following Requena et al. 2012). Then, we daily monitored all clutches, conducting intensive focal observations at night (19:00 – 02:00 h), when we used a flashlight covered with a red filter to minimize the disturbance to the individuals. In each survey, we spent at least 1 min

observing each clutch and looking for females in its vicinity (ca. 50 cm). If no female approached the clutch during this time, we moved to another clutch and started a new observation. When we found a female in the vicinity of a clutch, we conducted a focal observation with continuous record of the events (cf. Martin & Bateson 1994) until she walked away (> 1 m from the clutch). In these cases, we classified it as 'complete interaction' because we were able to follow female approach since the beginning. In contrast, when we found a male and a female already interacting at the time we started our observations, we classified it as 'partial interaction'.

During male-female interactions, we recorded whether both individuals accepted copulation, which individual initiated and ended copulation, which behaviors males and females performed during copulation, how long was copulation, whether individuals stayed together after copulation, how long was the period between copulation and oviposition, what individuals of each sex did between copulation and oviposition, and how long was the oviposition. We also individually marked all females after they walked away from the clutch to investigate whether they returned to the same clutch to copulate again or to lay more eggs.

RESULTS

We observed a total of 46 mating interactions, being 15 complete and 31 partial sequences. Every time a female approached a clutch, the caring male quickly moved toward her and tapped her with his front legs (n = 41; Fig. 2). In 15 of these occasions, males repelled adult females from their clutches, extending their spiny pedipalps towards them and grabbing their front legs (Fig. 2). Females never responded to the male's attack and always moved away from the clutches. In 26 cases in which females were not repelled, the male

approached the female frontally and grasped her pedipalps with his own pedipalps. Subsequently, the male raised the anterior portion of the female's body putting their ventral sides in close contact. In this position, the male everted his penis and inserted it into the female's genital opening (Fig. 2). This pre-mating stage was always fast, never lasting more than one minute, and resulted either in female rejection or copulation.

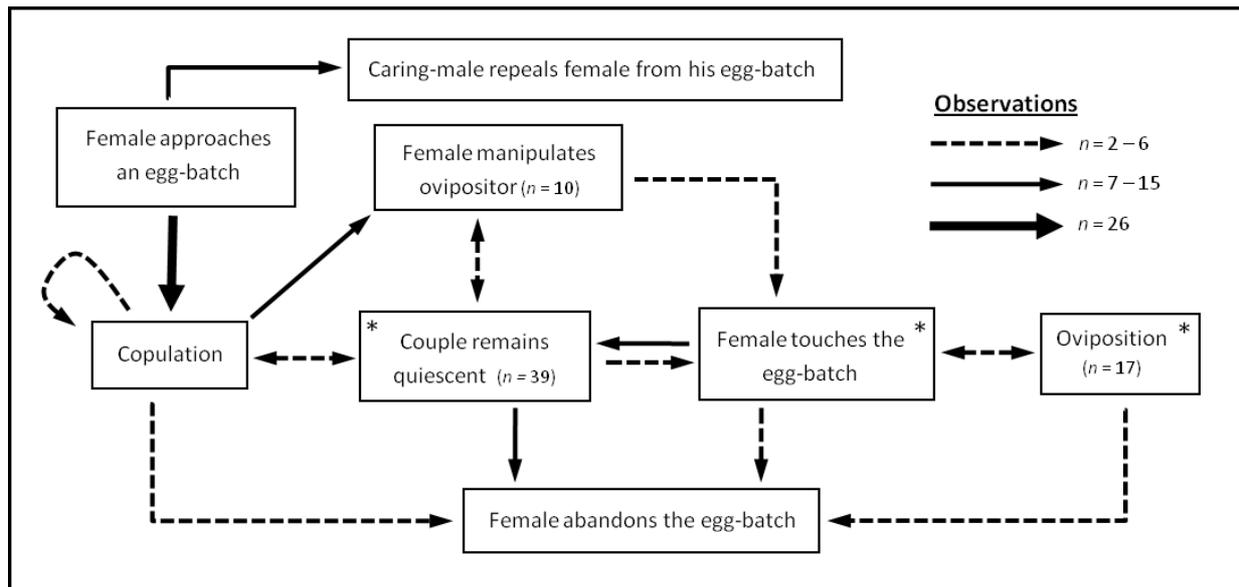


Fig.2. Flow diagram showing the sequence of male-female interactions in the harvestman *Iporangaia pustulosa*. The frequency of each behavior is indicated in parenthesis inside the boxes and the frequency of each transition is proportional to the arrow width. Some behavioral sequences were not observed since the beginning and, as they are included in this flow diagram, sample sizes are not cumulative. Initiation of partial observations is pointed out by asterisks.

Intromissions lasted up to 180 s (mean \pm SD = 139.8 \pm 30.2 s, n = 10). During this period, the male kept holding the female with his pedipalps, occasionally also holding females' legs using the tip of his second pair of legs. Furthermore, males intensely and repeatedly touched the basis of their penis and the first pair of legs of their partners using their own first pair of legs. At the end of the intromission, females always pushed back their bodies promoting the removal of the penis from their genital opening, and the subsequent behavior of males and females did not follow a rigid sequence (Fig. 2).

After copulation, males usually stayed quiescent, with their second pair of legs extended forward, gently touching females ($n = 32$ observations; Fig. 2). In three occasions, the couple stayed quiescent for up to 130 s, but after this period, the male resumed intromission. Females, on the other hand, exhibited a series of different behaviors after copulation (Fig. 2). Most of the times, they also stayed quiescent for up to 5 h ($n = 39$ observations, Figs. 2 and 3a). Females also everted their ovipositor and manipulated them using their chelicerae, just after the end of the intromission or after they walked around the leaf containing the clutch ($n = 10$ observations; Fig. 2). After this manipulation, females immediately started the oviposition ($n = 2$ observations) or stayed quiescent again, and finally abandoned the clutch ($n = 7$ observations) or laid eggs ($n = 5$ observations) (Fig. 2).

Just before oviposition, females touched the clutch for the first time with their first pair of legs. They also manipulated the mucus coat around the eggs with their front legs, pedipalps, and chelicerae (Figs. 2 and 3b). During the oviposition, females everted the ovipositor and approached their bodies to the previous eggs present at the border of the clutches, thus making difficult to quantify the amount of time invested in each oviposition event. Therefore, we were able to time only three ovipositions, that lasted up to 8 min per egg and, after every egg laid, females retracted their ovipositor and either abandoned the clutch or touched the eggs again and resumed oviposition (Fig. 2). Although caring males usually hold their partners using the curled tip of their second pair of legs ($n = 19$), this behavior prevented females from leaving the clutch in only two occasions. After females left the clutches, caring males touched and inspected the newly oviposited eggs for a period that exceeded three hours.

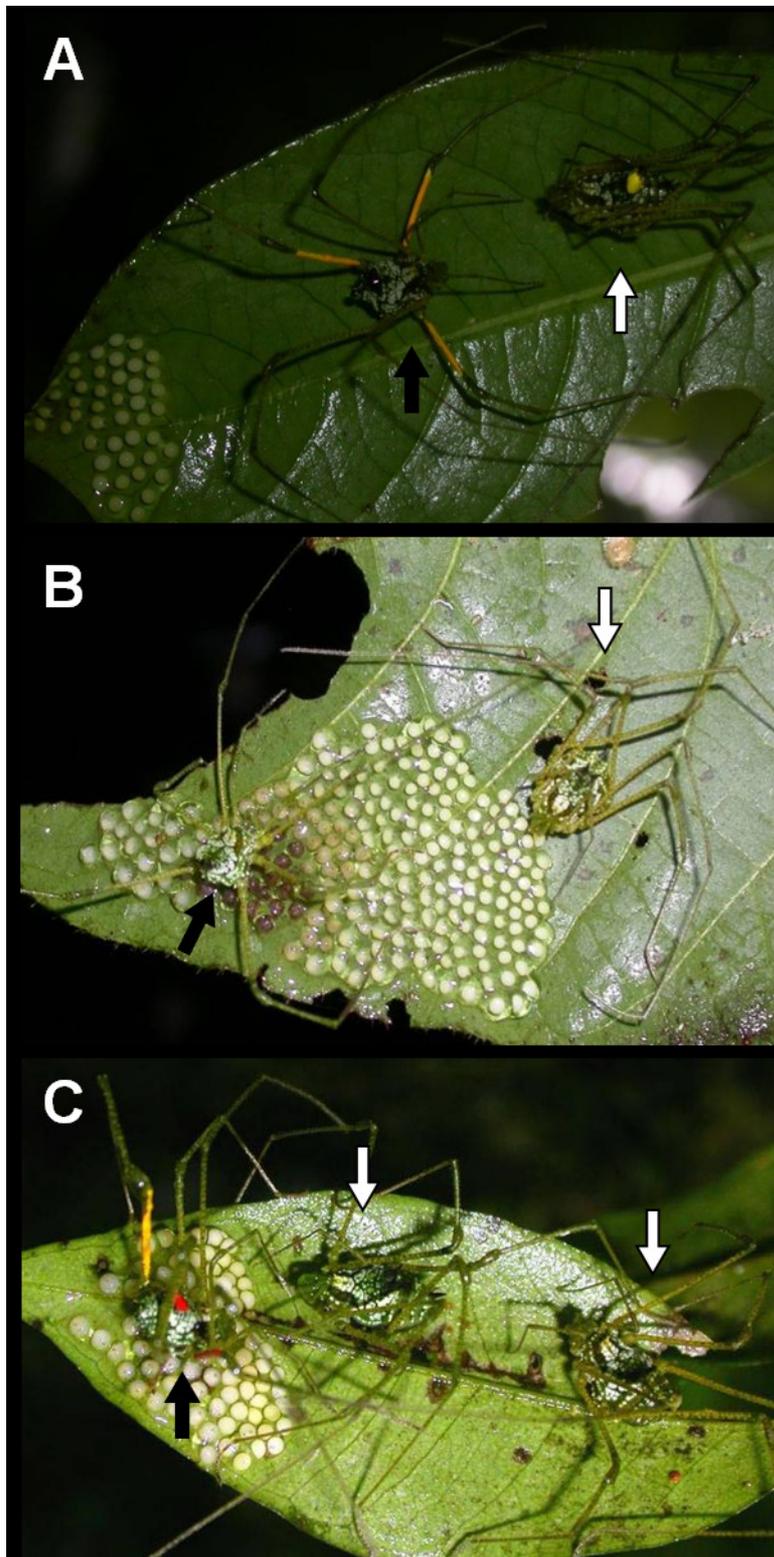


Fig. 3. Mating behavior of the harvestman *Iporangaia pustulosa*. (A) After copulation, the couple may stay quiescent for a while, when male gently touches female's legs with his second pair of legs. (B) In cases when female does not abandon the clutch after the quiescent period, she may approach the clutch and touch the previous eggs and the mucus coat with her first pair of legs and mouthparts. (C) In some occasions, caring males may interact with more than one female simultaneously. The male is indicated by a black arrow and females are indicated by white arrows.

In 17 occasions, we observed more than one female at the vicinity (ca. 30 cm) of a clutch (Fig. 3c). However, we never observed females fighting each other for access to caring males, either close to the clutches or in their vicinity. On the contrary, in 14 of those occasions, the caring male copulated with two females sequentially, in two observations, the male interacted with three females and, in one case, the male was surrounded by six different females. After copulating with a first female, the male mated the second female while the first one was observed quiescent or manipulating her ovipositor. Eventually, two or more females were found prostrated close to the same clutch during the quiescent period before oviposition (Fig. 3c).

DISCUSSION

Our observations on the mating behavior of the harvestman *Iporangaia pustulosa* clearly show that both males and females select mating partners. Although pre-copulatory courtship and copulatory courtship seem to be performed exclusively by males, mate search is performed exclusively by females, which approach stationary caring males on the vegetation. Upon arrival, more than 30% of the visiting females were repelled by caring males before copulation. After copulation, on the other hand, nearly 50% of the females abandoned the male without ovipositing. These results support our prediction that both males and females of *I. pustulosa* would exhibit some degree of mate choice. In the following paragraphs we compare our results with another well-studied arthropod species exhibiting paternal care, highlighting both convergences and divergences among them. We also discuss which features males and females may be selecting in their partners and what are the possible benefits of this selection for individuals of each sex.

Although sex role reversal in paternally caring arthropods has been described for water bugs (Smith 1997), a few sea spiders (Bain & Govedich 2004), and at least one harvestman species (Mora 1990), unequivocal evidence of mutual mate choice is available only for the last one. Males of the Neotropical harvestman *Zygopachylus albomargis* build and protect cup-like mud nests where visiting females leave their eggs (Rodríguez & Guerrero 1976; Mora 1990). In this species, females actively court males when they enter a nest and approximately 14% of the visiting females are aggressively rejected by males. Attacked females never respond to the male's bites (Mora 1990), a passive behavior similar to that reported here for *I. pustulosa*, suggesting that, contrary to males, harvestman females are unable to mate coercively (Machado & Macías-Ordóñez 2007). Accepted females in both harvestman species are also selective and carefully inspect the nest or the clutch before oviposition, sometimes leaving males without ovipositing (Mora 1990; this study). Indeed, among species with paternal care, females are predicted to take into account the expected quality of the caring behavior exhibited by males during mate choice (Hoelzer 1989; Tallamy 2001; Wagner Jr. 2011; Alonzo 2011). Not surprisingly, females in at least three arthropod species, including *I. pustulosa*, select males based on the possession of eggs (Gilbert et al. 2010; Nazareth & Machado 2010; Chapter 5 in this dissertation).

The reasons why some females are accepted whereas others are rejected remain unknown, but it is likely that *I. pustulosa* males are able to evaluate visiting females through chemical information based on tegumentary hydrocarbons. It has already been demonstrated for different arachnid species that, even during brief pre-copulatory interactions, males may evaluate potential mates according to their mating status (virgin vs. non-virgin), fecundity, or body condition (see examples in Aisenberg et al. 2011). Given that *I. pustulosa* females undergo continuous gonadotrophic cycles and continuously

mature eggs over time (Machado et al. 2004), it is likely that they copulate with several males over the course of the long breeding season and store sperm in their multiple spermathecae (Moya et al. 2007). Therefore, it is unlikely that caring males exclusively mate with virgin females, chasing away non-virgins potential partners. Field observations conducted with *Z. albomarginis* showed that nearly 90% of females visit only one or two nests during the entire mating season that lasts up to six months. A small proportion of these females remain close to the nests during the entire breeding season and may even care for the eggs if the guarding male is experimentally removed (Mora 1990). Thus, *Z. albomarginis* males could base their mate decisions on the specific identity of females, recognizing their frequent partners and repelling newcomer females, which probably represent high risk of sperm competition and egg cannibalism. However, this explanation does not seem to hold to *I. pustulosa*, since females actively search for mates in the vegetation and can dislodge large distances (Requena et al. 2012), probably accessing and copulating with several males.

Other putative explanation for female rejection in *I. pustulosa* is that males select good quality partners based on their body condition or fecundity, which are strongly correlated with body volume (Fig. 1). How males evaluate female body volume is an open question that deserves further investigation, but the front legs in this species are known to have sensory organs responsible for the detection of tactile and chemical stimuli under contact (Willemart & Chelini 2007) that may be used in a sexual selection context. However, given that *I. pustulosa* males do not have to deal with space limitation for receiving additional eggs into their clutch, as it is the case for water bugs (Smith 1997) and sea spiders (Bain & Govedich 2004), why should they select mates based on fecundity? Moreover, given that the presence of eggs increases male attractiveness (Chapter 5 in this

dissertation), why should males reject additional eggs, especially when they are caring for a small clutch that can be attacked and quickly consumed by predators? We suggest that the answer for these questions relies at least in part on sperm depletion, a completely unexplored subject in harvestmen reproduction.

We collected the data presented here during the peak of the reproductive season, when adults are more active (Requena et al. 2012). During this period, receptive females are searching for mates on the vegetation and the high encounter rate between mates is likely to increase male mating frequency. In fact, caring males were observed interacting sequentially with more than two females (Fig. 3), with a special situation in which up to six females were seen simultaneously close to a single caring male. Moreover, paternal care in *I. pustulosa* is known to impose severe energetic costs to caring males (Requena et al. 2012), which may contribute to sperm depletion over the course of the caring period. Although *I. pustulosa* males produce aflagellate sperm (Moya et al. 2007), which is probably cheap to produce (Macías-Ordóñez et al. 2010), the potentially high mating frequency and the low food intake of caring males may act together compromising future mating opportunities with high-quality females (e.g. Härdling et al. 2008). Therefore, sperm depletion may provide a mechanistic explanation for female rejection in *I. pustulosa*. One prediction of this hypothesis is that males in which the mating frequency is experimentally decreased or males that are experimentally fed during the caring period would exhibit lower frequency of female rejection when compared to the natural situation reported here.

In conclusion, both males and females show some degree of choosiness in *I. pustulosa*. Given that most harvestmen are non-visual organisms (Willemart et al. 2009), chemical and tactile cues probably play a decisive role in mutual evaluation during and after

mating. These chemical cues may come from the mating partner, signaling body condition or fecundity, and also from the clutch, honestly indicating the presence or the quantity of eggs. Based on chemical information, males may reject females with low fecundity, and both copulated and non-copulated females may abandon males without laying eggs. Although we found observational evidence of partial sex-role reversal in an arthropod exhibiting exclusive paternal care, female preference seems to be important, dictating the reproductive dynamics in *I. pustulosa*. Male mating distribution is highly skewed and more than half of the male population has never been observed caring for clutches (Chapter 5 in this dissertation), which suggests strong male-male competition. The strength and direction of sexual selection on male attributes have broad implications for understanding the evolution and maintenance of paternal care, and some of these attributes are currently under investigation in *I. pustulosa*.

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CAPÍTULO 5

Paternal care increases male attractiveness in a Neotropical harvestman (Arachnida: Opiliones)

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ABSTRACT

In species with paternal care, females are predicted to take into account not only traits that indicate genetic quality of potential partners during mate choice, but also the expected quality of the parental effort exhibited by the males. Here, we addressed two main questions about male attractiveness in the paternally caring harvestman *Iporangaia pustulosa*: (1) What is the relative importance of oviposition site quality, male quality, and male parental state in female mate choice process? (2) If caring males are more attractive to females, are non-caring males willing to adopt unrelated offspring? The results indicate that parental state is the most important attribute related to male mating success, since non-caring males show low probability of acquiring a new clutch. However, caring male attractiveness does not increase indefinitely, so that individuals caring for long periods are avoided by females. It may happen because egg-guarding in *I. pustulosa* imposes severe energetic costs to caring males, and old care-givers are generally in poor body condition, which may compromise the future quality of parental care they can provide. Finally, most males that visited experimentally unattended egg-batches cannibalized eggs and did not exhibit any aggressive response toward other individuals. We suggest that vagrant males in poor body condition have higher chances of finding unattended egg-batches than stationary males with good body condition, which may explain why there was no instance of egg adoption in the field.

Keywords: Body condition, Egg adoption, Energetic costs, Mate choice, Oviposition site, Sex pheromone, Sexual selection.

INTRODUCTION

Numerous observational and experimental studies using different model organisms have already demonstrated a causal link between variation in male traits and male mating success, showing that males possessing certain attributes have mating advantages arising from female mate choice (reviews in Ryan and Keddy-Hector 1992; Andersson 1994; Johnstone 1995). Females may select males based on (i) secondary sexual characteristics, which are presumable indicators of genetic quality that may increase offspring viability and/or attractiveness, and (ii) direct material benefits, such as nuptial gifts or male parental care, which may enhance female fecundity and offspring survival (reviews in Vahed 1998; Tallamy 2001; Andersson and Simmons 2006; Gwynne 2008; Wagner Jr. 2011). In species with paternal care, particularly, females are predicted to take into account not only traits that indicate genetic quality of potential partners during mate choice, but also the expected quality of the parental effort exhibited by the males (Hoelzer 1989; Alonzo 2011). Among paternally caring fishes, for instance, large males with high energetic reserves care for the offspring for longer periods than small males (e.g., Mackereth et al. 1999), resulting in a higher survival rate of the embryos (Suski and Ridgway 2007).

Besides direct benefits of paternal care on offspring survival, the additional influence of sexual selection on the evolution and maintenance of male parental behavior has been suggested in several theoretical studies (Baylis 1981; Gross and Sargent 1985; Hoelzer 1989, Tallamy 2001, Alonzo 2011). Empirical data of fishes and arthropods support some fundamental predictions of the most recent theories. First, parental males exhibit specific behaviors that increase offspring survival, providing protection against egg predators, provisioning oxygenated water, and cleaning the nest from pathogens (fishes: Pampoulie et al. 2004 and references therein; arthropods: Mora 1990; Munguía-Steyer and Macías-

Ordóñez 2008; Requena et al. 2009). Second, females from several species prefer to copulate with males exhibiting parental behaviors and oviposit in nests already containing eggs (fishes: Östlund and Ahnesjö 1998; Pampoulie et al. 2004; Lindström et al. 2006; arthropods: Gilbert et al. 2010; Nazareth and Machado 2010). In fact, the evolution of morphological and behavioral male traits, such as egg mimicry, egg thievery, and nest takeovers, has been attributed to female preference for egg-containing nests (Tallamy 2001; Porter et al. 2002).

Females of the Neotropical harvestman *Iporangaia pustulosa* lay their eggs on the underside surface of leaves of different shrubs growing at the margin of streams and abandon the offspring under males' protection (Machado et al. 2004). Paternal care in this species is crucial to offspring survival, preventing egg predation by conspecifics and other arthropods (Requena et al. 2009). However, paternal care decreases males' foraging activity and erodes their body condition over the course of the caring period (Requena et al. 2012). The distribution of males' mating success is strongly skewed given that more than 50% of males have never been observed caring for an egg-batch, while some of them copulated with more than 10 females (Fig. 1A-B). Successful males guard egg-batches composed of multiple clutches laid asynchronously, which may extend the caring period to four months (see Chapter 4 in this dissertation). Therefore, the great variation in male reproductive success and the cumulative costs associated to prolonged caring period observed in *I. pustulosa* provide an ideal scenario to investigate what factors influence female mate choice in a species exhibiting paternal care.

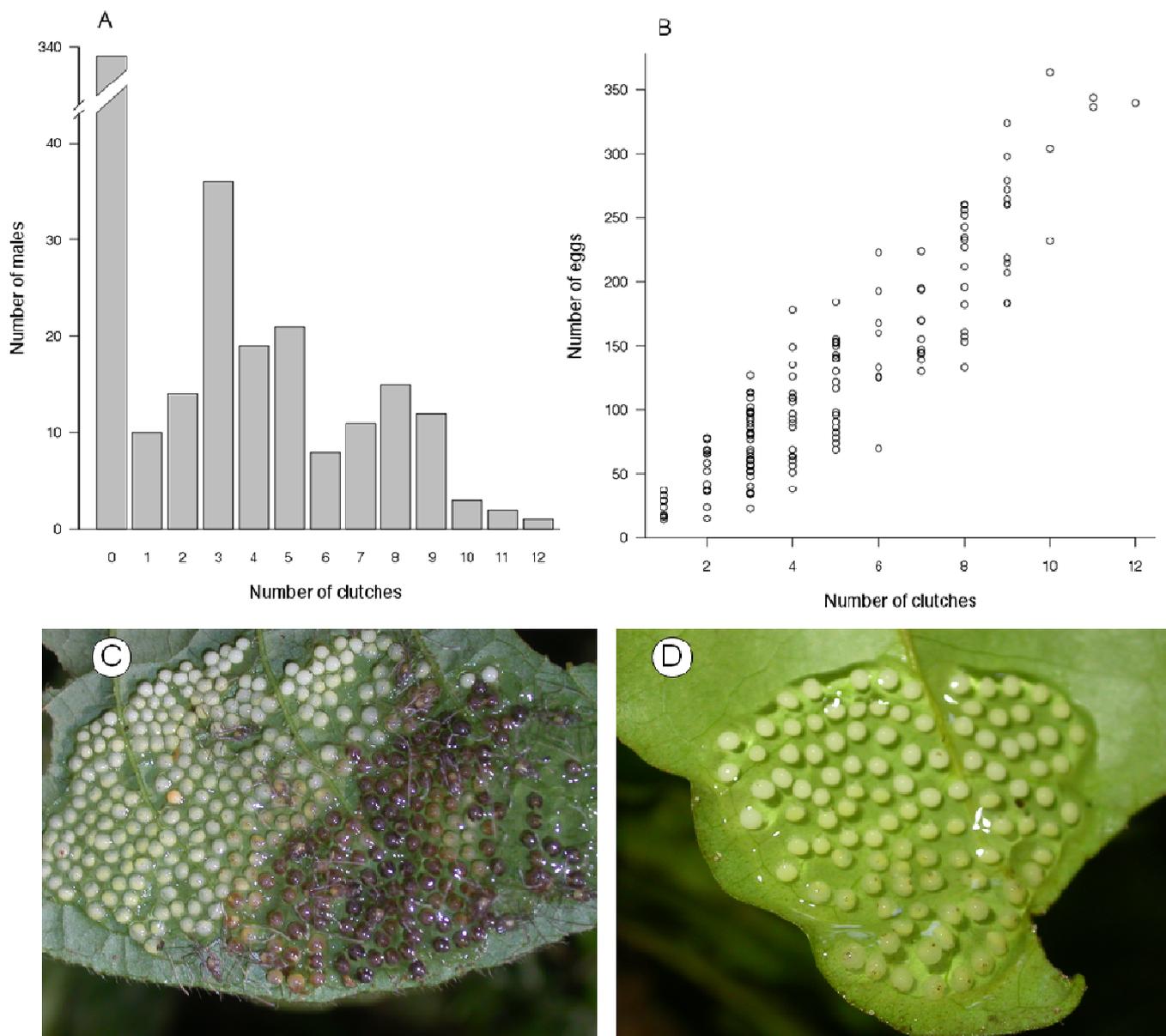


Figure 1. Distribution of mating success among males of the harvestman *Iporangaia pustulosa*. (A) Number of males captured between August 2003 and July 2004 in a 200 m-long transect (N = 479 males). Non-caring males correspond to the category “0 clutches”, while caring males are divided depending on the estimated number of clutches they were observed guarding, based on egg size and coloration, assuming eggs in different stages were oviposited at different moments (as described in Requena et al. 2012). (B) Correlation between the estimated number of clutches into an egg-batch and its observed number of eggs (N = 152 egg-batches). Pictures of egg-batches classified as (C) ‘old’, in which is visible an empty space at the tip of the leaf and usually containing nymphs, or (D) ‘new’, without the empty space.

In this paper, we used an information-theoretic approach to address two main questions about *I. pustulosa* male attractiveness. (1) What is the relative importance of oviposition site quality, male quality, and male parental state in female mate choice process? We predict that males of better quality (i.e. large males) and established in the preferred host plants should be selected by females as mating partners. Furthermore, we also predict that males signaling their willingness to care for the offspring, i.e., those exhibiting egg guarding behavior, should be more attractive than non-guarding males. However, given caring males' body condition continuously decay over the course of the caring period, the expected quality of future care would also decrease. Thus, we specifically investigated whether females' decisions are based only on the presence of eggs under males' protection or also take into account the time caring males already invested in parental activities. (2) Are non-caring males willing to adopt unrelated offspring? If the presence of eggs increases male attractiveness, we predict that non-caring males should adopt unrelated eggs because the prospects of acquiring mates should overcome the costs of caring for unrelated offspring (Tallamy 2001). On the other hand, if the presence of eggs does not increase male attractiveness, we predict that males should not adopt the eggs and eat at least some of them.

MATERIAL AND METHODS

Study site

We carried out fieldwork in an Atlantic Forest fragment at Intervales State Park (24°14' S; 48°04' W; 800 m of altitude), located in the state of São Paulo, southeastern Brazil. The region has high precipitation levels, with average annual rainfall varying between 2,000 and 3,000 mm/year, and mean annual temperature ranging from 12-20 °C. The weather is

mainly subtropical, with a warm-wet season lasting from October to March, and a cold-dry season from April to September. To collect our observational data, we monthly visited the study site during four consecutive days per month between August 2003 and July 2004, inspecting a 200 m transect along the vegetation flanking both margins of a stream three times a day (from 08.30 to 12.00 h, from 14.30 to 18.00 h and from 20.30 to 00.00 h).

Male quality and paternal care

In each daily survey, we captured *I. pustulosa* adults using the active searching method and marked all adults individually with enamel color paint (Requena et al. 2012). At the first time we captured a male, we measured his dorsal scute length (*DSL*) using an electronic caliper (precision of 0.01 mm). Given that harvestman do not molt after adulthood and the dorsal scute does not change in size with food acquisition, we used *DSL* measure as proxy of 'male quality' in the subsequent analyses (see Chapter 4 in this dissertation). After marking and measurement procedures, we released all individuals at the same place where we had captured them. Furthermore, every time we captured males, we classified them according to their parental state as *caring males* (i.e., those that were guarding an egg-batch) or *non-caring males* (i.e., those that did not have an egg-batch). Parental state is a fluctuating condition because caring males may become non-caring males after the dispersion of all nymphs, while non-caring males may copulate and obtain their first clutch, thus becoming caring males.

Given that the oviposition behavior of *I. pustulosa* follows a rigid pattern, with older eggs laid closer to the leaf apex and more recent laid eggs laid closer to the leaf base (Machado et al. 2004), we classified caring males as old or recent care givers based on features of their egg-batches. Old care givers were those males guarding egg-batches with

an empty space close to the leaf apex, indicating that nymphs from old reproductive events have already hatched and dispersed (Fig. 1C), and that the guarding males were in the caring state for at least a month. Recent care givers were those males guarding egg-batches without an empty space at the leaf apex (Fig. 1D), indicating that they were in the caring state for less than one month. We considered these two categories of time males have already invested in egg protection as a proxy of parental investment.

Oviposition site quality

We identified 36 plant species (hereafter called *host plants*) that were used at least once as an oviposition site by *I. pustulosa* in our transect between August 2003 and July 2004 (Table A1). Then, we quantified the availability of the host plants by counting the number of individuals of each species in the study area (two 200 m long and 1 m wide plots at each margin of the river). Next, we calculated the selection index for each host plant according to the formula $w_i = u_i / a_i$, where u_i is the proportion of the total 300 egg-batches that were laid on the host plant i , and a_i is the proportion of the total 2,534 available host plants in the transect that corresponds to the host plant i . Finally, we calculated the standardized plant quality index (*PQI*) for each host plant, according to the following formula:

$$PQI_i = \frac{w_i}{\sum_{i=1}^n w_i}$$
, where n represents the total 36 host plant species (modified from Manly et al. 1993).

Although the aim of this study was not to evaluate preferences of males and females for specific oviposition sites, we used *PQI* to explore variation among host plants and provide a proxy of oviposition site quality. Details on the identity of the host plants, as well as their availability, use as oviposition site, and corresponding *PQI* are presented in

the supplemental material (Table A1). Given that non-caring males are constantly moving on the vegetation and usually do not stay on a specific area for long periods (Requena et al. 2012), we could not to associate a host plant for these individuals. Therefore, we explored the information on *PQI* only in the analysis of female investment in number of eggs on pre-existing egg-batches, not in the analysis for the probability of acquiring a first clutch (see details below).

Mating success

We exhaustively searched the transect for all egg-batches we could find and took pictures of them at least twice a month to quantify the number of eggs caring males were guarding each month and the number of eggs each egg-batch received between consecutive sampling visits. Given that the four-day intervals in each month was not long enough to observe significant egg addition on egg-batches, we used the information of one-month intervals between two consecutive visits to access the number of eggs each male received during this period. Furthermore, males may receive more than one clutch during this one-month interval, and we adopted the following strategies to better access the number of eggs laid in different oviposition events: (1) we classified the eggs in different stages of embryonic development according to egg size and coloration, assuming that eggs in different stages were oviposited at different moments (as described in Requena et al. 2012); and (2) we discarded data of ovipositions containing more than 48 eggs (maximum fecundity observed to *I. pustulosa*, Machado et al., 2004) in the same stage of development to avoid overestimating the number of eggs in a given oviposition event. We also monthly classified males as: (a) successful, if they were non-caring males in i^{th} month and were observed caring for eggs in the $(i+1)^{th}$ month, or if they were caring males in the i^{th} month

and had new eggs added to their egg-batches in the $(i+1)^{th}$ month; or (b) unsuccessful, if they were found as non-caring males both in the i^{th} and $(i+1)^{th}$ months or if there was no addition of eggs to their egg-batches between the i^{th} and the $(i+1)^{th}$ month.

Evaluation of female mate choice

To investigate female mate choice, we formulated a series of generalized linear mixed models (GLMMs) and compared them using a model selection approach (Burnham and Anderson 2002). We implemented all GLMMs using the package 'lme4' (Bates et al. 2011) in R 2.13.1 (R Development Core Team 2011). Depending on sample size of the data used in each analysis, we used either the Akaike Information Criterion (AIC) or its small sample size bias-corrected version (AIC_c) to rank and calculate the relative weight of all models fitted to the data. We selected the model with the lowest AIC or AIC_c value as the most parsimonious model describing the data and computed the difference in the AIC or AIC_c value between the selected model and all other models in the ranking (Burnham and Anderson 2002).

First, we evaluated the probability of an *I. pustulosa* male become successful during the interval between consecutive visits, comparing alternative GLMMs that established that this probability depends on male's body size (*DSL*), male's parental state (non-caring vs. caring), parental investment (non-caring vs. recent care giver vs. old care giver), as well as their additive and interaction effects. For all models, we considered the errors following a binomial distribution and, due to the fact that the same male could receive more than one clutch between consecutive months, we included male identity as a random variable. Furthermore, we also included the i^{th} month as a random variable to account for variations in the population density, sex ratio, and reproductive activity throughout the year.

In a second analysis, we evaluated the number of eggs laid by females in each oviposition event. We compared alternative GLMMs in which the number of eggs laid by females in each oviposition event depended on *DSL*, host plant quality (*PQI*), male's parental state, parental investment, as well as their additive and interaction effects. Models in this second analysis considered errors following a Poisson distribution and, consequently, to minimize the over-dispersion problem usually associated with this distribution, we included an observation-based factor as a random variable. For the same reasons described above, we also included male identity and the i^{th} month as random variables.

Egg adoption experiment

To evaluate the willingness of *I. pustulosa* non-caring males to exhibit an adoption strategy, we conducted a field experiment removing caring males from their egg-batches for four consecutive days. We visited each egg-batch every 3 h throughout the entire experimental period. In each visit, we recorded the presence, permanence time, and behavior of conspecific males and females on the unprotected eggs. To avoid any effect of the number of the eggs on the variables observed, we artificially removed eggs from the egg-batches so that all of them contained 50 eggs before the experiment started. Furthermore, given that climatic conditions vary along the year (Requena et al. 2012), we conducted the experiment at two different moments: (a) in January and February 2004, during the wet and warm season and in the peak of the reproductive season (Requena et al. 2012), when we removed 23 caring males from their egg-batches; and (b) during July 2004, during the dry and cold season and when the number of caring males is very low (Requena et al. 2012). In this second occasion, we removed only seven caring males from their egg-

batches.

We built specific generalized linear models (GLM) or GLMMs establishing the influence of sex on each response variable, and then made a deviance analysis on every model. To compare the number of visiting males and females that cannibalized or did not cannibalize eggs from experimental egg-batches, as well as the number of individuals of each sex that remained prostrated on those eggs, we built GLMs with errors following a binomial distribution. To compare the number of eggs cannibalized by individuals of each sex, as well as the time they were observed close to the experimental egg-batches, we built GLMMs with errors following a Poisson distribution. In these cases, we also included an observation-based factor as a random variable. If the paternal care itself increases males' attractiveness, we expected that non-caring males visiting the experimental eggs-batches would stay with the eggs for longer periods and would not cannibalize them, perhaps exhibit parental activities such as aggressive behaviors against potential egg predators (Tallamy 2001). On the other hand, females are one of the main egg predators of *I. pustulosa* (Requena et al. 2009) and we expected that they would cannibalize eggs, stay for short periods on the egg-batches, and would not protect the eggs against potential predators.

RESULTS

Female mate choice

Both the model considering the sole effect of parental investment and the model considering the additive effect of this variable and male body size (*DSL*) were equally plausible ($\Delta AIC_c < 2.0$, Table 1) to explain the observed data on males' success. According to this result, there is a uncertainty about the influence of *DSL* on female mate decision,

with a subtle positive relationship between body size and the probability of acquiring a new clutch in the best-ranked model (Fig. 2). On the other hand, recent care givers are consistently more attractive than old care givers, followed by non-caring males, which is the least attractive male category (Fig. 2).

Table 1: Summary of hierarchical model selection statistics for models evaluating *Iporangaia pustulosa* females mate choice ($N = 1227$ observations).

Predictors	AIC _c	K	ΔAIC _c	Weight
DSL + male category	1013.7	6	0.0	0.590
Paternal investment	1015.3	5	1.6	0.269
DSL * paternal investment	1016.6	8	2.9	0.141
DSL + parental state	1073.3	5	59.6	< 0.001
DSL * parental state	1074.0	6	60.3	< 0.001
Parental state	1074.5	4	60.8	< 0.001
DSL	1215.0	4	201.3	< 0.001
Null model	1220.6	3	206.9	< 0.001

Models were ranked by increasing order of their small sample size bias-corrected version of the Akaike Information Criterion (AIC_c). K = number of parameters. ΔAIC_c = difference between the AIC_c value of each model and the AIC_c value of the most parsimonious model. Weight = AIC_c weight of each model. The more supported models (whose $\Delta AIC_c < 2.0$) are in bold. *DSL* is the dorsal scute length, a proxy for male quality. The symbols + and * represent additive effect and interaction between variables, respectively.

The number of eggs laid in each oviposition is explained by four equally plausible models ($\Delta AIC_c < 2.0$, Table 2), considering the effect of male parental state, its additive effect with *DSL*, its interaction effect with *DSL*, or the sole effect of parental investment. Once again, regardless the selected model, it is clear that there is a consistent difference between caring and non-caring males, with the later receiving fewer eggs than former (Fig. 3 and Fig. A1). Similar to the results on males' mating success, two selected models predict only a weak positive effect of *DSL* on the number eggs laid (Fig. 3 and Fig. A1), and the

other two models do not even include *DSL* among the predictor variables (Table 2). Given that all predicted values are quantitative and qualitatively similar (compare Fig. 3A and Fig. A1), we will not attain to little differences among selected models in the discussion and will focus on general patterns.

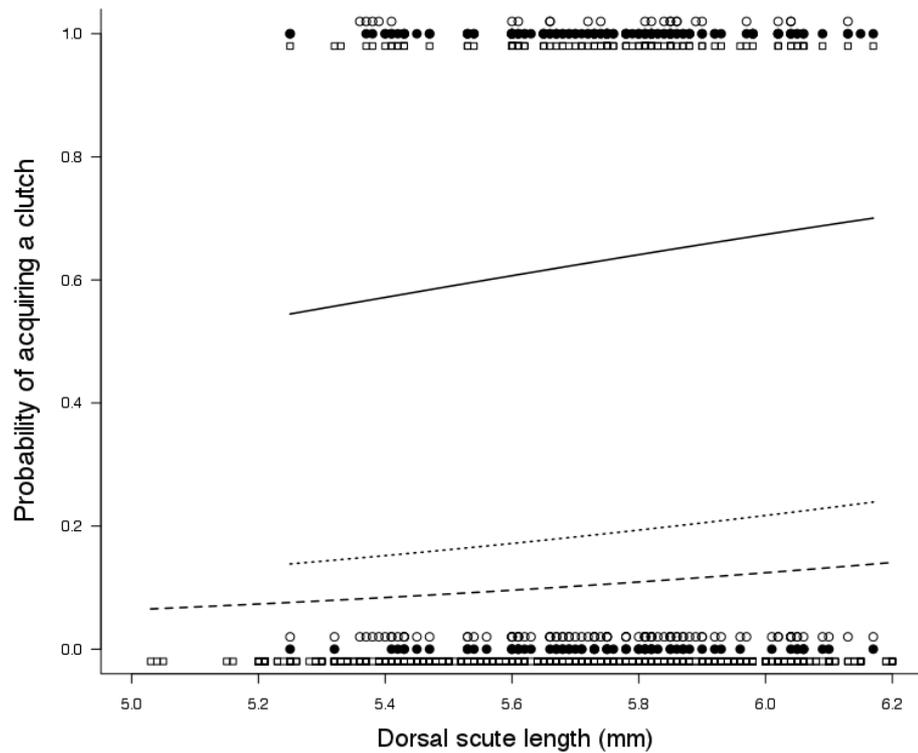


Figure 2. The effect of *Iporangaia pustulosa* males' quality on females' mate choice. The probability of acquiring a new clutch depends on males' body size (dorsal scute length in millimeters) and males' category based on time already invested in paternal activities. Open squares and dashed line respectively represent data for non-caring males and their predicted probabilities, while solid circles and solid line represent data for recent care-givers guarding recently laid eggs, and open circles and dashed line, for old care-givers guarding old egg-batches. Predicted probabilities were obtained using coefficients estimated by the most parsimonious selected model (see text and Table 1 for additional details).

Table 2: Summary of hierarchical model selection statistics for models evaluating *Iporangaia pustulosa* female investment in number of eggs ($N = 402$).

Predictors	AIC_c	K	ΔAIC_c	Weight
Parental state	1026.0	5	0.0	0.230
Paternal investment	1026.4	6	0.4	0.188
<i>DSL</i> * parental state	1027.7	7	1.7	0.098
<i>DSL</i> + parental state	1027.9	6	1.9	0.090
<i>PQI</i> + parental state	1028.1	6	2.1	0.082
<i>DSL</i> + paternal investment	1028.3	7	2.3	0.074
<i>PQI</i> + paternal investment	1028.5	7	2.5	0.067
<i>DSL</i> * paternal investment	1029.5	9	3.5	0.040
<i>PQI</i> * parental state	1029.6	7	3.6	0.037
<i>DSL</i> + <i>PQI</i> + parental state	1029.9	7	3.9	0.032
<i>DSL</i> + <i>PQI</i> + paternal investment	1030.4	8	4.4	0.026
Null model	1032.0	4	6.0	0.012
<i>PQI</i> * paternal investment	1032.1	9	6.1	0.011
<i>DSL</i>	1033.9	5	7.9	0.004
<i>PQI</i>	1034.0	5	8.0	0.004
<i>DSL</i> * <i>PQI</i>	1034.5	7	8.5	0.003
<i>DSL</i> + <i>PQI</i>	1035.9	6	9.9	0.002

Models were ranked by increasing order of their small sample size bias-corrected version of the Akaike Information Criterion (AIC_c). K = number of parameters. ΔAIC_c = difference between the AIC_c value of each model and the AIC_c value of the most parsimonious model. Weight = AIC_c weight of each model. The more supported models (whose $\Delta AIC_c < 2.0$) are in bold. *DSL* is the dorsal scute length, a proxy for male quality. *PQI* is an index for oviposition site quality. The symbols + and * represent additive effect and interaction between variables, respectively.

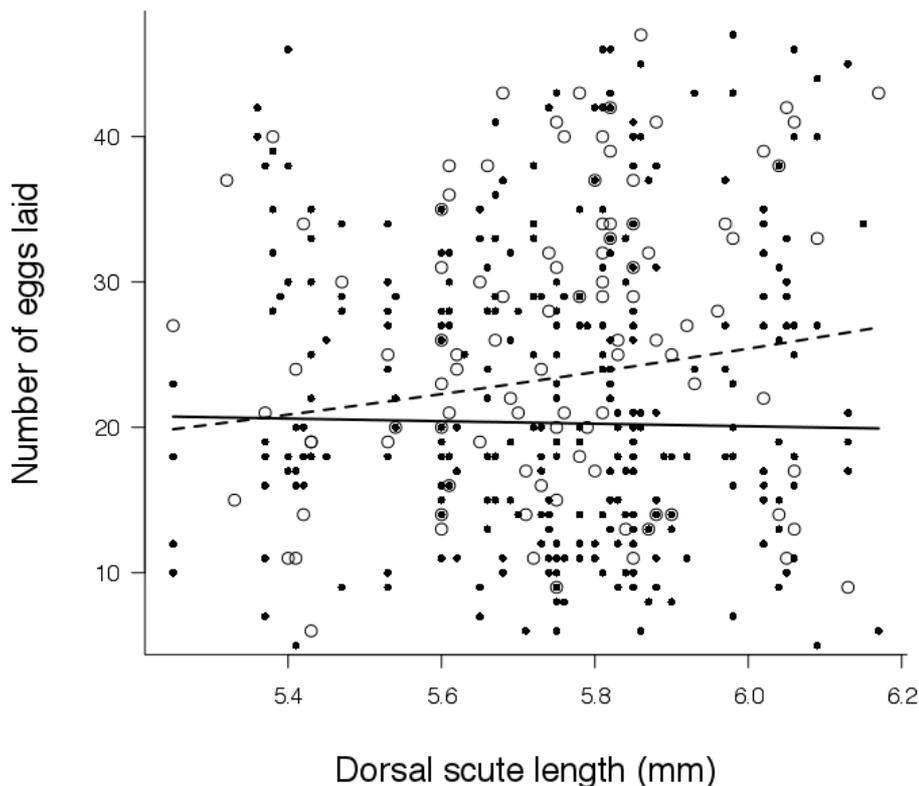


Figure 3. The effect of *Iporangaia pustulosa* males' characteristics on females' investment in number of eggs laid. The number of eggs received by males depends on males' body size (dorsal scute length in millimeters) and males' parental state. Open, big circles and dashed line represent, respectively, observed data and predicted values for non-caring males, while solid, small circles and solid line, for caring males. Predicted number of eggs were obtained using coefficients estimated by the model considering the interaction effect between males' characteristics (for additional details, see text, Table 2, and figure A1).

Egg-adoption

During the wet and warm season, the experimentally unattended egg-batches received 24 visits of males and 24 of females, during which individuals exhibited similar behaviors (Table 3). More than half of all observed males ($n = 13$) and females ($n = 15$) cannibalized at least one egg, with no sexual differences on the probability of cannibalism or on the number of consumed eggs (Table 3). Individuals of both sexes usually stayed on the

experimental egg-batches for short periods (males: median = 17.5 min; females: median = 19.0 min). One male, however, remained more than 24 h on the same egg-batch without cannibalizing any egg (Table 3). Males and females showed the same probability of remaining prostrated on the eggs (Table 3), but no defensive behaviors toward newcomers was exhibited by any individual during our observations.

During the dry and cold season, probably due to the low activity of the individuals, the seven experimental egg-batches received visits of only two males and one female. One of the males stayed 3 min with the unattended eggs and did not consume any egg. The other male stayed almost 30 min on the egg-batch and, during this time, cannibalized one egg. The female was also observed cannibalizing one egg, but her permanence time could not be estimated because she was apparently disturbed by the head-lamp during the nocturnal observation.

Table 3: Comparison between *Iporangaia pustulosa* males (n = 24) and females (n = 24) behavioral responses to experimental unattended egg-batches during wet and warm season (January and February 2004).

	Males	Females	Analysis of deviance
Permanence time (min)	1 - 1440	4 - 330	Δ deviance = 0.290, $P = 0.590$
% of individuals that cannibalized eggs	54.2	62.5	Δ deviance = 0.343, $P = 0.558$
Number of eggs consumed	1 - 5	1 - 6	Δ deviance = 0.477, $P = 0.490$
% of individuals that remained prostrated on the eggs	29.1	33.3	Δ deviance = 0.097, $P = 0.755$

DISCUSSION

The results reported here indicate that male parental state is the most important attribute related to mating success, since non-caring males show low probability of acquiring a new clutch, which in their case is the first one. More than just the dichotomy between caring and non-caring states, mating success seems to also depend on the time caring males have already invested in paternal activities: recent care-givers are more attractive than old care-givers. However, we have not observed any instance of egg adoption by vagrant males in experimentally unattended clutches, a behavior that would be expected under situations in which caring males are considerably more attractive than and non-caring males. In the following paragraphs, we integrate information from our previous studies on the reproductive behavior of *I. pustulosa* to understand the patterns of female mate choice reported here. Moreover, we discuss the discrepancy between theoretical expectations and empirical results regarding egg adoption in this harvestman species.

Contrary to other harvestman species exhibiting maternal care (e.g., Buzatto and Machado 2008; Osses et al. 2008), oviposition site quality does not affect female preference. We suggest that females base their ovipositing decisions mostly on micro-climatic conditions of the selected sites, regardless the identity of the host plant species. During the first days after oviposition, eggs of several harvestman species absorb water through the highly permeable chorion (Gnaspini 2007). Therefore, physiological requirements for harvestmen egg development, as moisture levels (e.g., Machado and Oliveira 1998), may have also played a central role in the reproductive biology of *I. pustulosa* and other representatives of the subfamily Progonyleptoidellinae, since individuals breed exclusively at the margin of streams (Machado et al. 2004; Requena et al. in press). Moreover, females of all species in this subfamily also deposit an abundant hygroscopic

mucus coat on the eggs, which is suggested to protect the eggs against dehydration, buffering variations in air humidity during the embryonic development (Machado and Macías-Ordóñez 2007).

Regarding male traits, the effect of parental state is definitely more important than the effect of their body size in female choice. In Figure 2 we can see, for instance, that the predicted mating probability of the biggest non-caring male is much lower than the mating success of the smallest caring male. Thus, the attractiveness of a given male seems to fluctuate over the course of breeding season, depending on the presence of eggs under his protection. In several fish species with paternal care, females prefer to copulate with and leave their eggs in the nests of males exhibiting high rates of parental activities, such as oxygenating movements and cleaning behaviors (e.g., Östlund and Ahnesjö 1998; Pampoulie et al. 2004; Lindström et al. 2006). Furthermore, males in some of these fish species intensify their parental activities and reduce filial cannibalism as the number of eggs in their nests increases (Sargent and Gross 1993; Pampoulie et al. 2004), suggesting that female choice for successful nests is an adaptive response to the improved quality of male care towards large broods (Alonzo 2008). Among paternally caring arthropods, experimental manipulations conducted in the laboratory with the harvestman *Magnispina neptunus* (Nazareth and Machado 2010) and the assassin bug *Rhinocoris tristis* (Gilbert et al. 2010) have also demonstrated female preference for egg-guarding males. To our knowledge, however, the results obtained here for *I. pustulosa* provide the first field evidence of the positive influence of paternal care on male attractiveness in an arthropod species.

When paternal care may be evaluated under the sexual selection context, the consistency and reliability of male care behavior are crucial to determine female decisions (Stiver and Alonzo 2009; Alonzo 2011). In some fish species, males adjust parental investment according to the size of the offspring, as mentioned above (e.g. Pampoulie et al. 2004). However, whenever parental care is depreciable, i.e., there is a negative relationship between offspring number and caring quality (Clutton-Brock 1991), paternal activities are expected to get worse as offspring number increases. These fluctuations in parental care quality have profound implications on male attractiveness because females should avoid males guarding large egg-batches. In the sea spider *Achelia simplissima*, a marine species in which males carry egg-masses using a specialized pair of legs (Bain and Govedich 2004), females clearly avoid males carrying a large number of egg-masses and mate more often with non-parental than parental individuals (Burriss 2011). In this case, the energetic costs associated to specific male parental activities, as well as the extra-weight gained by the egg-masses, are suggested to negatively affect the future quality of paternal care and, consequently, male attractiveness (Burriss 2010). We hypothesize that a similar explanation can be used to explain female preference for recent care-givers when compared with old care givers of *I. pustulosa*.

Due to asynchronous oviposition events, the caring period in *I. pustulosa* may last from one to up to four months (see Chapter 4 in this dissertation). During the caring period, male body conditions deteriorates, mainly because foraging activities of egg-guarding males are considerably reduced (Requena et al. 2012). Furthermore, even with a great variation in the initial amount of energetic reserves among males, severe energetic costs imposed to them by the long caring period deprived from food erodes the variation in body condition among males guarding old egg-batches (Requena et al. 2012). Under the

female perspective, therefore, old care-givers are always the worst mate choice because the future prospects of the caring quality are expected to be lower than of any recent care-giver. It is worth noting that our previous observational results on mating interactions revealed that females only touch the eggs after mating and just before oviposition (see Chapter 4 in this dissertation). Therefore, although we have used the age of the egg-batches as a predictor variable in our models, it is just a proxy of males' body condition, which is likely to be good when they are recent care-givers, and bad when they are old care-givers.

The most conspicuous sexual dimorphism in *I. pustulosa* is the presence of a thickened basitarsus on the first pair of legs and a thickened metatarsus on the fourth pair of legs of males (Kury and Pinto-da-Rocha 1997). Although the functional role to these forms of sexual dimorphism remains unknown, glandular openings have already been identified on both thickened areas of males' legs (Willemart et al. 2010). Information for other harvestman species demonstrated that male tegumentary glands may be directly involved in reproductive context, either providing nuptial gifts to females (Martens 1969) or depositing pheromones on the substrate that mediate communication between males and females (Willemart and Hebets 2011). Given that we have not observed direct contact between females and the thickened areas of males' legs before and during copulation (see Chapter 4 in this thesis), we suggest that the male leg glands release sex pheromones related to long-range mate attraction and / or mate assessment. Chemical signals used in this context usually advertise not only the identity and location of the sender, but also its potential quality as a mate, assessed by quantitative or qualitative intra-sexual differences in the pheromonal blend (Johansson and Jones 2007). Due to the costs associated to the pheromone production, condition-dependent expression of such substances has been

demonstrated for several arthropod and vertebrate species (review in Johansson and Jones 2007). Assuming that *I. pustulosa* males release costly sex pheromones that are also used by females to evaluate mate quality, the deterioration of male body condition over the course of the caring period may explain why old care-givers have lower chances of acquiring mates and why they receive a lower number of eggs when compared to recent care-givers.

The hypothesis of female attraction based on a sex pheromone has empirical support in our field behavioral observations on sexual interactions. In *I. pustulosa*, mate search is performed exclusively by females (see Chapter 4 in this thesis), which are constantly moving on the vegetation (Requena et al. 2012). Caring males, on the other hand, remain stationary most of the time, except when they temporarily abandon their egg-batches to forage (see Chapter 3 in this dissertation). After all eggs have hatched, males in poor body condition have to search for food on the vegetation to replenish energetic reserves. In our proposed scenario, these former caring males should feed until they reach good body condition, and thus they would probably remain stationary in favourable sites and start releasing sex pheromones to attract females. Therefore, we suggest that vagrant males in poor body condition have higher chances of finding unattended egg-batches than stationary males with good body condition, which may explain why there was no instance of egg adoption in our field experiment. Although cannibalized eggs may provide energy to non-caring males, the production of costly pheromones generally depends on multiple nutrient types, including proteins obtained only from specific food items (Tillman et al. 1999). The need for proteins that are not available in the eggs may also explain why caring *I. pustulosa* males do not practice filial cannibalism as a form of maintaining good body conditions and possibly high attractiveness to females.

In conclusion, the highly skewed mating success distribution among *I. pustulosa* males is probably the result of intense female mate choice, which we here demonstrated to be directed towards caring males, rather than oviposition site characteristics or male body size. However, caring male attractiveness does not increase indefinitely, so that individuals caring for long periods are avoided by females. It may happen because egg-guarding in *I. pustulosa* imposes severe energetic costs to caring males, and old care-givers are generally in poor body condition, which may compromise the future quality of parental care they can provide. Our findings suggest that, due to direct benefits provided by paternal care, female mate choice is based on honest signals of male condition. We argue that costly sex pheromones are good candidates for such indicators, and only well-fed males, which had access to a varied diet, could afford to be stationary and produce chemical compounds to attract females and announce their quality. Therefore, a possible trade-off between parental and mating efforts may explain some details of the reproductive biology of the harvestman *I. pustulosa* that still remains obscure, as the absence of filial cannibalism or egg adoption strategies.

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SUPPLEMENTARY MATERIAL

Table A1. Identity, frequency of use, availability, and the standardized index for quality (*PQI*) for the 36 host plant species that were used as oviposition site by females of the harvestman *Iporangaia pustulosa* between August 2003 and July 2004.

Host plant species	Number of egg-batches	Number of individuals	<i>PQI</i>
DRYOPTERIDACEAE			
<i>Didymochlaena truncatula</i> (Swartz) J. Smith	1 (0.33)	25 (0.99)	0.004
<i>Diplazium cristatum</i> (Desr.) Alston.	5 (1.67)	164 (6.47)	0.003
<i>Olfersia cervina</i> (L.) Kunze	32 (10.67)	136 (5.37)	0.024
Dryopteridaceae sp.	3 (1.00)	89 (3.51)	0.003
ACANTHACEAE			
<i>Justicia</i> sp.	5 (1.67)	47 (1.85)	0.011
AMARANTHACEAE			
<i>Amaranthus</i> sp.	16 (5.33)	107 (4.22)	0.015
ARACEAE			
<i>Philodendrum ochrortemon</i> Schott	9 (3.00)	59 (2.33)	0.015
BEGONIACEAE			
<i>Begonia</i> spp.	3 (0.99)	90 (3.55)	0.003
CELASTRACEAE			
<i>Maytenus</i> sp.	1 (0.33)	3 (0.001)	0.034
COMMELINACEAE			
<i>Dichorisandra thyrsoflora</i> Mikan	16 (5.33)	464 (18.31)	0.003
CYPERACEAE			
<i>Pleurostachys gaudichaudii</i> Brongn.	1 (0.33)	33 (1.30)	0.003
LAURACEAE			
<i>Endlicheria paniculata</i> (Spreng.) Macbr.	3 (1.00)	5 (0.20)	0.061
<i>Ocotea puberula</i> (Rich.) Nees	1 (0.33)	18 (0.71)	0.006
LEGUMINOSAE			
<i>Inga</i> sp.	1 (0.33)	21 (0.83)	0.005
<i>Zollernia ilicifolia</i> (Brongn.) Vogel	4 (1.33)	8 (0.82)	0.051
<i>Dahlstedfia pinnata</i> (Benth.) Malme	3 (1.00)	6 (0.24)	0.051
MARANTACEAE			
<i>Calathea communis</i> Wanderley and Vieira	33 (11.00)	447 (17.64)	0.007
MELASTOMATACEAE			
<i>Leandra</i> spp.	7 (2.33)	43 (1.70)	0.016
<i>Miconia</i> spp.	2 (0.67)	22 (0.86)	0.009
<i>Salpinga margaritacea</i> (Naudin) Triana	1 (0.33)	8 (0.32)	0.013

Cont. Table A1

MONIMIACEAE			
<i>Mollimedia schottiana</i> (Spreng.) Perkins	5 (1.67)	20 (0.79)	0.025
MYRSINACEAE			
<i>Ardisia guyanensis</i> (Aubl.) Mez	19 (6.33)	56 (2.21)	0.034
MYRTACEAE			
<i>Calycorectes acutatus</i> (Miq.) Toledo	1 (0.33)	3 (0.12)	0.034
<i>Campomanesia guaviroba</i> (DC.) Kiaersk.	8 (2.67)	9 (0.36)	0.090
<i>Gomidesia schaueriana</i> O. Berg	43 (14.33)	37 (1.46)	0.117
<i>Gomidesia tijucensis</i> (Kiaersk.) D. Legrand	2 (0.67)	21 (0.83)	0.010
<i>Myrceugenia</i> spp.	13 (4.33)	32 (1.26)	0.041
NYCTAGINACEAE			
<i>Guapira opposita</i> (Vell.) Reitz	3 (1.00)	19 (0.75)	0.016
PIPERACEAE			
<i>Piper</i> spp.	10 (3.33)	124 (5.30)	0.008
RUBIACEAE			
<i>Alseis floribunda</i> Schott	6 (2.00)	6 (0.24)	0.101
<i>Bathysa australis</i> (A.St.-Hil.) Benth. and Hook.f.	24 (8.00)	40 (1.58)	0.061
<i>Psychotria</i> spp.	2 (0.67)	19 (0.75)	0.011
<i>Rudgea jasminioides</i> (Cham.) Müll. Arg.	5 (1.67)	6 (0.24)	0.084
SAPOTACEAE			
<i>Chrysophyllum</i> sp.	2 (0.67)	31 (1.22)	0.006
URTICACEAE			
<i>Pilea astrogramma</i> Miq.	6 (2.00)	298 (11.76)	0.002
UNIDENTIFIED	4 (1.33)	18 (0.72)	0.022

NOTE: Numbers in parenthesis indicate the relative use (number of egg-batches) and the relative availability (number of individuals) of each host plant.

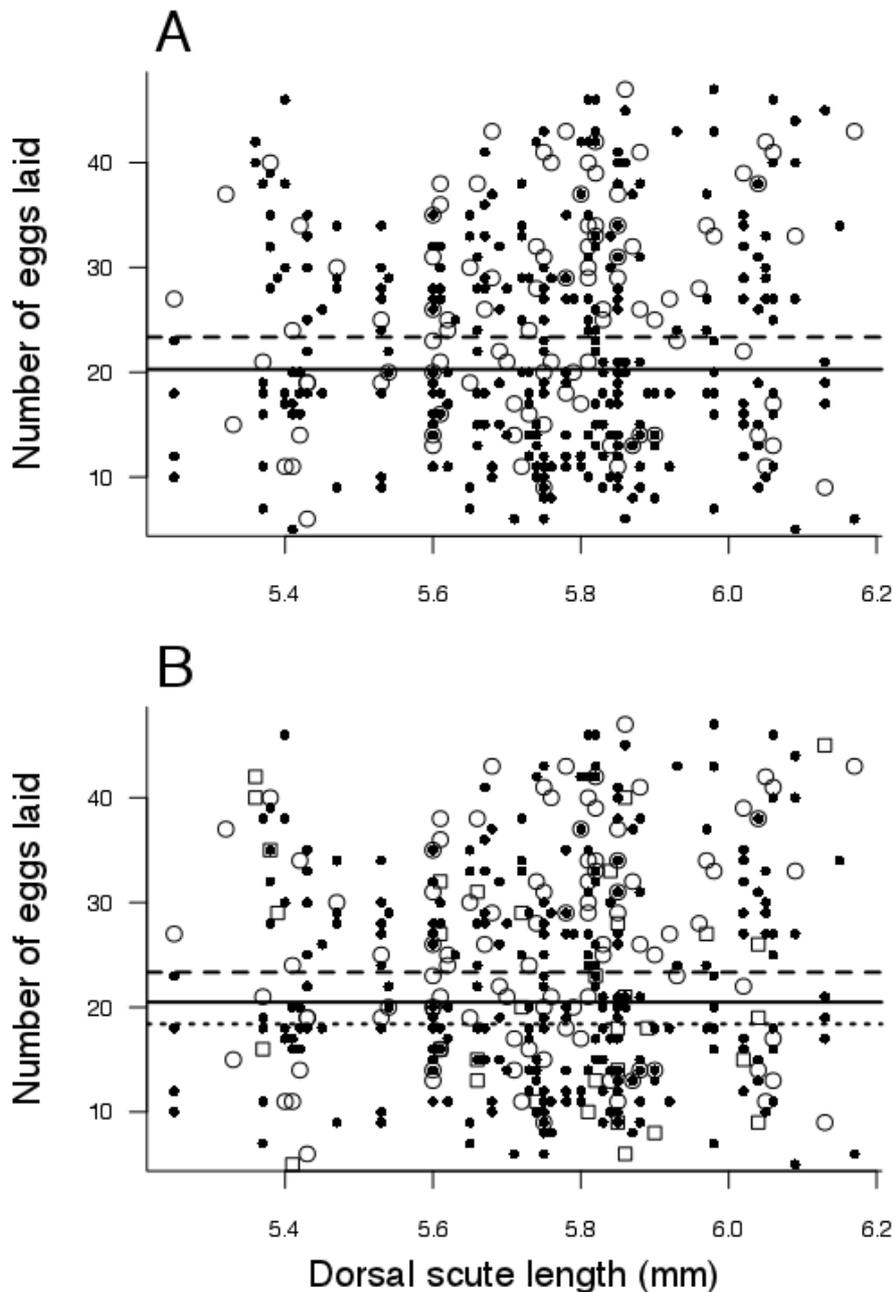


Figure A1. The number of eggs laid by *Iporangaia pustulosa* females predicted by two other equally plausible selected models. The effect of (A) males' parental state and (B) individuals category based on the time males have already invested in parental activities on females investment in number of eggs. Graphics were built using estimated coefficients for every variable, predicted by the best-ranked and the second best-ranked models (for additional details, see text and Table 2). Open, big circles and dashed line represent, respectively, observed data and predicted values for non-caring males; solid, small circles and solid line, for recent care-givers guarding recently laid eggs; and squares and dotted line, for old care-givers guarding old egg-batches.

CONCLUSÕES GERAIS

Como citado anteriormente, apenas o segundo capítulo desta tese havia sido aceito antes da redação do Capítulo 1, que revisa as informações então disponíveis sobre cuidado paternal em artrópodes. Os outros capítulos empíricos desta tese (Capítulos 3-5), entretanto, trazem contribuições importantes e adicionais ao nosso conhecimento sobre o tema. Grande parte dessas contribuições e conflitos entre expectativas teóricas e dados empíricos foi apresentada e discutida nos próprios capítulos. Aqui, faço uma síntese em português dos principais pontos, cujo objetivo é salientar nossas lacunas de conhecimento e indicar direções promissoras para futuros estudos teóricos e empíricos sobre investimento parental, papéis sexuais e seleção de parceiros em artrópodes.

Praticamente todos os modelos propostos para explicar a evolução de cuidado paternal e papéis sexuais, desde os trabalhos clássicos (e.g. Trivers, 1972; Williams, 1975; Maynard-Smith, 1977) até os modelos teóricos mais recentes (e.g. Manica & Johnstone, 2004; Kokko & Jennions, 2008; Barry & Kokko, 2010; McNamara et al., 2010; Alonzo, 2011), consideram o saldo entre custos e benefícios dos comportamentos exibidos por machos e fêmeas. Apesar do intuitivo benefício do cuidado em termos de aumento na sobrevivência da prole ser amplamente reconhecido (Clutton-Brock, 1991), os custos pagos pelos indivíduos parentais não apresentam um padrão assim tão claro. Para alguns grupos animais, como é o caso de aves e mamíferos, existem, de fato, grandes conflitos entre o esforço reprodutivo e o esforço parental (Stiver & Alonzo, 2009). Para artrópodes, no entanto, embora o conjunto de informações esteja restrito a apenas poucas espécies, o suposto custo em termos de aumento nos riscos de mortalidade dos machos enquanto desempenham atividades parentais não apresenta um padrão geral. Enquanto os machos guardiões do percevejo *Rhinocoris tristis* parecem ser mais atacados por predadores do que

machos não guardiões (Gilbert et al., 2010), o estado parental não afeta a sobrevivência de machos na barata d'água *Abedus breviceps* (Munguía-Steyer & Macías-Ordóñez, 2007). Os resultados que apresentamos aqui são ainda mais drásticos: nas duas espécies de opilião estudadas (*Iporangaia pustulosa* e *Zygopachylus albomarginis*) os riscos de mortalidade são mais altos para os indivíduos, tanto machos quanto fêmeas, que não estão associados a uma desova (Capítulos 2 e 3).

O que discutimos é que, dado que machos guardiões permanecem estacionários no ambiente por muito mais tempo que machos e fêmeas que se locomovem enquanto forrageiam ou buscam parceiros, o padrão de deslocamento dos indivíduos deve estar associadas à maior ou menos exposição a inimigos naturais. Essa ideia encontra paralelos com trabalhos desenvolvidos com besouros que já demonstraram que a mortalidade também é mais intensa para os indivíduos com maiores áreas de vida (McCauley & Lawson, 1986; Polis et al., 1998). Embora não se aplique aos resultados observados para o percevejo *R. tristis*, a nossa hipótese poderia servir também como uma explicação *ad hoc* para o caso da barata d'água *A. breviceps*, pois os machos guardiões são capazes de se locomover mesmo no estado parental, o que os tornaria igualmente sensíveis a seus inimigos naturais quando comparados a machos não guardiões. Obviamente esta é uma hipótese que ainda precisa ser especificamente abordada, e não usada como explicação para resultados já obtidos. Nesse sentido, destacamos dois grupos de espécies que são candidatas apropriadas para este teste formal. As espécies *Magnispina neptunus* (Nazareth & Machado 2010), *Gonyleptes saprophilus* e *Neosadocus* sp. (Machado et al., 2004), cujos machos cuidam dos ovos em cavidades naturais e estariam ainda melhor protegidos em seus ninhos do que machos de *Z. albomarginis*. Já opiliões da família Podoctidae (Martens, 1993; Machado & Macías-Ordóñez, 2007) e picnogonidos (Bain & Govedich, 2004), cujos

machos guardiões carregam ovos e podem se locomover livremente pelo ambiente, deveriam apresentar padrões similares aos observados na barata d'água *A. breviceps*.

Visando um teste rigoroso dessa hipótese proposta e uma comparação entre espécies, métodos de captura-marcação-recaptura associados a manipulações experimentais em condições de campo seriam os mais apropriados. Embora necessite de um desenho amostral detalhado, com acompanhamento dos indivíduos no campo e de um ferramental analítico específico, essa recomendação agrega diversas vantagens. Primeiramente por produzir resultados diretamente comparáveis entre estudos ao usar uma mesma metodologia criteriosa. Em segundo lugar, diferenças na pressão de predação ou parasitismo entre indivíduos, assim como no acesso a recursos, dificilmente são replicados com precisão em condições de laboratório. Por fim, a abordagem de modelos de marcação e recaptura, por utilizar históricos de encontro individuais, são robustos o suficiente para dissociar os efeitos de detectabilidade (influenciados, por exemplo, por padrões de atividade dos animais e pela habilidade do pesquisador) dos efeitos de sobrevivência, melhorando a precisão na estimativa do parâmetro de interesse (Lebreton et al., 1992).

O cuidado parental pode impor também diversos custos energéticos, tanto quanto a restrições na obtenção de alimento durante o período de cuidado quanto nos gastos associados a comportamentos específicos direcionados à prole (Gross & Sargent, 1985; Clutton-Brock, 1991). Novamente, essa situação parece ser o padrão para aves, nas quais o alto metabolismo dos filhotes requer que os indivíduos parentais estejam constantemente buscando alimento para a prole (Stiver & Alonzo, 2009). No caso de artrópodes com cuidado paternal exclusivo à prole, existe uma grande diversidade de sistemas e de comportamentos parentais interagindo sobre o balanço energético final do cuidado parental. Em baratas d'água e picnogonidos, por exemplo, embora os machos guardiões

possam se locomover enquanto cuidam da prole, potencialmente acessando recursos alimentares (Burris, 2010), eles também exibem uma série de comportamentos de oxigenação dos ovos que são tidos como energeticamente custosos (Munguía-Steyer et al., 2008; Burris, 2010). Em opiliões e percevejos, os machos guardiões permanecem estacionários sobre as desovas por um período relativamente longo de tempo, porém os dados disponíveis sugerem que este comportamento restringe o forrageamento de machos de algumas espécies (Nazareth & Machado, 2010; Requena et al., 2012), mas não afeta o acesso desses machos a alimento em outras (Capítulo 3), sendo que eles podem inclusive canibalizar ovos da própria desova (Thomas & Manica, 2005). Dessa maneira, quantificações precisas e padronizadas do investimento energético de indivíduos parentais são fundamentais para gerar resultados comparáveis entre estudos. Nesse sentido, embora algumas ressalvas sejam feitas, medidas de volume e massa, controladas por medidas de tamanho estrutural (i.e. aquelas que são fixas e não se alteram com mudanças no estado nutricional), assim como densidade corporal, estão cada vez mais ganhando notoriedade como bons proxies de condição corporal (Moya-Laraño et al., 2008).

Embora não exista um padrão claro quanto aos custos energéticos associados ao cuidado paternal, eles parecem ser mais importantes no sistema de acasalamento dessas espécies do que os custos em termos de sobrevivência. Nesse sentido, a categorização de cuidado depreciável ou não-depreciável pode agregar informações mais refinadas e talvez com melhor poder preditivo. Clutton-Brock (1991) define essas categorias baseado na relação negativa direta entre a qualidade do cuidado oferecido pelo indivíduo parental e o número de prole sob o seu cuidado, porém esta divisão foca apenas em diferenças no grau de atendimento dos indivíduos parentais a uma dada prole única. Por outro lado, desovas múltiplas são comumente encontrada em espécies com cuidado paternal (peixes: e.g.

Manica & Johnstone, 2004; artrópodes: revisões em Tallamy, 2001; Requena et al., in press). Nesses casos, a combinação da assincronia entre oviposições e o tempo de cuidado requerido pelos ovos individuais até completarem seu desenvolvimento inserem um componente temporal não apreciado pelas definições originais. Por exemplo, no caso de *I. pustulosa*, nós argumentamos que o cuidado seja depreciável, não porque o macho guardião diminua o atendimento individual que oferece a cada ovo quando está cuidando de uma desova grande, mas porque, com o passar o tempo de cuidado, ele adquire desovas adicionais (Capítulo 5) ao mesmo tempo que sua condição física decai (Capítulo 2). Embora o padrão observado possa ser o mesmo nos dois casos, i.e. qualidade do cuidado despendido diminuindo com o aumento do número de ovos, essa ligação parece ser apenas indireta no caso de *I. pustulosa*.

Sob essa nova perspectiva, a categorização do cuidado em depreciável ou não-depreciável passaria a considerar se a qualidade do cuidado paternal futuro depende não apenas de habilidades inatas dos machos, mas de sua condição corporal. Quando depreciável, a previsão é que a condição corporal do macho decairia ao longo do tempo (nos casos de longo tempo de desenvolvimento dos ovos e/ou desovas múltiplas assincronicamente ovipostas) ou de acordo com o tamanho da desova (nos casos de comportamentos parentais direcionados individualmente para cada ovo). No caso de cuidado não depreciável, por outro lado, a condição corporal dos machos guardiões apresentaria variações independentes das características da desova. Em espécies com cuidado paternal, espera-se que as fêmeas avaliem seus potenciais parceiros considerando o benefício direto da transferência de cuidado para o macho (Hoelzer, 1989; Alonzo 2011). Entretanto, distinções entre cuidado depreciável ou não-depreciável podem mudar

radicalmente quais características específicas elas devem avaliar para acessar com precisão a qualidade do cuidado futuro (Wagner Jr., 2011).

Quando o cuidado é não-depreciável, as chances de sobrevivência da prole sob o cuidado de machos não diminui ao longo do tempo ou com o tamanho da desova. Na verdade, machos de alguns peixes até intensificam seus comportamentos parentais e diminuem a taxa de canibalismo de ovos quando cuidando de desovas grandes (e.g. Pampoulie et al., 2004). Assim, variações entre machos ou entre sítios de oviposição são provavelmente mais importantes no sucesso da prole e fêmeas devem avaliar exatamente essas características. Como se tratam de características relativamente fixas ao longo de um curto intervalo de tempo, o sinal de que outra fêmea copulou com um determinado macho recentemente poderia servir como um sinal avaliado pelas fêmeas durante suas decisões. Em diversas espécies de peixes, padrões de escolha não independente de parceiros por fêmeas já foram descritos (e.g. Dugatkin, 1992; Witte & Ryan, 2002; Alonzo, 2008). Mais do que isso, se a característica avaliada é o ninho ou a presença / quantidade de ovos no território de machos, a evolução de estratégias de briga por territórios, ocupação de ninhos desabrigados e até mesmo adoção de desovas seriam favorecidos (Tallamy, 2001). Essas predições parecem estar de acordo com o observado no percevejo *R. tristis* e no opilião *Z. albomarginis*, cujos machos têm acesso a alimento durante o período de cuidado (Thomas & Manica, 2003; 2005; Capítulo 3) e estão dispostos a adotar desova (Gilbert et al., 2010) ou apenas invadir os ninhos de outros machos bem sucedidos (Mora, 1990).

Por outro lado, quando o cuidado é depreciável, o comportamento paternal atual não é um sinalizador honesto da qualidade do cuidado no futuro. Neste caso, fêmeas que avaliassem diretamente a característica que determina a qualidade do cuidado, ao invés da simples presença de ovos em territórios de machos, estariam em vantagem (Wagner Jr.,

2011). Como a qualidade do cuidado decairia em função da diminuição da condição física dos machos ao longo do tempo de cuidado, esperamos que a seleção feminina em casos de cuidado depreciável seja baseada em características condição-dependente. Além disso, como o sinalizador da qualidade do cuidado estariam estritamente relacionadas com a condição pontual do macho, estratégias de adoção de ovos não teriam pressão evolutiva nenhuma a favor, pois esses machos invasores apenas investiriam energia em atividades parentais, diminuindo sua atratividade e ainda aumentando a sobrevivência de ovos não relacionados geneticamente a eles. No caso do picnogonido *Achelia simplissima*, é sugerido que a presença de massas de ovos nas pernas ovígeras dos machos por si só sejam suficientes para indicar o desgaste físico dos indivíduos parentais, dados os efeitos do peso extra adquirido com os ovos e da maior taxa de comportamentos de oxigenação dos ovos (Burris, 2010). No opilião *I. pustulosa*, por sua vez, o efeito parece ser misto, já que existe uma janela temporal na qual machos guardiões são extremamente atrativos, passando para um estágio subsequente em que são quase tão evitados por fêmeas quanto machos não guardiões (Capítulo 5). Associado a isso, existe uma variação muito grande na condição física de machos não guardiões e que estão guardando desovas em estágios iniciais de desenvolvimento, ao passo que machos com ovos prestes a eclodir apresentam condições físicas muito parecidas entre si, além de estarem em pior estado do que não guardiões (Requena et al., 2012).

Apesar de interessante, esta hipótese não deve ser vista como uma explicação para os resultados observados aqui ou em outros trabalhos. Ela está apoiada em uma série de premissas que parecem plausíveis para alguns sistemas, porém ainda faltam muitas informações básicas para a maioria das espécies. Uma conclusão geral, entretanto, é que investigar restrições energéticas às quais machos guardiões podem ou não estar sujeitos

parece o primeiro passo em direção ao entendimento dos sistemas de acasalamento em espécies com cuidado paternal, não apenas artrópodes. Entretanto, para testes formais desta e de outras hipóteses subsequentes, avaliações mais finas e experimentais do efeito da condição física de machos guardiões ou não guardiões em sua atratividade são fundamentais. Mais do que investigar esse efeito, hipóteses interessantes podem se basear nas vias mecânicas de como indivíduos acessam a condição de parceiros durante interações reprodutivas. Aqui, nos dois capítulos sobre papéis sexuais e seleção de parceiros (Capítulos 4 e 5), sugerimos que a comunicação química seja uma via importante de transmissão de informações entre opiliões, especulando especificamente sobre o potencial papel de hidrocarbonetos de cutícula e feromônios sexuais nessa dinâmica. Apesar das vias de acesso à informação utilizada por indivíduos de outras espécies poderem ser outras, a hipótese geral ainda se sustenta e investigações em grupos diferentes forneceriam testes independentes dessa mesma proposição.

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