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Bruno Alves Buzatto

BIOLOGIA REPRODUTIVA DO OPILIÃO *ACUTISOMA PROXIMUM* (ARACHNIDA: OPILIONES): ESTRATÉGIAS ALTERNATIVAS DE ACASALAMENTO EM MACHOS E CUIDADO PARENTAL EM FÊMEAS

Este exemplar corresponde à redação final
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Glauco Machado
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RESUMO

A presente dissertação investigou a biologia reprodutiva do opilião *Acutisoma proximum* no Parque Estadual Intervales, sul do estado de São Paulo. No Capítulo 1, o sistema de acasalamento da espécie é descrito em detalhes. Fêmeas depositam seus ovos sob folhas na vegetação que margeia riachos de interior de mata e utilizam preferencialmente determinadas espécies de plantas como sítio de oviposição. No começo da estação reprodutiva, machos lutam entre si pela posse de territórios na vegetação onde fêmeas se reproduzirão. Alguns meses mais tarde, na mesma estação reprodutiva, essa poliginia por defesa de recursos muda para uma poliginia por defesa de fêmeas, e machos passam a guardar cada fêmea individualmente em seqüência. O opilião *A. proximum* é o primeiro aracnídeo não-acarino que apresenta uma mudança em seu sistema de acasalamento ao longo da estação reprodutiva.

No Capítulo 2, são descritas duas estratégias alternativas de acasalamento entre os machos de *A. proximum*, e a morfologia e o comportamento dos machos que adotam cada estratégia é investigado. Os machos de *A. proximum* se dividem em dois morfos distintos, de acordo com a relação alométrica do comprimento da perna II, que é também mais longa nos machos do que nas fêmeas. A diferença na morfologia dos dois morfos só é detectável quando muitos machos são medidos e a relação entre o comprimento do segundo par de pernas e o tamanho do corpo são analisados, o que se encaixa na definição de dimorfismo intra-sexual críptico. Machos com pernas II longas defendem territórios na vegetação, brigando e repelindo outros machos que se aproximam das fêmeas dentro desses territórios. Machos com pernas II curtas nunca defendem territórios ou brigam. Eles se

deslocam entre os territórios dos machos grandes, invadindo-os e copulando com as fêmeas que estão dentro deles. Este trabalho é o primeiro a descrever, com dados comportamentais e morfológicos, a existência de estratégias alternativas de acasalamento na ordem Opiliones.

No Capítulo 3, o enfoque passa a ser as fêmeas e os custos e benefícios do cuidado maternal em *A. proximum*. Em um experimento de remoção de fêmeas guardiãs, os ovos desprotegidos sobreviveram 75,6% menos que os ovos protegidos pelas fêmeas, revelando a importância da proteção materna. Em outro experimento, as desovas de metade das fêmeas foram removidas e o sucesso reprodutivo delas foi monitorado por dois anos. Fêmeas impedidas de cuidar da prole produziram novas desovas mais frequentemente e tiveram um sucesso reprodutivo 18% maior que o das fêmeas que cuidaram da prole. Adicionalmente, o estudo de captura-marcação-recaptura não demonstrou nenhuma diferença entre a sobrevivência de fêmeas que foram impedidas de cuidar da prole e fêmeas que cuidaram da prole. Pesando os custos e benefícios do comportamento de guarda de ovos, uma estratégia de abandono da prole implicaria em uma redução média de 73,3% no sucesso reprodutivo total das fêmeas. Apesar dos custos da guarda de ovos para a fecundidade das fêmeas, o cuidado maternal aumenta o seu sucesso reprodutivo devido à crucial proteção aos ovos fornecida pelas fêmeas.

INTRODUÇÃO GERAL

> “Welcome to planet earth, a world positively rippling with life. In the cosmic perspective it is, for the moment, unique. The only world in which we know with certainty that the matter of the cosmos has become alive and aware.”
>
> Carl Sagan

> “Isn't it enough to see that a garden is beautiful without having to believe that there are fairies at the bottom of it too?”
>
> Douglas Adams

Esta introdução geral à dissertação foi escrita pensando-se em dois tipos de leitores: os leigos e os estudantes que estão se iniciando em ecologia comportamental. Entretanto, leitores do tipo "especialistas" também poderão encontrar alguma informação de interesse neste texto, já que ele introduz a estrutura geral da tese, direcionando os assuntos que serão tratados em cada capítulo. A introdução geral expõe brevemente o contexto teórico da dissertação e, em seguida, sintetiza as perguntas levantadas em cada um dos capítulos. Como o objetivo principal desta parte da tese foi comunicar seu conteúdo aos "não-especialistas", evitou-se o jargão técnico quase totalmente. Espera-se que para os leigos o texto sirva como um exemplo do tipo de pesquisa que se faz em ecologia comportamental, salientando que há outras razões para se estudar a biologia dos artrópodes além dos possíveis benefícios ou malefícios que eles trazem ao homem. Para os estudantes iniciantes, espera-se que o texto tenha um valor educativo, pois integra aspectos básicos de comportamento animal sob a luz da teoria da evolução.

A teoria da evolução pela seleção natural (Darwin 1859), popularmente conhecida e ensinada como a "luta pela sobrevivência", é o paradigma central de toda a biologia moderna. Porém, o assunto principal abordado nesta dissertação deriva de um outro mecanismo evolutivo, a seleção sexual — também proposto por Charles Darwin, e provavelmente tão importante quanto a seleção natural. Para introduzir esse assunto, começarei descrevendo dois animais que ilustram eficientemente um importante fenômeno. Entre os inúmeros animais classificados pelo pioneiro naturalista sueco Carlos Lineu (1758), estão dois patos completamente distintos. Uma dessas espécies possui uma plumagem de coloração marrom e branca bastante heterogênea, além de uma mancha azul nas asas. Lineu a nomeou como *Anas platyrhynchos*. A outra espécie possui uma plumagem de coloração em geral cinza pálido, mas com o peito marrom escuro, a cabeça verde metálica e uma mancha azul nas asas. Lineu a nomeou como *Anas boschas*. Hoje em dia, sabe-se que esses dois patos muito distintos aos nossos olhos, na verdade pertencem à mesma espécie, sendo *A. boschas* nada mais do que o macho de *A. platyrhynchos*, em português conhecido como "pato-real".

O fenômeno natural responsável por diferenças tão conspícuas entre machos e fêmeas, como as existentes no pato-real, foi um dos principais motivos que levaram Darwin a escrever o livro "A origem do homem e a seleção em relação ao sexo (1871)". Nesse livro, Darwin formaliza a teoria da seleção sexual, que enfatiza a importância da "luta pela reprodução", atuando simultaneamente com a "luta pela sobrevivência", na evolução das espécies. Desde então, a diversidade morfológica e comportamental entre machos e fêmeas, chamada de dimorfismo sexual, vem sendo entendida como o resultado de diferentes pressões de seleção sexual atuando em cada sexo, predominantemente devido à seleção sexual mais intensa sobre os machos (Andersson 1994).

Todo o desenvolvimento da teoria de seleção sexual se baseou em inferências sobre a forma e a intensidade com que a seleção sexual atua sobre machos e fêmeas. Isso só foi possível através da compreensão das estratégias que machos e fêmeas adotam para se reproduzir. Dessa forma, a própria construção da teoria de seleção sexual deve-se fundamentalmente ao estudo de sistemas de acasalamento em animais. Em 1977, Emlen & Oring categorizaram os sistemas conhecidos na época e sintetizaram toda informação sobre o assunto em um único contexto teórico. Dentre as categorias descritas por eles, está a poliginia, que ocorre sempre que alguns machos conseguem acasalar-se com múltiplas fêmeas, cada um deles frequentemente lutando com outros machos pelo controle e monopólio das fêmeas.

Apesar da importância teórica da classificação dos sistemas de acasalamento, o ato de designar uma dada espécie para apenas uma das categorias raramente é uma tarefa simples. De fato, a fronteira que separa poliginia por defesa de recursos e poliginia por defesa de fêmeas, por exemplo, é tão sutil que a maioria dos animais estudados até o presente não se encaixa perfeitamente em nenhuma dessas categorias (Ostfeld 1987). Uma compreensão mais adequada da diversidade de sistemas de acasalamento em animais pode ser atingida quando se imagina que a maioria dos sistemas reais estudados se situa em algum ponto ao longo de um contínuo entre as categorias (Shuster & Wade 2003). Além disso, machos de várias espécies já estudadas parecem mudar entre uma categoria de poliginia e outra de acordo com variações temporais em seu ambiente social ou físico (Tsukamoto et al. 1994).

O objetivo do Capítulo 1 desta dissertação foi estudar o sistema de acasalamento do opilião *Acutisoma proximum*. As fêmeas dessa espécie depositam seus ovos sob folhas na vegetação que margeia pequenos riachos do Parque Estadual Intervales. Nesse capítulo, são investigadas a preferência das fêmeas por certas espécies de plantas como sítio de oviposição e a estratégia principal utilizada pelos machos para tentar monopolizar as fêmeas. Em seguida, é fornecida uma descrição do cuidado paternal ocasional oferecido à prole, seguida de uma discussão sobre as implicações deste comportamento para a compreensão da evolução do cuidado paternal exclusivo em opiliões.

Após a compreensão do sistema de acasalamento de *A. proximum* fornecida pelo Capítulo 1, o Capítulo 2 possui um enfoque exclusivo nos machos desta espécie. Além da diversidade morfológica e comportamental que pode ser encontrada entre os sexos, como descrito no início desta introdução, também pode existir uma diversidade intra-sexual (Gross 1996). Assim, machos de uma mesma população podem apresentar uma variação descontínua em sua morfologia e comportamento. Essas diferenças podem resultar da evolução de distintas estratégias de acasalamento entre eles, fenômeno aparentemente comum quando a competição entre machos pelas fêmeas é bastante intensa (Gadgil 1972). Em algumas espécies, as diferentes estratégias dos machos resultam em um sucesso reprodutivo médio similar entre elas, de forma que esse polimorfismo representa uma estratégia evolutivamente estável mista (Maynard Smith 1988). Nesse caso, a seleção dependente de freqüência regula a proporção de machos que adota uma ou outra estratégia na população. Em outras palavras, as duas ou mais estratégias existentes em uma mesma população são igualmente vantajosas paras os machos que as adotam, contanto que um ponto de equilíbrio entre a proporção delas seja mantido. Alternativamente, as diferentes estratégias adotadas pelos machos podem ser dependentes de sua condição física, de forma que cada alternativa é mais vantajosa sob determinadas circunstâncias ou restrições (Eberhard 1982).

Assim, o objetivo do Capítulo 2 foi descrever a variação morfológica e comportamental presente entre machos do opilião *A. proximum*. Nesse capítulo, é descrita a existência de duas morfologias alternativas associadas a distintas estratégias de acasalamento em machos da espécie. Além disso, é proposta uma inovação metodológica para detectar o sutil dimorfismo morfológico entre machos de uma mesma população. O Capítulo 2 testa também uma previsão da teoria de competição espermática, que diz que, em espécies que apresentam estratégias alternativas de acasalamento, indivíduos do morfo que sofre um maior risco de competição espermática devem produzir maior quantidade de esperma (Parker 1990).

Ao contrário dos machos de *A. proximum*, as fêmeas desta espécie não apresentam estratégias reprodutivas alternativas. Todas elas visitam o território dos machos na vegetação que margeia pequenos riachos (conforme descrito no Capítulo 1), copulam com esses machos e, em seguida, começam a depositar seus ovos sob folhas dentro desses territórios. Após a deposição dos ovos, as fêmeas permanecem sobre eles até que as ninfas eclodam e se dispersem, aproximadamente 37 dias depois. Essa guarda de ovos é um importante aspecto da biologia reprodutiva das fêmeas de *A. proximum* e por isso ela foi o enfoque do Capítulo 3 desta dissertação.

Poucos estudos até o presente tiveram sucesso em quantificar os custos e benefícios da guarda de ovos por fêmeas em artrópodes (mas veja Zink 2003 e referências). Além disso, também existem poucos estudos de longa duração que investigam separadamente os custos do cuidado maternal em termos de sobrevivência e de fecundidade das fêmeas. O único estudo até o momento que avaliou os efeitos da guarda de ovos sobre a sobrevivência das fêmeas guardiãs em uma espécie de artrópode encontrou exatamente o oposto do que prevê a teoria. Tallamy & Denno (1982), estudando o percevejo *Gargaphia solani*, encontraram que o investimento em defesa dos ovos por fêmeas desta espécie aumentou, ao invés de diminuir, a longevidade das fêmeas.

No Capítulo 3, manipulações experimentais são aliadas a um estudo de captura-marcação-recaptura em uma tentativa de identificar e quantificar os custos e os benefícios do cuidado maternal por fêmeas do opilião *A. proximum*. Além disso, para elucidar a questão do efeito da guarda de ovos sobre a sobrevivência das fêmeas, os custos da guarda de ovos são decompostos em dois componentes: custos ecológicos (que afetam a sobrevivência) e custos fisiológicos (que afetam a fecundidade). Finalmente, no Capítulo 3 também é simulada uma estratégia alternativa de abandonar os ovos para fêmeas dessa espécie, e o sucesso reprodutivo resultante desta estratégia é comparado com o sucesso reprodutivo das fêmeas que guardam seus ovos.

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CAPÍTULO 1

Resource defense polygyny shifts to female defense polygyny over the course of the reproductive season of the Neotropical harvestman *Acutisoma proximum* (Arachnida: Opiliones): a unique mating system in arachnids

Cópula no opilião *Acutisoma proximum*: o macho está à esquerda e a fêmea à direita.

Abstract

The categorization of a given species' mating system is seldom clear, and in particular the difference between female defense and resource defense polygyny can be slight. In addition, males of some species shift between different types of polygyny in response to temporal variation in social and environmental conditions. Here we describe the mating system of the harvestman *Acutisoma proximum*, whose females lay their eggs on the underside of the leaves of certain host plant species. At the beginning of the reproductive season, males fight other males for the possession of territories on the vegetation where females will later oviposit, as is typical of resource defense polygyny. Later on the reproductive season, however, males reduce their patrolling to focus on individual females that are ovipositing inside their territories. Therefore, the mating system becomes more similar to female defense polygyny. This is the first described case of an arachnid that exhibits a shift in mating system over the reproductive season. Finally, our data on the occasional paternal care presented by territorial males shed light on the evolution of exclusive post-zygotic paternal care in Opiliones.

Keywords: Gonyleptidae, harem, host plant selection, male territoriality, paternal care.

Introduction

The evolution of animal mating systems was a central issue of Darwin's (1871) theory of sexual selection and a matter of interest for evolutionary biologists ever since. Three decades ago, Emlen and Oring (1977) provided a unified theoretical framework for mating systems evolution that focused on the ecological constraints affecting the reproductive strategies of males and females. Emlen and Oring's (1977) central argument was that individuals from one sex (usually males) could increase their fitness by attempting to control access to the members of the other sex (usually females). According to the authors, the degree to which this monopoly is possible depends on environmental factors, such as the availability and spatial dispersal pattern of critical resources used by the limiting sex.

Polygyny is the mating system in which a minority of males control or gain access to multiple females leaving most other males without access to females (Shuster & Wade 2003). This mating system is usually favored by selection when individual males are able to monopolize a clumped group of receptive females (Emlen & Oring 1977). Polygyny can be further divided into female defense polygyny and resource defense polygyny. Resource defense polygyny occurs when males control females indirectly by defending territories or resources against conspecific males, whereas female defense polygyny occurs when males control females directly by defending them against conspecifics males. Thus, the spatial and temporal distribution of females and resources will determine whether males defend females or resources that attract receptive females (Shuster & Wade 2003). However, the boundary between female defense and resource defense polygyny is actually slight and most species does not fall neatly into neither of the categories (Ostfeld 1987). There are also numerous described cases of animal populations that shift between different types of polygyny according to temporal variations in the social and environmental conditions (Tsukamoto et al. 1994).

Among arthropods, resource defense polygyny is widespread (e.g., Thornhill & Alcock 1983). Examples of male insects that defend resources include the hylaeine and wool-carder bees that defend the flowers that provide pollen and nectar for females (Alcock & Houston 1996, Starks & Reeve 1999), coreid bugs and grasshoppers that defend host food-plants (Mitchell 1980, Greenfield 1997, Eberhard 1998), bark beetles that defend burrows where females feed and reproduce (Reid & Stamps 1997), and carrion beetles that defend small carcasses attractive to females (Scott 1998, Suzuki et al. 2006). In pollen wasps, males may defend either water collection sites or flowers, both important reproductive resources for females (Groddeck et al. 2004). Male dragonflies, robber flies, and tephritid flies defend the oviposition sites preferred by females (Wolf et al. 1997, Hastings et al. 1994, Aluja et al. 2001).

In contrast to resource defense polygynists, males from species that engage in female defense polygyny directly defend groups of females (e.g., Poston 1997) or individual females in sequence (e.g., Robinson 1986). This kind of polygyny is fairly common among mammals, and also occurs in a small number of bird species (see Webster 1994, Poston 1997, Webster & Robinson 1999 and references within). Although female defense polygyny is not particularly common within arthropods, it has been reported for at least seven insect orders, namely Hemiptera (Fujisaki 1981), Orthoptera (Gwynne & Morris 1983), Zoraptera (Choe 1994, 1997), Thysanoptera (Crespi 1988), Coleoptera (Hamilton et al. 1976, Kirkendall 1983, Kirkendall et al. 1997), Diptera (Wilkinson & Dodson 1997), and

Hymenoptera (Eberhard 1975, Alcock et al. 1977, Smith & Alcock 1980, Gwynne 1980, Lloyd 1981), and for the crustacean order Amphipoda (McCurdy et al. 2000).

Although many studies classify the mating system of arthropod species as female defense or resource defense polygyny, the boundary between these two categories is slight and most species does not fall neatly into neither of them (Ostfeld 1987). There are also numerous described cases of animal populations that shift between different categories of polygyny according to temporal variations in the social and environmental conditions (Tsukamoto et al. 1994). The purpose of this study was to examine the mating system of the Neotropical harvestman *Acutisoma proximum* (Gonyleptidae), for which the dichotomy between female defense and resource defense polygyny can be specially misleading. In the beginning of the reproductive season, males defend territories on the vegetation where females lay their eggs, resembling a resource defense polygyny. Over the course of the reproductive season, however, the mating system shifts to female defense polygyny, since males start guarding sequentially individual females inside their territories. To the best of our knowledge, this is the first time that this shift in a polygynous mating system is described for arachnids. The study also provides an experimental test for the hypothesis that the association between territorial males and offspring through the defense of an oviposition site constitutes the basis for the evolution of paternal care (Williams 1975).

Materials and methods

Study site

The study was conducted in an Atlantic Forest fragment at Intervales State Park, southern São Paulo state, south-eastern Brazil. The region has high precipitation levels, with the average annual rainfall varying between 2,000 and 3,000 mm/year, and the average annual temperature varying between 17°C and 19°C. The altitude in the Park ranges from 60 to 1,095 m, and the weather is mainly subtropical, with a warm-wet season lasting from October to March and a cold-dry season from April to September. The *Acutisoma proximum* population studied here was found on the vegetation at the Caçadinha Track (24°14′ S; 48°04′ W; 800 m a.s.l.), a trail that follows a small river. The river is approximately 5 m wide and is flanked by abundant vegetation that sometimes partially covers the river's bed.

Study species

The reproductive behavior of *A. proximum* females was firstly described by Ramires & Giaretta (1994) for a population from the foothills of Serra de Cubatão, Santos, São Paulo state, south-eastern Brazil. At that location, *A. proximum* females lay their eggs among gaps in granitic stones on river margins. At Intervales State Park, however, females oviposit mainly on the underside of leaves on the vegetation hanging above the river (Buzatto et al. 2007). Maternal care in *A. proximum* lasts approximately 37 days, and the costs and benefits of female egg-guarding have been investigated with experimental manipulations and capture-mark-recapture methods in the field (Buzatto et al. 2007). The authors found that, although egg-guarding in the species has fecundity costs for females, this behavior increases female fitness because female presence prevents egg predation. Even though maternal care in *A. proximum* has already been intensely studied, the oviposition site selection by females and its consequences to the mating system have not yet been explored.

Behavioral observations

Between July 2003 and December 2005, we studied the reproductive behavior of *A. proximum* by inspecting monthly a 200 m long transect in the Caçadinha Track. Each monthly sample consisted of monitoring the study site for four consecutive days, each of which comprising approximately 12 hours of observations, carried out between 08:00 h and 00:00 h. We conducted nocturnal observations with a flashlight covered by a red filter to avoid disturbing the harvestmen (Machado & Oliveira 1998). For the whole period of 30 months, the total amount of naturalistic observations in the field was almost 1,440 hours.

During the study, we measured (to the nearest 0.01 mm) every adult *A. proximum* for the dorsal scute length, fourth right leg femur length, and second right leg length. At the time of the first capture, we also recorded if the individuals had any amputated legs or pedipalps. Next, we individually marked them applying enamel paint (Testors Co., Rockford, U.S.A.) to the dorsal scute and to the femur of the third and/or fourth pair of legs. This marking procedure has been widely used in harvestmen studies since it does not affect the behavior of these organisms (see Buzatto et al. 2007 and references within). Throughout the field observations, we continuously recorded all relevant behavioral events as suggested by Martin and Bateson (1986).

In order to achieve a better understanding of the mating system in the species, we conducted approximately 800 hours of additional observations on 33 *A. proximum* harems between December 2006 and March 2007. These harems are generally composed of one territorial male and up to six egg-guarding females (see Results). Furthermore, we intensively monitored four of these harems, carrying out eight scan samplings per day (08:00, 11:00, 14:00, 17:00, 20:00 and 23:00 h) for approximately 20 consecutive days, starting when the first female of each harem began to oviposit. During each scan, we recorded how close the territorial male was from each of the egg-guarding females in his harem. The distances recorded were categorized as following: male nowhere at sight (score 0), at less than 1 m from the female (score 1), at less than 20 cm from the female (score 2), at less than 5 cm from the female (score 3), or copulating with the female (score 4). We later plotted the daily average of these distance categories for each female in four charts (one for each harem), in order to visualize how the territorial male divided his patrolling activity along the females from his harem.

Oviposition sites availability

In the 200 m transect of the Caçadinha Track where we had previously carried out all the naturalistic observations, we identified all plant species (thereafter called host plants) that were used at least once by females during the reproductive season that lasted from September 2003 to April 2004. Afterwards, in order to assess the availability of oviposition sites for females, we sampled the relative abundance of all host plants by recording the number of individuals of each host plant in our transect. Finally, we used a G test to contrast host plant abundance with their use by *A. proximum* females.

Results

Reproductive seasonality

Between July 2003 and December 2005, we found 424 clutches of *Acutisoma proximum* in the study transect. Each clutch was guarded by a female, and the average (±SD) number of eggs per clutch was 129.7±30.3 (range: 36-217 eggs; n=350 clutches). Eggs in the initial stage of embryonic development are cream colored and have an average (±SD) diameter of

1.44±0.05 mm (range: 1.37–1.48 mm; n=10 eggs). During the embryonic development, eggs gain water, growing and getting darker until they reach an average (±SD) diameter of 1.98±0.06 mm (range: 1.91–2.1 mm; n=10 eggs) and a spotted black coloration soon before hatching, approximately 30 days later. In contrast to other harvestman species exhibiting maternal care (Machado & Macías-Ordóñez 2007), *A. proximum* females do not lay their entire clutch in a short period of time. In the first three days 95.4±8.0% (mean±SD) of the eggs are laid, but the female may take up to 14 days to complete oviposition. That results in a developmental heterogeneity within the eggs of a clutch, with small young eggs and large old ones occurring side by side in the same clutch. Over several days, the smaller and younger eggs develop and hatch normally, but the asynchrony in the oviposition results in a longer period of egg guarding for females, given that more time is required for all the eggs to hatch.

The reproductive activity of *A. proximum* is markedly seasonal: one minor peak occurs between October and November (beginning of the warm-wet season), while a major peak takes place in February, which corresponds to the end of the warm-wet season (Fig. 1a). This pattern was observed in both reproductive seasons studied (2003-2004 and 2004-2005), and the number of clutches per month was positively related to the local monthly rainfall (Spearman, rs=0.820, P=0.001; n=12 for the 2003-2004 season; and rs=0.843, P<0.001; n=12 for the 2004-2005 season). There was also a positive relationship between the number of clutches found each month and the monthly average local temperature (Spearman, rs=0.664, P=0.018; n=12 for the 2003-2004 season; and rs=0.737, P=0.006; n=12 for the 2004-2005 season).

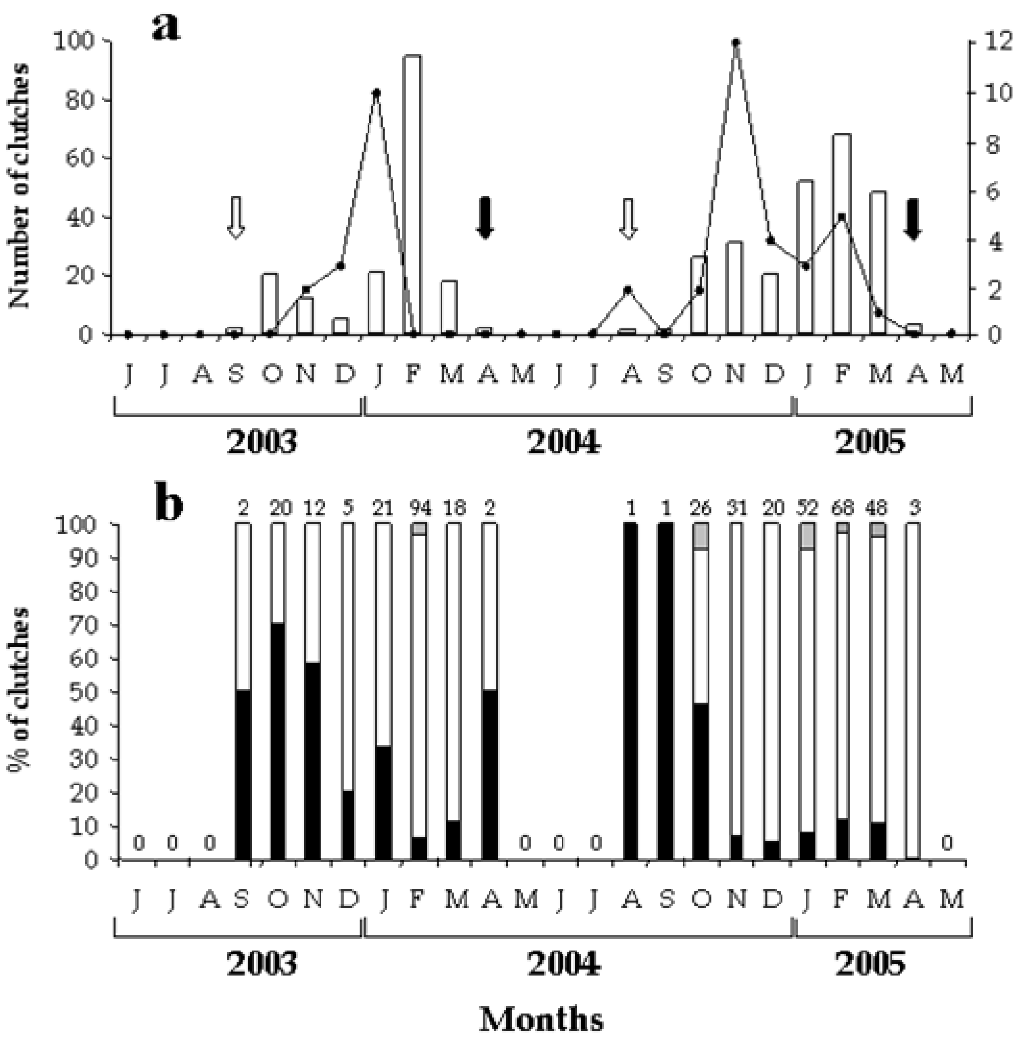


Figure 1: Reproductive seasonality of the harvestman *Acutisoma proximum* at Intervales State Park, south-eastern Brazil. (a) The white bars represent the number of clutches found each month (from June 2003 to May 2005). The white arrow indicates when males' territorial patrolling activity began in both reproductive seasons, and the black arrow indicates when such activity ended. The line represents the number of male territorial fights recorded each month. Note that in both reproductive seasons fights were most common before the peak of oviposition activity in February. (b) The proportion of *A. proximum* females that laid eggs on boulders (black bars), on the underside of leaves (white bars), and on the surface of tree trunks (grey bars). The numbers above bars represent the total number of clutches found each month.

Host plant selection

Among all females found guarding eggs, 338 had clutches laid on the underside of leaves, 73 had clutches laid on boulders, and 13 had clutches laid on the surface of tree trunks, always very close to the river margins (20-300 cm). The proportion of clutches found on leaves, boulders, and trunks varied monthly during the seasons of 2003-2004 and 2004-2005, without a clear pattern (Fig. 1b). There was no difference between the average (±SD) number of eggs in clutches found on boulders (122.3±28.3 eggs) and on leaves (129.7±30.7 eggs) (t=1.810, df=341, P=0.073). Clutches found on tree trunks (130.4±26.0 eggs) were left out of the analysis due to the small number found in this particular microhabitat.

Regarding the clutches laid on leaves, *A. proximum* females used 25 different host plant species as oviposition sites, but the frequency of utilization varied among host plant species (Table 1). Three species comprised 50% of the used host plants: the ferns *Olfersia cervina* (16.4%) and *Blechnum binervatum* (10.2%), and the tree *Gomidesia schaueriana* (23.4%). Although these species were frequently used as oviposition sites, they were not the most abundant host plant species in the transect (Fig. 2). On the other hand, some very abundant host plant species, such as *Dichorisandra thyrsiflora* and *Calathea communis*, were seldom used as oviposition sites (Fig. 2). Thus, host plants were clearly not used according to their abundance in the study transect (G-test=226.94; df=24; P>0.0001), further confirming that females show preference for some plant species.

Table 1. Number of clutches of the harvestman *Acutisoma proximum* found on each host plant species used by egg-guarding females at Intervales State Park.

Host plant species	**Number of clutches**	**Number of individuals**
FILICOPSIDA		
Blechnaceae		
Blechnum binervatum (Poiret) C. Morton & Lellinger	13	16
Dryopteridaceae		
Diplazium cristatum (Desr.) Alston.	8	101
Dryopteridaceae sp.	3	31
Lastreopsis sp.	1	65
Olfersia cervina (L.) Kunze	21	81
Hymenophyllaceae		
Trichomanes radicans Swartz	6	14
Polypodiaceae		
Campyloneurum minus Fée, Gen. Fil.	1	78
ANGIOSPERMA		
Amaranthaceae		
Amaranthus sp.	6	64
Araceae		
Philodendrum ochrortemon Schott	6	52
Begoniaceae		
Begonia sp.	1	35
Commelinaceae		
Dichorisandra thyrsiflora Mikan	1	316
Lauraceae		
Ocotea puberula (Rich.) Nees	1	13

Table 1. Continuation.

Host plant species	**Number of clutches**	**Number of individuals**
Leguminosae		
Zollernia ilicifolia Vog.	1	5
Marantaceae		
Calathea communis Wanderley & Vieira	3	192
Myrsinaceae		
Ardisia guyanensis (Aubl.) Mez	6	20
Myrtaceae		
Calyptranthes grandifolia O.Berg	3	1
Gomidesia schaueriana O.Berg	30	26
Myrceugenia myrcioides (Cambess.) O.Berg	6	26
Nyctaginaceae		
Guapira opposita (Vell.) Reitz	1	16
Piperaceae		
Piper sp.1	2	45
Piper sp.2	1	16
Rubiaceae		
Bathysa australis (A.St.-Hil.) Benth. & Hook.f.	2	25
Psychotria sp.	1	8
Rudgea jasminioides (Cham.) Müll.Arg.	1	6
Sapotaceae		
Chrysophyllum sp.	3	25

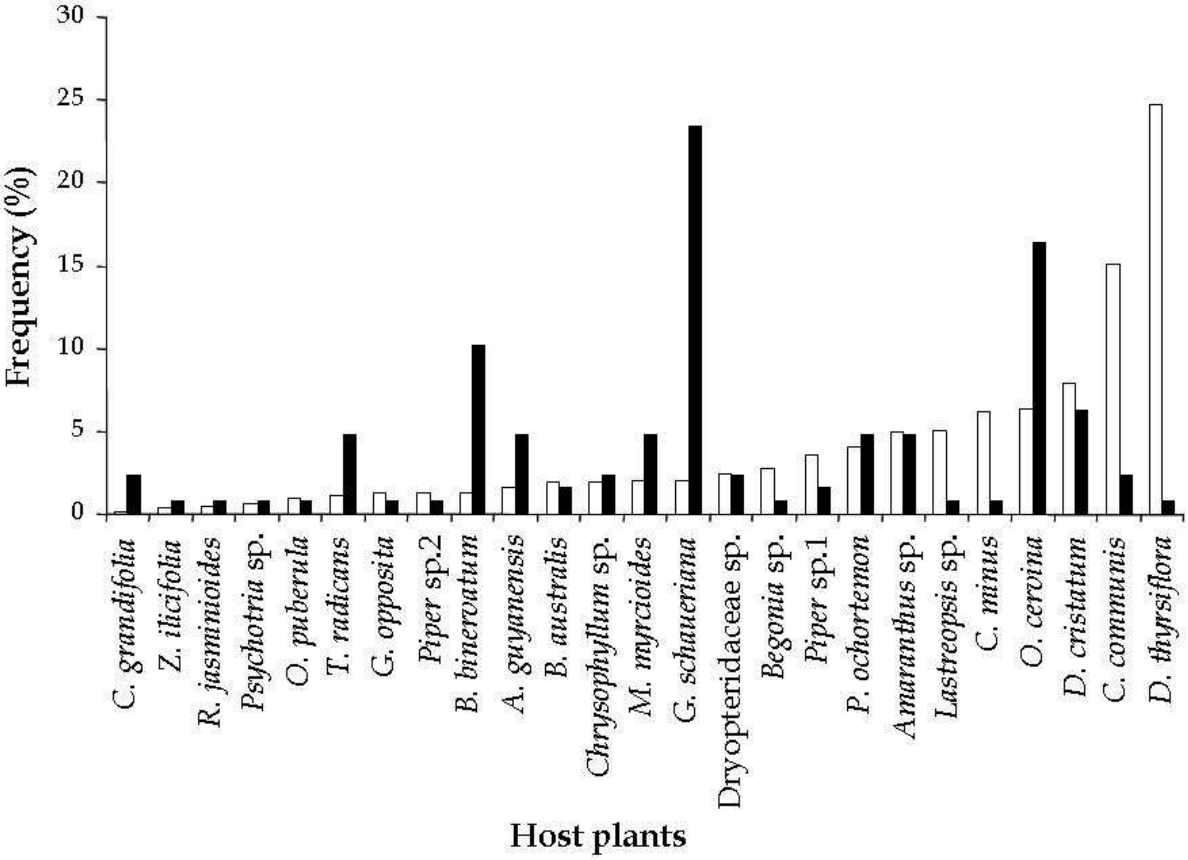


Figure 2: Host plant selection by ovipositing *Acutisoma proximum* females. The white bars represent the relative abundance of each host plant species, whereas the black bars represent their relative frequency of utilization by females. Black arrows indicate the host plant species that are most frequently used.

Male territoriality

In spite of the fact that *A. proximum* belongs to a subfamily of typically nocturnal harvestmen, many *A. proximum* males were found during the day standing still on exposed places of the vegetation, waving their extended sensorial second pair of legs (Fig. 3a). Besides standing still and waving the second pair of legs, they also walked around, apparently patrolling areas on the vegetation, which averaged 3.3 m2 (SD=1.8 m2; n=57 territories). The beginning of the diurnal activity of males, which is associated with patrolling behavior, occurred in September (reproductive season 2003-2004) and August (reproductive season 2004-2005) (Fig. 1a). Individual males spent on average 61.1 days (SD=44.9 days; n=78) in territorial activities on the vegetation. After April, which corresponded to the end of the reproductive season both in 2004 and 2005, males are no longer found on the vegetation during the day (Fig. 1a). From May to July, individuals of *A. proximum* (both males and females) are rarely found in the study area, and the few active individuals are only found at night.

During the reproductive season, whenever two large males met, they engaged in ritualistic fights (n=42), during which they faced each other, holding the second pair of legs laterally extended, the first pair of legs retreated above the body, and the pedipalps raised (Fig. 3b). In this position, each male repeatedly hit the opponent with the tip of both second legs, using them as whips. We have also observed males attacking the opponent's first pair of legs with rapid pedipalp strikes (n=14). In one of the fights, the opponents turned their backs to each other and intertwined their powerful and spiny legs IV. After some period of leg locking, both contenders fell from the vegetation to the ground, where the fight continued for a few more minutes. Male fights took on average 31.0±35.0 min (n=22 fights followed since the beginning), and 27 out of 42 fights occurred during daylight. Male fights occurred mainly in the first months of the reproductive season, with a marked peak before the period of most intense oviposition activity (Fig. 1a). Nine out of all observed fights occurred without any reproductive female nearby.

Most fights ended with a winner (n=25), which had usually behaved more aggressively during the interaction, i.e., striking the opponent with the pedipalps more frequently. Moreover, winner males remained in place where the fight occurred during the following days, and sometimes even months. Loser males left the area soon after the fight ended, sometimes (n=14) with the winner in pursuit for up to 3 m. There were no significant size differences between loser and winner males (Table 2).

Table 2. Size features (mean±SD) of winner and loser males in territorial fights (n=20) of the harvestman *Acutisoma proximum*.

Male trait (mm)	**Winner**	**Loser**	**Statistics**
Dorsal scute length	7.82 ± 0.34	7.80 ± 0.36	t = 0.533, *P* = 0.300
Right fourth femur length	25.87 ± 1.12	26.17 ± 1.49	t = 0.668, *P* = 0.256
Second right leg length	132.48 ± 6.98	132.50 ± 9.27	t = 0.126, *P* = 0.450

Although we have never observed a male physically injuring another one in a territorial fight, we did find several *A. proximum* adults with amputated legs and pedipalps. If those injuries were caused by male fights, we would expect to find them more frequently on males than on females. In fact, amputations were much more common in males (10.8% with amputations; n=484) than in females (2.7% with amputations; n=547) ($\chi2$=22.063, df=1, P<0.0001). This sexual bias in the frequency of amputations could also be caused by the increased exposure of males to predators due to their territorial activities. However, all known predators of Goniosomatinae harvestmen, including opossums, wandering spiders, and toads (Cokendolpher & Mitov 2007), are active at night, when both territorial males and females are found exposed on the vegetation.

Figure 3: (a) Male of the harvestman *Acutisoma proximum* standing still during the day on an exposed part of the vegetation. (b) Two *A. proximum* males in fighting position, facing each other with the second pair of legs laterally extended, the first pair of legs retreated above the body, and the pedipalps raised.

Mating and female defense by males

During the period of most intense fighting activity, but mainly later in the season, males' territories are visited by ovigerous females at night. These females visit the territories, mate with the territory owners, and subsequently oviposit inside the territories, constituting harems that range from one to six females (median of two females; n=33 harems). Males with longer legs II posses a greater number of females within their harems (R^2=0.408; F=8.972; n=15 harems; P=0.01; Fig. 4). Within the harems, we observed 141 copulations, which lasted on average 345±233 seconds (n=32 copulations followed from the beginning). While mating, male and female face each other, and the male grasps the base of the female pedipalps using his own pedipalps. Locked together by the male's pedipalps, both the male and the female lower the posterior part of their bodies, straightening the angle formed by the ventral part of their bodies. Next, while the male uses his chelicerae to massage the upper side of the female's chelicerae, the penis is everted and enters the female's genital opening. During the first seconds of the copulation, the male touches the female's dorsal scute with his first and second pair of legs. Occasionally, the female starts moving in what seems to be an attempt to interrupt the penetration, and at these moments the male resumes the touches on the female dorsum. Penetration seems to be terminated by the female, when she moves suddenly backwards releasing her body from the male's grasp.

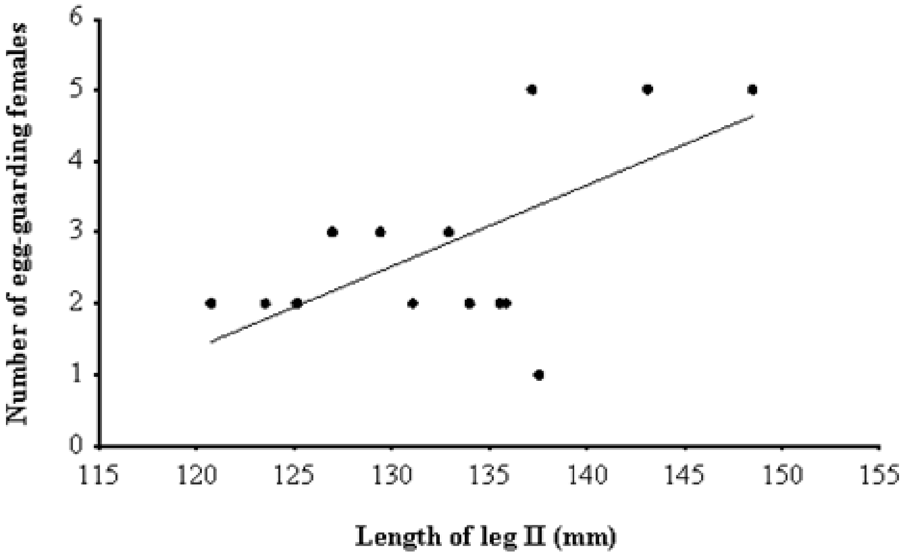


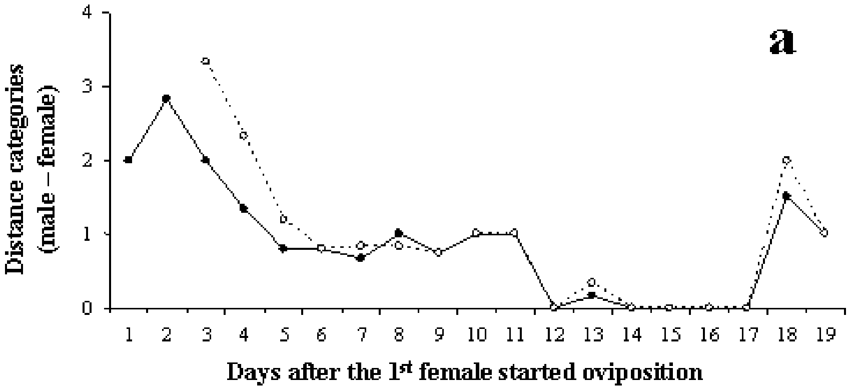
Figure 4: The size of the sexually dimorphic leg II of *A. proximum* males is positively related to the number of egg-guarding females within the harems defended by those males.

After copulation, the male is generally driven away by the female, but he stays next to her apparently mate guarding for a median time of 8 hours (range: 4-42 h; n=51) (Fig. 5a). During mate guarding, the male occasionally touches the female dorsum and/or legs with his second pair of legs (Fig. 5a). Even when territorial males are not mating nor performing close mate guarding, they stay near the females without touching them, patrolling the harem for up to three months. During this period, territorial males seem to divide their patrolling activity among the females from their respective harems. In the four harems in which we have conducted quantitative observations, it was clear that territorial males spent most of the reproductive season very close to the females that had recently laid eggs in their territories. As the days pass and the females that have arrived first finish their oviposition activities, the territorial males quit visiting these females and start spending more time close to the newer females that arrive in their territories (Fig. 6).

Figure 5: (a) A male of *Acutisoma proximum* (right) mate guarding an ovipositing female (left). The male's second pair of legs (pointed out by the arrow) is extended towards the female, and periodically touches her. (b) After the clutch of an egg-guarding *A. proximum* female had been experimentally removed, the territorial male resident in the area took care of the clutch for up to eight days.

Occasional paternal care

In four harems, one of the females disappeared during the period of maternal care, probably due to the mortality caused by parasitoids that commonly attack egg-guarding females in the area (unpublished data). In all these cases, the territorial males were later found resting on the clutches in a typical egg-guarding position (Fig. 5b). Those males guarded the clutch from one to six consecutive days, apparently protecting the eggs. One of these males was seen consuming a few eggs from the clutch he was standing on. However, this cannibal male only consumed eggs during the first hours after female disappearance, and then he later stayed five more days on the clutch without eating more eggs. In February 2007, we experimentally removed 11 egg-guarding females from their clutches and kept them alive in plastic vials. In six cases, after 4–192 hours from female removal, the territorial males took care of the females' eggs in the same way as described above. Those males stood with the clutches for up to eight days, but all of them temporarily abandoned their clutches a few times during this period. We experimentally introduced a female of the harvestman *Promitobates ornatus* (a known predator of *A. proximum* eggs, Buzatto et al. 2007) to four of these egg-guarding males. Only one of the males responded aggressively to the approach of this potential egg predator, attacking the female *P. ornatus* in the same way egg-guarding females were seen repelling conspecifics in Buzatto et al. (2007). The other three males abandoned the clutches without showing any aggressive reactions towards the "intruder", but later returned to their clutches.


a
Distance categories (male – female)
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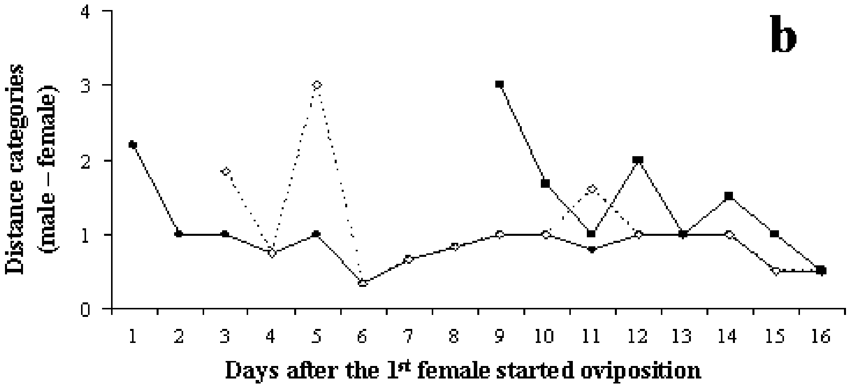
Days after the 1st female started oviposition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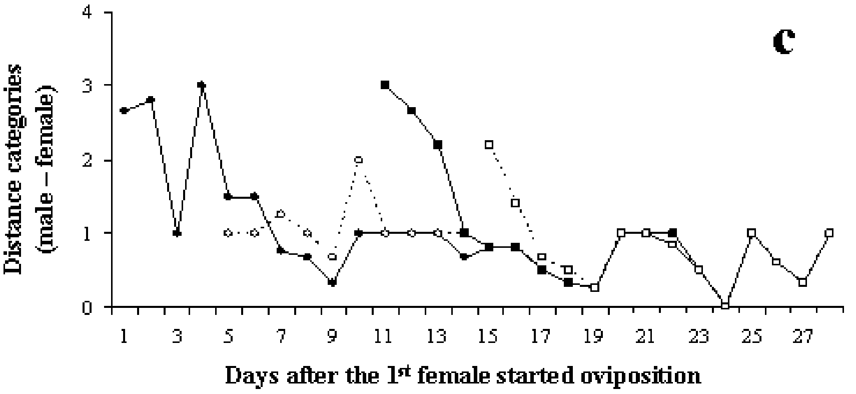
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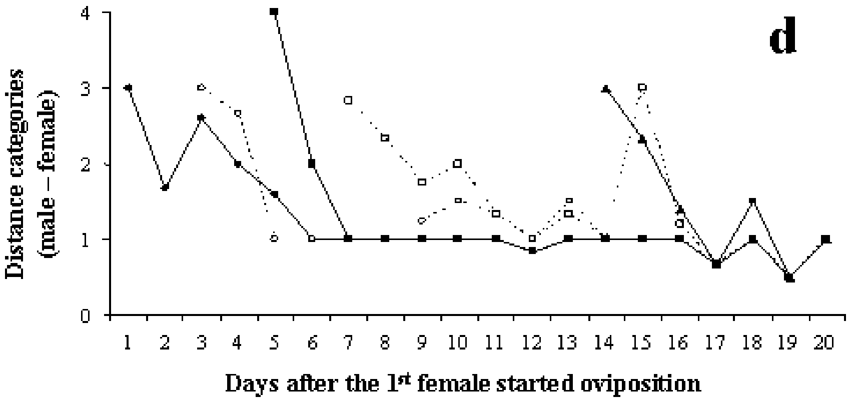
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Days after the 1st female started oviposition

Figure 6: The daily average distance between the territorial male and each female in his harem varies along the days of the reproductive season in the harvestman *Acutisoma proximum*. The distances were categorized as following: male nowhere at sight (score 0), at less than 1 m from the female (1), at less than 20 cm from the female (2), at less than 5 cm from the female (3) or copulating with the female (4). We monitored four different harems, with respectively: (a) two, (b) three, (c) four, (d) and five egg-guarding females. Dark circles linked by a continuous line represent the first female to arrive in the harem, open circles linked by a dashed line represent the second female, dark squares linked by a continuous line represent the third female, open squares linked by a dashed line represent the fourth female, and dark triangles linked by a continuous line represent the fifth female to lay eggs in the harem.

Discussion

Reproductive seasonality and host plant selection

Reproductive seasonality seems to be fairly common in tropical harvestmen, and the pattern recorded here for *Acutisoma proximum* is similar to what was previously described for *Goniosoma spelaeum* (Gnaspini 1995), *Acutisoma longipes* (Machado & Oliveira 1998), and *G. albiscriptum* (Willemart & Gnaspini 2004), three closely related species of the subfamily Goniosomatinae. These species have two peaks of reproductive activity throughout the year: a minor one in the beginning of the warm-wet season and a longer one at the end of the same season. Furthermore, similarly to what was observed for *A. longipes* (Machado & Oliveira 1998), the number of clutches of *A. proximum* found on each month at the study site was strongly correlated with specific environmental conditions, such as local monthly rainfall and temperature. However, when compared to the other goniosomatine mentioned above, *A. proximum* has the shortest reproductive period, lasting from October to March. Given that *A. proximum* is the only species in the group that reproduces outside caves in relatively exposed microhabitats, females of this species are probably more sensitive to the climatic harshness of the cold-dry season, and hence can only reproduce during a narrower period.

The population of *A. proximum* studied here is unique among goniosomatines on using three different kinds of oviposition sites, namely leaves, boulders, and tree trunks. Studying another *A. proximum* population, Ramires and Giaretta (1994) found females laying eggs solely on rocky surfaces next to river margins, what is probably the ancestral state among species of the subfamily (see Machado 2002). At Intervales State Park, most individuals of *A. proximum* spend the cold-dry season inside caves (M.C. Chelini pers. comm.), but leave the caves to reproduce on the vegetation close to the rivers. We hypothesize that the reproductive migration is an adaptation to avoid the extremely intense predation pressure that other goniosomatine harvestmen face inside caves (see Machado & Oliveira 1998). On the vegetation, females can temporarily abandon their clutches to forage nearby (Buzatto et al. 2007) without having to go long distances to find food as the cavernicolous species are obligated to do. This opportunity to feed close to the clutch would allow females to secure at least some food during the period of maternal care. Indeed, *A. proximum* guarding females have been occasionally observed foraging next to their clutches (n=2). Females that can sporadically forage during the egg guarding period should be able to gather more energy and reproduce again more frequently than females that starve during the entire egg guarding period. Whereas almost all goniosomatine species studied so far concentrate their reproductive effort primarily in a single oviposition (Machado 2002), more than 18% of *A. proximum* females naturally reproduce twice in their lifetime (Buzatto et al. 2007).

Over the whole study period, leaves were the most frequent oviposition site used by *A. proximum* females. Additionally, females of this harvestman had a marked preference for certain host plant species (Table 1, Fig. 2). Females of other harvestman species also choose very specific oviposition sites (review in Machado & Raimundo 2001). Females of the Neotropical *Bourguyia hamata* (Gonyleptidae), for instance, oviposit almost exclusively inside the leaf-tube of the epiphytic bromeliad *Aechmea nudicaulis* (Machado & Oliveira 2002), and additionally prefer individuals with longer rosettes, which accumulate more water and have small amounts of debris inside them. These features probably promote a more adequate microhabitat for egg development and consequently may increase offspring survival (Osses et al. 2008). The reasons for the preference for some host plants exhibited by *A. proximum* females, however, is not that obvious. The three most often used host plants are not closely related (two ferns and one tree), nor do they possess similar leaf morphology. Moreover, the preferred host plants have also completely different habits: one of the ferns is herbaceous (*Olfersia cervina*), the other is epiphytic (*Blechnum binervatum*), and the tree (*G. schaueriana*) is woody. Consequently, it is unlikely that *A. proximum* females choose the host plants according to some conspicuous morphological trait they possess. An alternative hypothesis is that preferred host plants are the ones with more long-lived leaves, a feature that would minimize the risks of losing a clutch due to the abscission of the leaf on which the eggs were laid. Indeed, one of the preferred ferns (*O. servina*) and *G. schaueriana* seem to have quite long-lived leaves. Nevertheless, several abundant host plants in the study site, including *Calathea communis* and *Philodendrum ochrortemon*, also possess long-lived leaves, but were rarely used by *A. proximum* females. Thus, this hypothesis is also incapable of satisfactorily explaining the females' preference for certain host plants. The reasons why *A. proximum* females prefer some host plants over others deserve further investigation.

Nonetheless, assuming that host plant selection evolved before male territoriality in *A. proximum*, female preference for certain plant species has implications for the mating system of the species. Since females choose specific host plants for oviposition, these plants become a predictable resource where females will aggregate at the time of breeding. As a result, males may try to defend and monopolize the preferred host plants as a means of securing mates (Emlen & Oring 1977, Thornhill & Alcock 1983). Perhaps by aggregating, females intensify male competition and indirectly select the best males capable of succeeding in a harem-like mating system (Wiley & Poston 1996).

Mating system

In opposition to the strictly nocturnal activity of most harvestmen, males of *A. proximum* are also active during the day. Especially between November and January, males spent most of the day patrolling and defending territories on the vegetation. Territorial behavior has been previously described for *Acutisoma longipes*, whose males defend small areas on cave walls, places where females lay their eggs (Machado and Oliveira 1998). Although *A. longipes* males have been observed patrolling their territories, detailed descriptions of male fights in species of *Acutisoma* were not available until the present study. In spite of apparently being ritualized fights that normally consists of mutual assessment of the opponents' condition by each contestant, the fights of *A. proximum* may also result in physical damage to contestants judging from the fact that males in the study site were more frequently found with amputations than females. Moreover, males placed together in the same vial generally end up with leg amputations (unpublished data). Because winner males usually return to the original area where the fight begun, we conclude that males of this species possess well-defined territories during the reproductive season. Despite their aggressiveness, winners were not statistically larger than losers and it is likely that, as previously observed for the harvestman *Leiobunum vittatum*, whose males defend mating territories on rocks (Macías-Ordóñez 1997), residency is more important than the physical attributes of both contestants.

Males begin patrolling and fighting in some places at the beginning of the reproductive season, before females arrive to reproduce on these areas; some fights even occurred between males with no females in their territories. That would be expected only if males could predict where females would arrive, due to the spatial distribution of the females' reproductive resources. Thus, we propose that males are defending territories that contain potential oviposition sites, in an attempt to monopolize later mating access to ovigerous females. Although the leaves available on the vegetation at the study site are clearly not a limiting resource for females, their preference for a few host plant species must allow males to predict where they will arrive. This is a typical resource defense polygyny scenario, but future studies are still necessary to test if the abundance of the preferred host plants inside the males' territories is actually greater than what would be expected by chance.

After females arrive, *A. proximum* males concentrate their patrolling activity on that part of the host plant where individual females have laid their eggs, and we have observed the same host plant being occupied by three distinct territorial males, each of them defending a different harem in different parts of the host plant (unpublished data). At this stage male behavior suggests a female defense polygynous mating system, as described for the coreid *Acanthocoris sordidus*, in which males defend the small portions of the host plant where females aggregate (Fujisaki 1981). Each territorial *A. proximum* male defend a harem that may be composed of up to six females, and the longer the sexually dimorphic leg II of the males, the more egg-guarding females are found in their harems. Since leg length does not account for fight outcome, we speculate that this pattern is generated by female mate choice. Males with longer legs II may be preferred by ovigerous females that visit different territories, either because leg size is an indicative of male quality or because males with longer legs II perform copulatory and post-copulatory courtship more effectively. Soon after mating with a female, the male may guard her for almost two days, constantly touching her with his second legs. Given that *A. proximum* females may take several days to complete oviposition, the male mate guarding behavior is probably an adaptive trait that functions to secure the paternity over all the eggs laid by the female. Our intensive scan samplings on four different harems also revealed that mate guarding and patrolling by territorial males are focused on those females that have most recently arrived in their territories. This strategy is analogous to defending individual females in sequence, as in some other animals that present female defense polygyny (see Robinson 1986 for an example). Thus, we believe that *A. proximum* mating system can only be precisely described as follows: resource defense polygyny in the beginning of the reproductive season gives place to female defense polygyny after females arrive and aggregate on small parts of the host plants. To the best of our knowledge, this is the first described case of an arachnid that shifts its mating system over the reproductive season.

Finally, it is possible that the defense of reproductive territories predisposes *A. proximum* males to guard the clutches of females that abandon their eggs or die during the egg guarding period. In two other species of Goniosomatinae (*G. albiscriptum* and *A. longipes*), a few males have been seen temporarily taking care of unattended clutches. In the present study, we observed *A. proximum* males providing occasional paternal care under natural conditions, and we have also induced this behavior by experimentally removing egg-guarding females from several males' harems. Although male egg-guarding is temporary, this behavior may be crucial (especially just before hatching), since egg predators can consume entire batches in a few days (Buzatto et al. 2007). Machado and Raimundo (2001) suggested that the association between male and offspring through the defense of an oviposition site may constitute the basis for the evolution of paternal assistance in many harvestmen species. In fact, our results with *A. proximum* provide support to the notion that egg-guarding by male harvestmen, even for short periods, can occur when the males defend a territory that is also an oviposition site. The occasional male care recorded here has at least two behavioral similarities with the exclusive post-zygotic paternal care described for other harvestmen: (1) like most paternal species, males do not stay on their clutches all the time, and leave them unattended for some time (generally hours), probably in order to forage (Machado et al. 2004); (2) like *Iporangaia pustulosa* (Gonyleptidae), males may consume some eggs of the clutch (Requena & Machado unpublished. data), which may be viewed as a means of acquiring energy without abandoning the territory. In conclusion, we suggest that paternal care in harvestmen has evolved from ancestral maternal species with resource or female defense polygyny. This hypothesis is currently being investigated in our laboratory through a phylogenetic approach and using the family Gonyleptidae as model group.

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CAPÍTULO 2

Alternative mating tactics, cryptic male dimorphism, and sperm competition between sneaks and guards in a neotropical harvestman*

Macho territorial do opilião *Acutisoma proximum*.

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Abstract

Although not as well studied as sexual dimorphism, alternate behavioral or morphological traits also exist intraspecifically among members of the same sex. Here we describe alternative mating tactics in males of the harvestman *Acutisoma proximum*, including discontinuous size variation in the length of the sexually dimorphic legs II, a weapon used by major males in fights for territories. Male morphs are distinct but cryptic, and we suggest a new approach to detect intra-sexual dimorphism by applying an objective and statistically adequate method that classifies males into different morphs. Approximately 21% of the males in the population belong to a morph with relatively small second legs compared with body size. Minor males do not patrol, nor do they defend females and territories. Instead, they adopt an alternative mating tactic based on furtively invading large males' harems where they sneak copulations with egg-guarding females. We quantified ejaculate investment by both morphs to test the hypothesis that major males (guards) have lower sperm investment than minor males (sneaks). This hypothesis was not corroborated by our results and possible explanations are explored. This is the first case of alternative mating strategies in Opiliones, and also the first description of cryptic male dimorphism in arachnids.

Keywords: *Acutisoma*, allometry, harem, polygyny, sperm investment.

Introduction

Behavioral and morphological diversity between males and females of the same species (sexual dimorphism) is usually understood as the result of different selection pressures on the two sexes, largely due to stronger sexual selection on males (Andersson 1994). Although not as well comprehended as sexual dimorphism, intra-specific diversity also occurs in behavioral or morphological traits among members of the same sex (Gross 1996). In contrast to sexual dimorphism, intra-sexual morphological dimorphism is rarer, and restricted to species whose males bear sexual traits that increase mating success but decrease survival (Gadgil 1972). Male dimorphism is generally accompanied by alternative reproductive behaviors among males; larger males (majors) typically have more elaborate weaponry, and guard females or reproductive territories, while small males (minors) have reduced weaponry and sneak copulations (Gross 1996).

The classic examples of male dimorphism consist of extreme morphological divergence between male morphs, such as the case of the coho salmon *Oncorhynchus kisutch*, in which minor males are as small as 30% of the adult body size of the major males in the population (Gross 1985). However, in most male dimorphic species, the main difference between male morphs occurs in sexual traits that vary discontinuously with body size, such as the forceps of male earwigs (e.g., Tomkins & Simmons 1996). In such cases, whereas male morphs are clearly discrete concerning secondary sexual traits, they overlap greatly in overall body size. Actually, among male dimorphic species, it seems to exist a continuous spectrum of cases starting with no overlap in body size at all, and grading continuously into species with great overlap. In the later cases, male dimorphism may even be considered cryptic, i.e., the polymorphism is revealed only by investigating the allometry of body parts (sensu Cook & Bean 2006). Different methods of detecting dimorphisms have been proposed (Eberhard & Gutiérrez 1991, Eberhard et al. 2000, Kotiaho & Tomkins 2001, Cook & Bean 2006). All are based on scaling relationships (*sensu* LaBarbera 1989, Emlen & Nijhout 2000) of the presumably dimorphic sexual traits on overall body size.

In addition to differences in external morphology, distinct male morphs may also diverge internally, with respect to investment in testes and sperm production. In Parker's (1997, 1998) general sperm competition models, the probability of a sneak mating is directly proportional to the risk of sperm competition. Whenever minor males in a given population adopt sneaking tactics, it is expected that their matings will nearly always occur with females that have previously mated with a major male, and that the ejaculates of both males will compete for fertilization of her eggs (Parker 1970). Thus, theory predicts that sneak males will always be subject to sperm competition whereas the degree that guarding males will also be subject to sperm competition depends on the frequency of sneaks in the population (Parker 1990). As a consequence, guarding males should have an overall lower investment on their ejaculate than sneaks, as already found for many animal groups presenting male alternative mating tactics. The predicted associations occur in dung beetles (Simmons et al. 2007), ground-nesting bees (Simmons et al. 2000), mammals (Stockley & Purvis 1993), and fish (Gage et al. 1995, Vladic & Järvi 2001, Neff et al. 2003).

In arthropods, in which individual adult size is fixed, adult male morphological dimorphism has been documented for several insect orders: Coleoptera (Emlen & Nijhout 2000, Eberhard et al. 2000, Hanley 2001, Miller & Wheeler 2005, Tomkins et al. 2005, Okada & Miyatake 2007); Dermaptera (Tomkins & Simmons 1996, Tomkins 1999); Hymenoptera (Alcock 1996, Cook et al. 1997, Jousselin et al. 2004, Longair 2004); and Thysanoptera

(Kranz et al. 2001). In the crustacean *Paracerceis sculpta* (Shuster 1987, 1992) there are three distinct adult male morphs which are genetically determined, and that yield similar reproductive success (Shuster 1989, Shuster & Wade 1991). For all other studies where behavioral data is available, the morphological intra-sexual dimorphism is coupled with the expression of alternative mating tactics, such as fighting, sneaking or dispersing, rather than genetic polymorphisms.

Even though arachnids present an enormous diversity of reproductive strategies (Thomas & Zeh 1984), male morphological dimorphism has been recorded only for two species of spider (Clark & Uetz 1993, Heinemann & Uhl 2000, Clark & Morjan 2001, Vanacker et al. 2003, Vanacker et al. 2004), and two species of mite (Radwan 1993, Radwan & Klimas 2001, Radwan et al. 2002, 2003). In the salticid spider *Maevia inclemens*, male morphs differ dramatically in coloration and presence of tufts of setae, and each morph shows distinct courtship behaviors (Clark & Uetz 1992, 1993, Clark & Morjan 2001). A dramatic male dimorphism is also expressed in the linyphiidae spider *Oedothorax gibbosus*, in which only one of the male morphs bear a nuptial fluid producing gland on the upper side of the cephalothorax (Heinemann & Uhl 2000, Vanacker et al. 2003, Vanacker et al. 2004). In the two species of mites with male dimorphism, male morphs can be easily distinguished by the morphology of the third pair of legs, which is modified as a weapon (thickened, with a sharp tip) in one male morph (Radwan 1993, Radwan & Klimas 2001, Radwan et al. 2002, 2003).

Here we describe the alternative mating tactics of males of the Neotropical harvestman *Acutisoma proximum* (Opiliones: Gonyleptidae). Large males use their sexually dimorphic legs II in ritualistic fights for the possession of territories where females lay eggs (Buzatto & Machado submitted). Major and minor males overlap greatly in body size measures, and we suggest a simple modification on the approach of Cook & Bean (2006) to detect cryptic male dimorphism (*sensu* Cook & Bean 2006) that allows the application of a more objective and statistically adequate method to classify males into distinct morphs. Furthermore, we also quantified ejaculate investment by both morphs to test predictions from the models of sperm competition games between sneaks and guards (Parker 1990, Simmons et al. 2007).

Materials and methods

Organism

The reproductive behavior of *A. proximum* females, including the costs and benefits of the maternal care have been studied (Buzatto et al. 2007, Buzatto & Machado submitted). *Acutisoma proximum* females lay their eggs mainly on the undersides of leaves hanging above the river (Buzatto et al. 2007). Following oviposition, females guard their eggs and the hatched nymphs for approximately 37 days (Buzatto et al. 2007). In the beginning of the reproductive season (which lasts from September to April), males fight for territories on the vegetation next to the river where females mate, lay, and guard their eggs. Territorial males then stay the entire reproductive season patrolling their territories (Buzatto & Machado submitted). During the ritualized fights, males hit each other with their elongated second pair of legs, which are much longer in males than in females. This sexual dimorphism was described in other species of the subfamily Goniosomatinae (Machado et al. 2003). The length of a male’s second pair of legs is positively related to the number of females in his harem (Buzatto & Machado submitted). The scaling relationship of this male weapon has not been investigated in any harvestmen so far.

Study site

The study was conducted in the Intervales State Park (24°14′ south, 48°04′ west; 800 m a.s.l.), an area covered by dense Atlantic Forest close to the city of Ribeirão Grande, southern São Paulo state, south-eastern Brazil. There is a marked seasonality, with a wet and warm period from October to March, and a dry and cold period from April to September, when frosts are common. The annual rainfall ranges from 2,000 to 3,000 mm and the mean annual temperature ranges from 17°C to 19°C. Fieldwork was carried out on 200 m transect with marks every 10 m in the vegetation flanking the river that follows the Caçadinha trail. The river here is approximately 5 m wide, and is flanked by abundant vegetation that sometimes partially covers the river bed.

Behavioral observations

To further investigate the sexual behavior of *A. proximum* males in the field, we used two different approaches. Firstly, we conducted an almost 2-year capture–mark–recapture study to monitor movements of males and females. Every adult found in the transect between September 2003 and December 2005 was measured to the nearest 0.05 mm for the dorsal scute length and right second leg length, and received a unique enamel paint mark. This marking procedure does not affect behavior and the paint marks last for over two years (Buzatto et al. 2007).

We followed the reproductive activity of marked individuals off and on for two years (comprising the reproductive seasons of 2003–04 and 2004–05). In each study period of four consecutive days each month the transect was inspected three times a day (from 08:30 to 12:00 h, from 14:00 to 17:30 h and from 20:30 to 00:00 h). We used visual searches to find individuals, and recorded the sex and location in transect of each one to the nearest

1 m. We estimated individual movements as the straight line distance between points where a harvestman was found. We compared the movements of major and minor males using a Mann-Whitney test. This analysis was done using only individuals that were captured at least twice during a given reproductive season.

After we determined the exact location of several males' territories in the transect, we conducted intensive observations within these territories during the reproductive season of 2006-07, more specifically from December 2006 to March 2007. Throughout the study, which comprised 2,300 h of field observations, we continuously recorded all relevant behavioral events, such as copulations and agonistic interactions between individuals.

Scaling relationships and male dimorphism

The method proposed by Eberhard & Gutiérrez (EG) (1991) to detect dimorphisms is based on the following model:

$$Y = \beta_0 + \beta_1 X + \beta_2 (X - X_D) D + \beta_3 D + \varepsilon, \qquad \text{eqn 1}$$

in which Y and X are linear measurements of the sexual trait size and body size, respectively; X_D is a switch point; D = 0 if $X < X_D$ or D = 1 if $X \geq X_D$; β are the regression coefficients; and ε is the error. The EG model allowed us to detect whether there is a size dependent switch point (X_D) between two discrete morphs. According to the model, if β_3 is significantly different from zero, then the distribution of the trait Y size is discontinuous at the estimated switch point. Likewise, if β_2 is significantly different from zero, then the linear slope between the trait size, Y, and body size, X, changes at the switch point. Whenever individuals from distinct morphs overlap in body size, searching for a switch point also in Y axis rather than only in the X axis may enhance the distinction of different male morphs (Eberhard et al. 2000). Kotiaho and Tomkins (KT) (2001) formalized this suggestion with the following model:

$$X = \beta_0 + \beta_1 Y + \beta_2 (Y - Y_D) D + \beta_3 D + \varepsilon. \qquad \text{eqn 2}$$

in which all terms are defined in the same way as for the EG model. The difference between EG and KT models is that the simple substitution of Y with X and X with Y results in setting the switch point in the Y axis (Y_D). However, when morphs overlap in both body size and sexual traits, Cook and Bean (CB) (2006) suggested classifying males into their morphs based on the ratio of the sexual trait to body size, rather than searching for a fixed switch point at the X or the Y axis.

We compared the switch points obtained with the EG and KT methods when investigating the scaling relationship of the second leg length of both males and females of *A. proximum*. EG and KT methods involve choosing a few possible switch points visually, and then selecting the one that returns the highest R^2 value. Similarly, the CB method designates males for each morph also by visually choosing a switch point, but this time based on a ratio (sexual trait/body size). Here we proposed an alternative and more objective method in which we calculate the Akaike Information Criterion (AIC, see Burnham & Anderson 1998, Johnson & Omland 2004) values for all possible models generated when each male in our sample was used as the switch point between morphs. These AIC analyses were repeated using values on the X axis (cf. the EG method), values on the Y axis (cf. the KT method), and using the ratio Y/X as a "switch point ratio" (cf. the CB method). Once we have found the individual that gave us the lowest AIC value, we formalized the CB method by applying the switch point ratio selected (here measured as second leg length/dorsal scute length) to the model:

$$Y = \beta_0 + \beta_1 X + \beta_2 D + \beta_3 XD + \varepsilon, \qquad \text{eqn 3}$$

in which D = 0 if the ratio of the second leg length to body size (Y/X) is bellow the selected switch point ratio, and D = 1 otherwise. With this model, we tested the significance of parameters β_2 and β_3, on an attempt to find differences between the two morphs' intercept and slope.

Sperm investment by majors and minors

A recent review by Snook (2005) showed that several sperm traits, both quantitative (such as sperm number) and qualitative (such as sperm mobility and longevity), interact in complex ways and are important in the outcome of sperm competition. However, whereas increased sperm number is a general and taxonomically widespread response to increased sperm competition, other sperm quality traits have not yet been shown to influence sperm competition (Snook 2005). For instance, in the bluegill sunfish (*Lepomis macrochirus*), a species with alternative male phenotypes, competition experiments involving equal volumes of ejaculate showed that fertilization success increased linearly with sperm number (Neff et al. 2003). Therefore, although male sperm investment can be measured in a number of different ways (Tomkins & Simmons 2002, Snook 2005), we decided to use simply the number of sperm cells inside the seminal vesicle of well-rested male to investigate sperm investment.

To assess the sperm expenditure, we used 13 major males and 11 minor males, all collected between March 15 and April 8, 2007. Each male was isolated in captivity for at least one week, with food and water *ad libitum*, thus assuring that all males had time and energy to replenish the sperm in the seminal vesicle. After this period in captivity, we dissected each male and removed his seminal vesicle. We macerated the seminal vesicle in 200 µl of distilled water. Next, we placed 12 µl of the solution in a Neubauer improved chamber, and estimated the sperm number under a light microscope. We counted the number of sperm cells inside 12 randomly chosen squares; the average was used as the sperm number for each male. We used the remaining diluted sperm solution for each male to repeat the counting procedure, in order to check the repeatability of the method. We used an ANCOVA to investigate differences between the sperm number inside the seminal vesicle of majors and minors, using the of log dorsal scute length as a covariate to correct for male body size (Tomkins & Simmons 2002).

Results

Scaling relationships and male dimorphism

The frequencies of second leg lengths, and of the ratios of second leg length to dorsal scute length were strongly bimodal for males (Figs. 1a,b), but not for females (Figs. 1c,d). For males, the switch points with lowest AIC value were at 7.20 mm on the X axis (AIC = 26,597.3) for the EG analysis and at 95.00 mm on the Y axis (AIC = 2,383.3) for the KT analysis (Fig. 2a). However, the overall lowest AIC value for males was obtained using the ratios of second leg length to dorsal scute length as possible "switch point ratios" (cf. Cook & Bean 2006, Fig. 3a). Therefore, the best fitting switch point occurred at the second leg length of 120.52 mm and at the dorsal scute length of 7.69 mm (second leg length/dorsal scute length at this point is 15.67, AIC = 2,346.3). Applying this switch point ratio between morphs (cf. Cook & Bean 2006), we tested the normality and the homogeneity of residuals and next fitted our suggested model (eqn 3), testing the significance of explanatory variables using t tests and an ANOVA (Tables 1 and 2, Fig. 3b). According to the model, the significance of β_2 and β_3 indicates that there are statistical differences between both the intercepts and the slopes of the two male morphs.

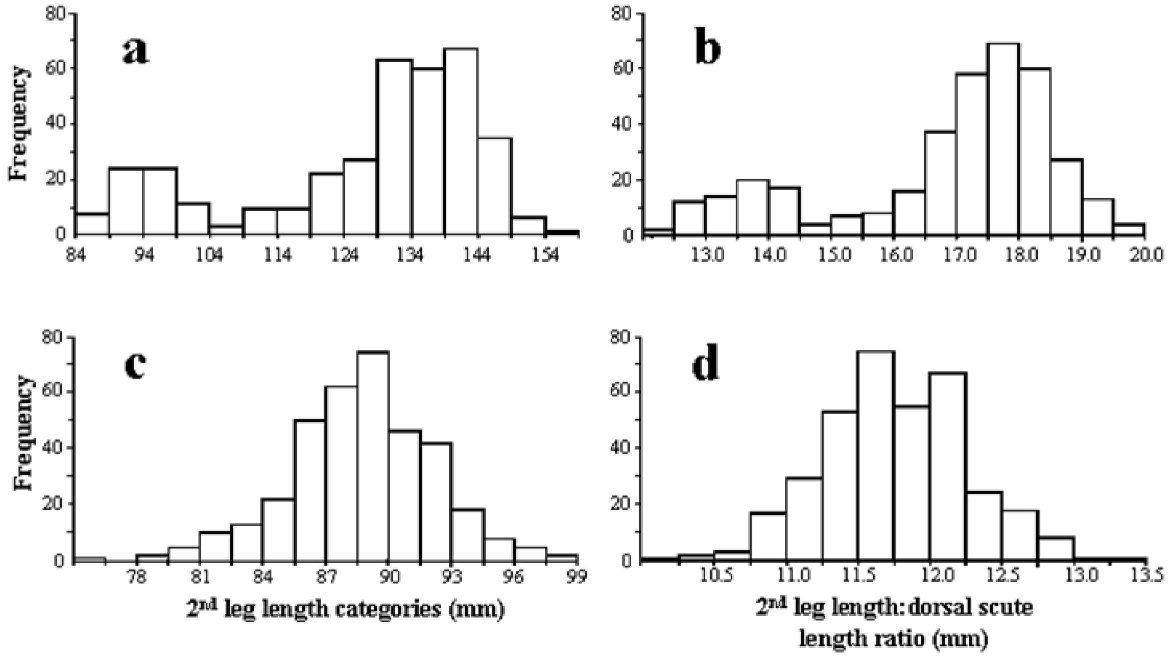

Figure 1: Among males of the harvestman *Acutisoma proximum,* (A) the distribution of the second legs length and (B) the distribution of the ratios of the second leg length to body size (measured as dorsal scute length) are both strongly bimodal. Conversely, (C) the distribution of the second legs length and (D) the distribution of the ratios of the second leg length to body size in females do not present the same bimodality.

Table 1. Statistical tests for intra-sexual dimorphism in the harvestman *Acutisoma proximum*: multiple regressions. The fitted model was (Y = -23.3 + 17.1X + 14.1D + 1.78X*D + з) for males and (Y = 21.4 + 8.60X + з) for females. In the males' model, D = 1 if Y/X (the ratio of second leg length to dorsal scute length) ≥ 15.67 and D = 0 if Y/X < 15.67. The D*X tests the change in the slope, and D tests the discontinuity at the switch point Y/X.

Source	β	**SE of** β	***t***	**Significance**
Males; best switch point (Y/X) = 15.67; dependent variable: second leg length (mm); R^2 = 0.887				
Constant	-23.294	7.892	-2.95	0.003
Dorsal scute length	17.138	1.120	15.30	< 0.001
D	14.095	5.223	2.70	0.007
D*X	1.7801	0.7097	2.51	0.013
Females; best switch point (Y/X) = 11.8; dependent variable: second leg length (mm); R^2 = 0.612				
Constant	21.427	4.874	4.40	< 0.001
Dorsal scute length	8.5965	0.6379	13.48	< 0.001
D	7.211	7.223	1.00	0.319
D*X	-0.2483	0.9600	-0.26	0.796

Table 2. Statistical tests for dimorphism in *Acutisoma proximum*: ANOVAs. The D*X tests the change in the slope, and D tests the discontinuity at the switch point Y/X.

Source	**df**	**SS**	**MS**	***F***	**Significance**
*Males; best switch point (Y/X) = 15.67; dependent variable: second leg length (mm); predictors: constant, dorsal scute length, D, D*X.*					
Regression	3	101,820	33,940	942.74	< 0.001
Residual	359	12,924	36		
Total	362	114,744			
*Females; best switch point (Y/X) = 11.8; dependent variable: second leg length (mm); predictors: constant, dorsal scute length, D, D*X.*					
Regression	3	2,629.75	876.58	187.13	< 0.001
Residual	356	1,667.59	4.68		
Total	359	4,297.34			

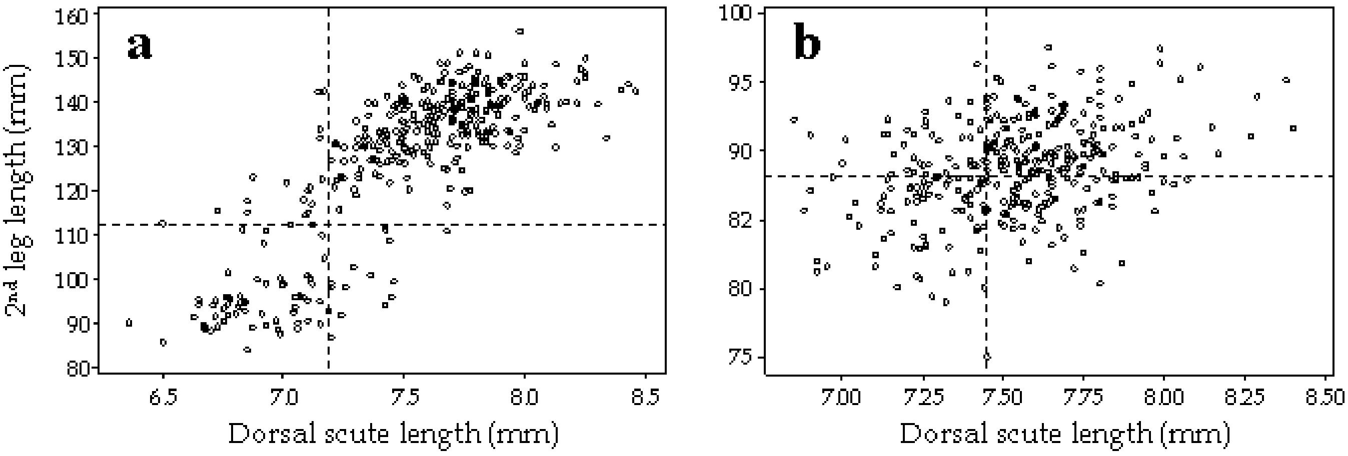

Figure 2: Allometry of second leg and dorsal scute length in (A) males and (B) females of *Acutisoma proximum*. The vertical dashed line indicates the proposed switch point in the EG model (selected by AIC analyses), whereas the horizontal dashed line indicates the proposed switch point in the KT model (also selected by AIC analyses).

In the case of females, the switch points with lowest AIC value were at 7.45 mm on the X axis (AIC = 1,872.4) for the EG analysis and at 88.1 mm on the Y axis (AIC = 1,575.2) for the KT analysis (Fig. 2b). Using the ratios of second leg length to dorsal scute length as possible "switch point ratios" (cf. Cook & Bean 2006, Fig. 4a), the best fitting switch point occurred at the second leg length of 88.57 mm and at the dorsal scute length of 7.50 mm (second leg length/dorsal scute length at this point is 11.81, AIC = 1,583.0). Conversely to what was found for males, the model (eqn 3) fitted to females revealed no dimorphism, given that β_2 and β_3 were not significant (Tables 1 and 2, Fig. 4b).

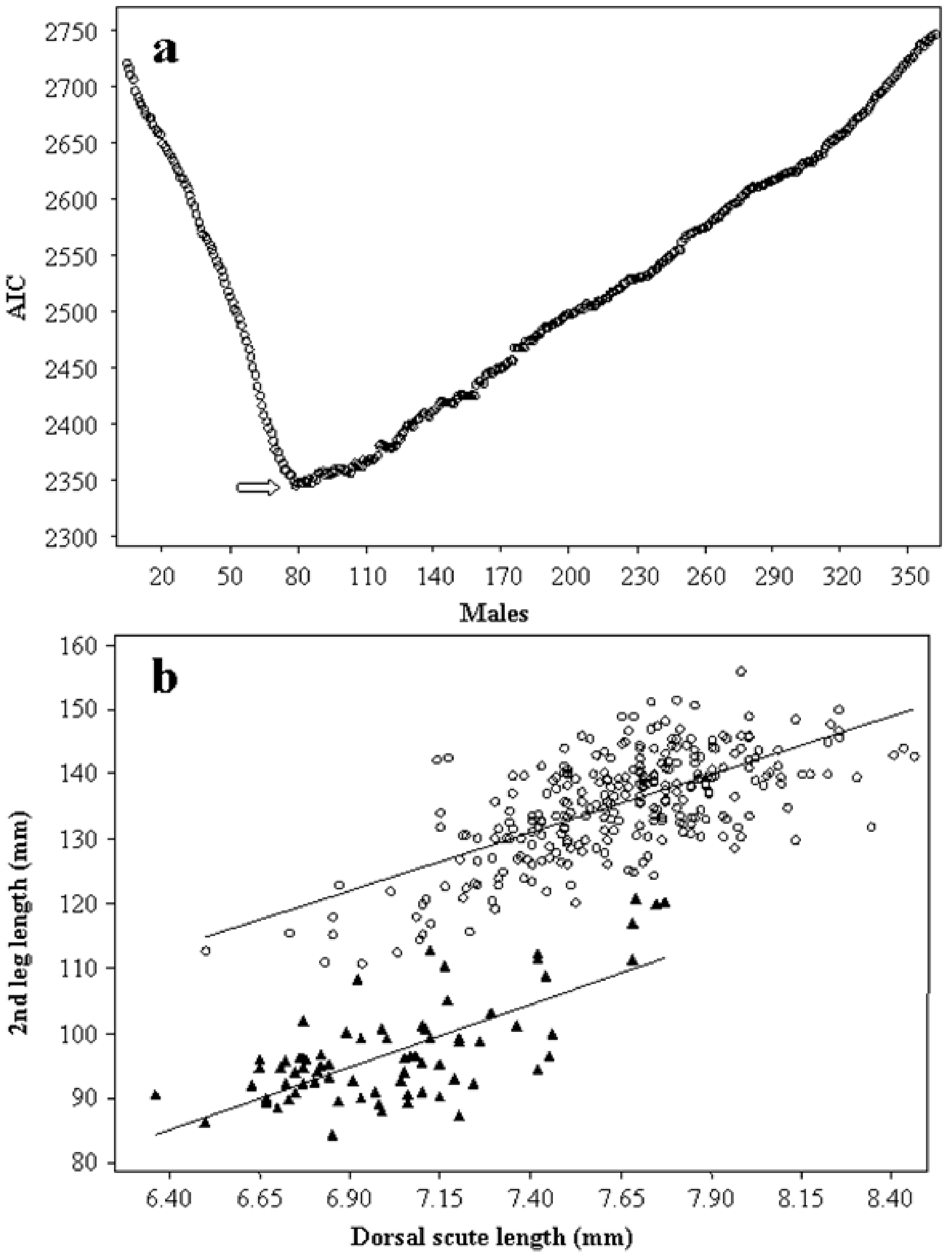

Figure 3: (A) The distribution of AIC values obtained for all possible models generated when each male in our sample was used as the switch point ratio between putative morphs. Choosing the male that gave us the lowest AIC value (arrow), the coefficients that tested for intra-sexual dimorphism in our model were significant. (B) The best model that fitted the allometry of second leg and dorsal scute length in males is represented by two continuous lines. Males from the major morph (second leg length/dorsal scute length ≥ 15.67) are represented by open circles, whereas males from the minor morph (second leg length/dorsal scute length < 15.67) are represented by dark triangles.

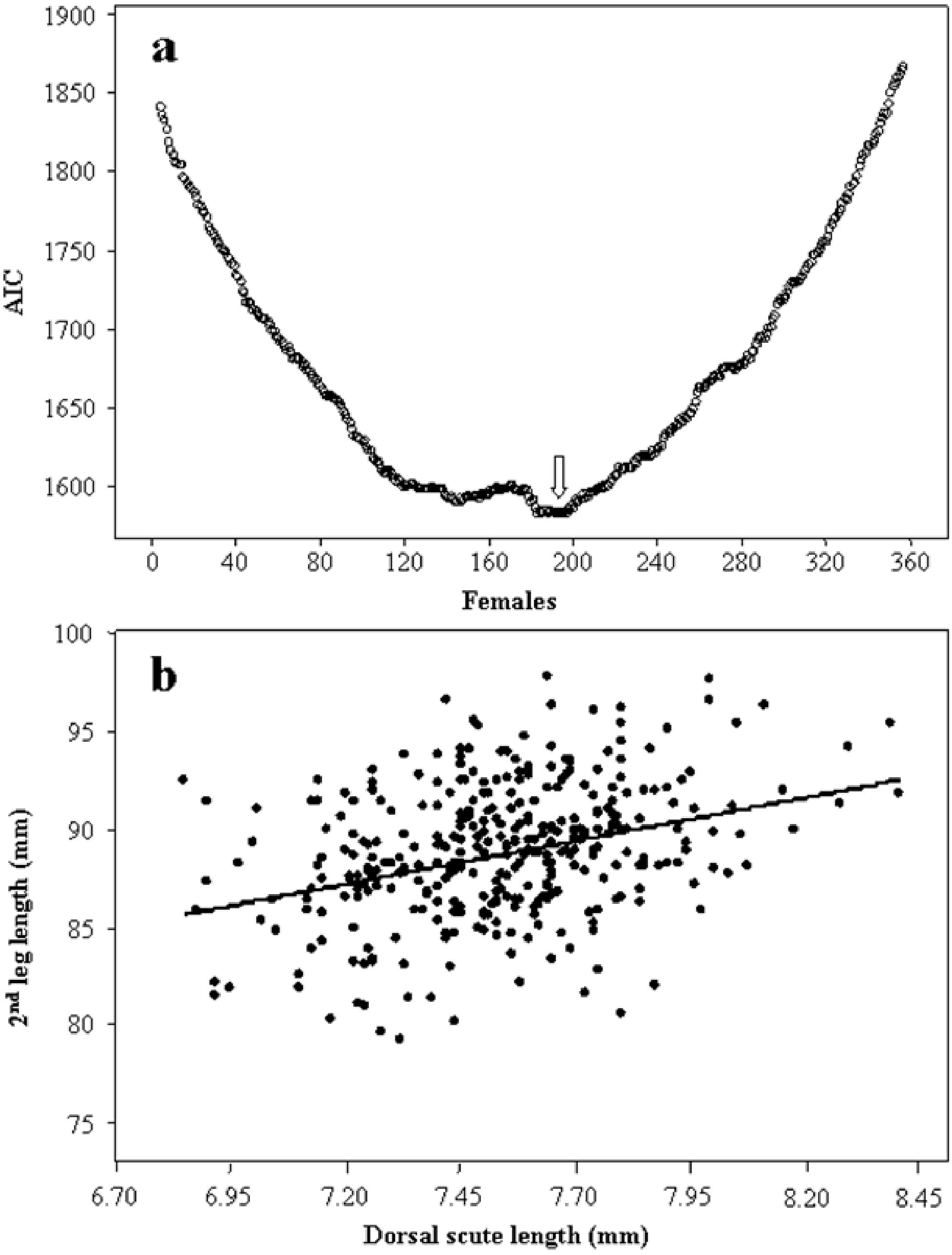

Figure 4: (A) The distribution of AIC values obtained for all possible models generated when each female in our sample was used as the switch point ratio between putative morphs. Even when we have chosen the female that gave us the lowest AIC value (arrow), the coefficients that tested for intra-sexual dimorphism in our model were non-significant. (B) The best model that fitted the allometry of second leg and dorsal scute length in females is represented by a single continuous line.

Behavioral differences between male morphs

During the field observations, we captured 363 *A. proximum* males, of which 285 (78.5%) belonged to the major morph (on the basis of second leg length) and 78 (21.5%) to the minor morph. Minors were never observed patrolling territories as major males do. All 42 male fights observed occurred between major males. Furthermore, after copulating, majors were observed mate guarding much more frequently (45 out of the 91 males, i.e. 49.5%) than minor males (only 1 out of 59 males, i.e. 1.7%) (Fisher exact test, $P < 0.001$). During the reproductive season, minors were more vagile (median displacement = 7 m; range 0 - 138 m; n = 32 males) than majors (median displacement = 4 m; range 0 - 171 m; n = 177 males) ($U = 2,202.5$; $P = 0.046$).

The pre-copulatory and the copulatory courtship behavior of minor was extremely similar to what has been previously observed for major (Buzatto & Machado submitted). The duration of copulation was not different for minors (median = 292 s; range: 20 – 967 s; n = 24 copulations) compared with majors (median = 380.5 s; range: 180 – 1200 s; n = 8 copulations) ($U = 72.0$; $P = 0.296$). Of all the 118 copulations observed during the study period, 72 (61%) were accomplished by majors and the remaining 46 (39%) by minors. However, that proportion was not different than what would be expected by the relative frequency of minors and majors in the studied population ($X2 = 0.494$, d.f. = 1, $P = 0.482$). Finally, majors were seen copulating only with egg-guarding females inside their territories, whereas some minors were seen copulating with females from more than one harem (Fig. 5). Instead of defending a small area on the vegetation as majors do, minors invaded one or more territories to mate. When a minor invaded a territory, the owner of the territory usually repelled him by attacking him with aggressive strikes of pedipalps and chelicerae (n = 19 observations).

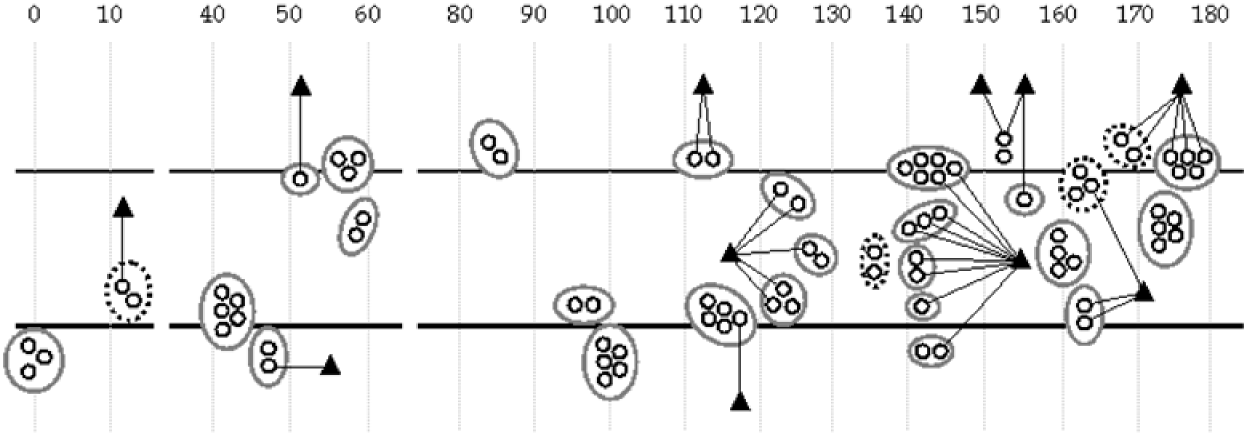

Figure 5: A schematic representation of an upper view of 29 harems of the harvestman *Acutisoma proximum* at the study site. The numbers indicate the distance (in meters) from the beginning of the study transect. The two dark horizontal lines represent the river margins, and harems drawn between them were located on the vegetation that hung above the river. The large grey circles represent the harems of guards, and the small open circles contained inside them represent egg-guarding females. Harems represented by a dashed dark circle are those whose ownership shifted between two different guards along the reproductive season. Sneaks are represented by small dark triangles, and the lines connecting them to females represent copulations by these males. Two females between 150 and 160 m were never seen guarded by a major male.

Majors and minors sperm investment

The sperm counts were highly repeatable ($F = 183.37$, $R^2 = 0.88$, $P < 0.001$ for all males). All males with a ratio of second leg length to dorsal scute length ≥ 15.67 were classified as guards, whereas those with a ratio of second leg length to dorsal scute length < 15.67 were classified as sneaks. The analysis of covariance revealed that sperm count was not related to male size (dorsal scute length) ($F = 0.142$, d.f. = 1, $P = 0.711$), and did not differ between sneaks and guards ($F = 0.015$, d.f. = 1, $P = 0.905$). There was also no interaction between dorsal scute length and male morph ($F = 0.018$, d.f. = 1, $P = 0.895$).

Discussion

Alternative mating tactics

Our data show that there is a morphological intra-sexual dimorphism coupled with alternative mating tactics in the Neotropical harvestman *Acutisoma proximum*. Whereas major males' mating tactic is based on territorial and female defense, minor males (21.5% of the males in the population) do not patrol nor defend females and territories. Instead, minor males adopt an alternative mating tactic completely based on furtively invading large males' harems and sneaking copulations with egg-guarding females inside them. But why are minor males seeking copulations with females that are already guarding eggs? The answer for this question probably relies on the fact that, unlike other harvestman species of the subfamily Goniosomatinae (Machado 2002), oviposition by *A. proximum* females is relatively asynchronous. Although nearly 95% of the eggs are laid in three days, females may take up to 14 days to complete oviposition (Buzatto & Machado submitted). Thus, sneaks that invade territories may have a chance to sire at least some of the last eggs that a female lays (Fig. 6). Successful copulations by sneaks are facilitated because males guard up to six females (see Fig. 6 in Buzatto & Machado submitted), so at any given moment, at least some females are not well-guarded. This same mating tactic has been described for elephant seals (Le Boeuf 1974) and boat-tailed grackles (Post 1992, Poston 1997) in which males also defend harems.

Figure 6: Clutch of the harvestman *Acutisoma proximum* on a leaf. The white arrow indicates a group of approximately 20 lighter eggs that are in younger stages of development, a result of the asynchronous oviposition by females.

Among the *A. proximum* males that guard territories, the length of the second legs is positively related to the number of females in their harems (Buzatto & Machado submitted). Thus, sneaking behavior has probably evolved in this harvestman species as an alternative by which small males can achieve a better reproductive success than their short second legs and overall small size would allow if they attempted to defend territories or females. Concerning the maintenance of such a polymorphism, it is still unknown if the alternative sneaking tactic in the species returns the same reproductive success than the territorial tactic. Regardless of the genetic or environmental maintenance of the male polymorphism though, our results show that the males' second legs do in fact show alternative forms that are not simply extremes of a continuous variation, but instead represent distinct body plans that resulted from different developmental programs (Eberhard 1980). Yet, the intra-sexual dimorphism in *A. proximum* is cryptic (*sensu* Cook & Bean 2006), since guards and sneaks do not express strikingly different morphologies. Given that sneaks are not subject to the same sexual selective forces that lead to the development of exaggeratedly long legs II in guards, cryptic male dimorphism is probably a consequence of the fact that natural selection has reduced unnecessary costs with leg development. This hypothesis was formalized by Lee & Bass (2006) as the ''reduced sexual selection for the exaggerated morphology''. Striking male dimorphism would be expected only if reduced characters in sneaks provided a functional advantage over exaggerated ones for their specific mating behavior, what Lee & Bass (2006) called ''sexual selection for reduced characters''.

Detecting cryptic intra-sexual dimorphism

The first statistical method for objectively detecting male dimorphism was proposed by Eberhard & Gutiérrez (1991) and consists on assigning males to one or another morph based on a switch point at the body size. This classification of individuals among morphs, however, misplaces a great number of males when individuals from distinct morphs overlap in body size (Tomkins & Simmons 1996, Tomkins 1999). In an attempt to improve the accuracy of classifying males into distinct morphs, Eberhard et al. (2000) proposed a modification of the EG model, later formalized by Kotiaho & Tomkins (2001), which consists on searching for a switch point also in the sexual trait rather than only in body size. In spite of the benefits of the KT method, if morphs overlap extensively in both body size and sexual trait, any approach that establishes a fixed switch point will misplace some males. More recently, Cook & Bean (2006) proposed a new technique that distinguishes male morphs based on the ratio of the sexual trait to body size, although they actually used the KT method to test for male dimorphism first.

In the present study, we formalized the suggestion of the CB method to use a switch point ratio by applying this technique to the model we described in eqn 3. Moreover, we have not chosen possible switch points visually, and then selected the one that returns the highest R2 value, as in the techniques of EG, KT, and CB. Instead, we compared the AIC values of all possible models generated when each individual in our sample was used to set a switch point or a switch point ratio. The establishment of a switch point ratio instead of a fixed switch point on body size or sexual trait size, coupled with testing all individuals in the sample for possible switch points or switch point ratios, allowed us to exclude any subjectivity present in the previous methods. We suggest that this slight modification should be used in future studies aimed on detecting male dimorphism. Finally, another advantage of our method was that we have also investigated the scaling relationships of females' second legs. This was only possible because the sexual trait analyzed is also present in females, contrary to the traditionally studied species of horned beetles (Eberhard & Gutierrez 1991, Simmons et al. 2007), where females usually lack horns. Thus, our analysis with females served us as a control for intra-sexual dimorphism, nearly absent in the current literature on the subject.

Sperm competition between sneaks and guards

Although theory predicts that the risk of sperm competition should be more intense for sneaks than for guards, sneak copulations in *A. proximum* were fairly often, and have been recorded in 19 out of 29 (65.5%) harems. Hence, our behavioral results suggest that the risk for sperm competition is probably high for both male morphs, what would explain why male morphs did not differ in sperm investment. An alternative hypothesis to explain why sneaks do not have higher sperm investment relies on the fact that the penis in harvestmen of the suborder Laniatores presents a striking diversity and complexity of ornaments, such as the ventral process of some gonyleptids (Macías-Ordóñez et al., in press). Although no information is available on the actual role of the ventral process during intromission, its shape and relative position suggests that it may penetrate the lumen of the ovipositor, smoothly brushing the inner walls on the way in, but scraping off the same surface on the way out. The ventral process is hardened by hydraulic pressure, thus potentially giving the male some extra control to maneuver this structure inside the female ovipositor (Macías-Ordóñez et al., in press). Males of *A. proximum* have the so called ventral process (M.B. DaSilva, pers. comm.) and sneaks could rely on some kind of sperm removal strategy to enhance their siring ability over the eggs of the previously inseminated females with which they copulate. Consequently, even if the risk of sperm competition is greater to sneaks, we would not expect them to invest in larger amounts of sperm production.

Concluding remarks

The present study represents the first case of alternative mating strategies in the order Opiliones. Moreover, this is also the first time that cryptic male dimorphism is described for arachnids. The harvestman *A. proximum* is a good model organism to understand the evolution of male dimorphism and sperm competition between male morphs. The species is also an appropriate model to investigate questions related to female promiscuity, since they not only mate several times with the same guard, but also often accept the copulation attempts of sneaks. By copulating with several males, females may benefit through increased fertility assurance (Ridley 1988, Sakurai 1996, but see Cook 1999) and/or through increased genetic diversity and fitness of their offspring (Sakurai 1996, Bateman 1998, Hosken & Blanckenhorn 1999). We believe that females' reproductive behavior has played an important role on the evolution of alternative mating strategies among males of *A. proximum*. In fact, the sneaking tactic in the species is only possible because females present an asynchronous oviposition behavior. If females completed the oviposition in a short period of time, as occurs with other harvestman species of the subfamily Goniosomatinae (Machado 2002), guards would probably be able to secure the fertilization of all females' eggs. Given that theory predicts benefits for females that mate more than once, it is likely that the asynchronous oviposition of *A. proximum* behavior has evolved as a females' strategy to overcome guards' monopoly, and gain an opportunity to mate with other males.

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CAPÍTULO 3

Effects of maternal care on the lifetime reproductive success of females in a Neotropical harvestman*

Fêmea do opilião *Acutisoma proximum* depositando seus ovos sob uma folha.

*Buzatto B.A., Requena G.S., Martins E.G. & Machado G. (2007). *Journal of Animal Ecology*, 76(5): 937-945.

Abstract

Few studies have experimentally quantified the costs and benefits of female egg-guarding behavior in arthropods under field conditions. Moreover, there is also a lack of studies assessing separately the survival and fecundity costs associated with this behavioral trait. Here we employ field experimental manipulations and capture-mark-recapture methods to identify and quantify the costs and benefits of egg-guarding behavior for females of the harvestman *Acutisoma proximum*, a maternal species from south-eastern Brazil. In a female removal experiment that lasted 14 days, eggs left unattended under natural conditions survived 75.6% less than guarded eggs, revealing the importance of female presence preventing egg predation. By monitoring females' reproductive success for two years, we show that females experimentally prevented from guarding their eggs produced new clutches more frequently and had mean lifetime fecundity 18% higher than that of control guarding females. Regarding survival, our capture-mark-recapture study does not show any difference between the survival rates of females prevented from caring and that of control guarding females. We found that females experimentally prevented from guarding their eggs have a greater probability to produce another clutch (0.41) than females that cared for the offspring (0.34), regardless of their probability of surviving long enough to do that. Our approach isolates the ecological costs of egg-guarding that would affect survival, such as increased risk of predation, and suggests that maternal egg-guarding also constrains fecundity through physiological costs of egg production. Weighting costs and benefits of egg-guarding we demonstrate that the female's decision to desert would imply an average reduction of 73.3% in their lifetime fitness. Despite the verified fecundity costs of egg-guarding, this behavior increases female fitness due to the crucial importance of female presence aimed to prevent egg predation.

Keywords: *Acutisoma*, costs of reproduction, Gonyleptidae, Opiliones, parental investment.

Introduction

In iteroparous species, parents are expected to decide how much they should invest in each brood, weighing the benefits of investing in current offspring against the costs in investing in future reproduction (Williams 1966, Trivers 1972, Clutton-Brock 1991). Even simple forms of parental care, such as the physical protection of eggs from predators and parasites (Tallamy & Wood 1986), should include both benefits (usually enjoyed directly by the offspring) and costs (invariably paid by one or both parents). In arthropods, the benefits of egg-guarding, without the occurrence of extended maternal care, have been investigated in several lineages of insects (e.g., Tallamy & Denno 1981, Kudo & Ishibashi 1995, Stegmann & Linsenmair 2002, Kudo 2002, Zink 2003) and spiders (e.g., Fink 1986, Gillespie 1990, Gundermann, Horel & Roland 1997), as well as in two species of harvestmen (Machado & Oliveira 1998, 2002) and one species of bromeliad-dweller crab (Diesel 1992). These studies have found that the female presence enhances the survival of the brood by reducing predation on eggs and/or early instars. On the other hand, the fitness costs paid by the female for defending her eggs are far from being that well documented in arthropods.

Maternal care is costly since females reduce their foraging time while allocating energy on parental activities not related to their own growth and maintenance, an unbalance which may negatively affect future reproduction (reviewed by Clutton-Brock 1991). To date, only few studies have managed to quantify experimentally the costs of egg-guarding in arthropods and all of them have dealt with species of the orders Araneae (Fink 1986, Gundermann et al. 1997) and Hemiptera (Tallamy & Denno 1982, Stegmann & Linsenmair 2002, Zink 2003). In these cases, females that were prevented from guarding their eggs had an increase in subsequent fecundity. Nevertheless, two of these studies were conducted under laboratory conditions (Tallamy & Denno 1982, Gundermann et al. 1997) while a third one used caged individuals (Fink 1986). As far as we know, the studies by Stegmann & Linsenmair (2002) and Zink (2003) are the only experimental quantifications of the costs of egg-guarding in arthropods conducted in the field, where predation pressure and environmental harshness may influence the performance of guarding females in a more realistic way.

Despite a number of methodological differences, all studies mentioned above have demonstrated that egg-guarding behavior compromises future reproduction. If egg-guarding behavior is costly for females, one might predict that this behavior diminishes parental survival as well. Paradoxically, the only study aimed to evaluate the effects of egg-guarding on survival in arthropods found exactly the opposite, i.e., as investment in maternal behavior increased, so did female lifespan (Tallamy & Denno 1982). The authors argued that females whose maternal care was experimentally prevented, spent all of their reproductive life in egg production and thus were subjected to physiological hardships more often than females that replaced egg production with some period of maternal care. Contrasting this suggestion with the widely accepted theory of parental investment (Trivers 1972) leads to two entirely opposite predictions: (1) maternal egg-guarding is costly and, therefore, diminishes female survival; (2) maternal egg-guarding prevents females from the costly overproduction of eggs and, therefore, increases female survival. Therefore, the remaining question is: does egg-guarding increase or decrease parental survival?

Opiliones, commonly known as harvestmen, are suitable organisms for addressing questions about the costs and benefits of parental care since this behavioral trait is widespread in the order and has evolved at least four times independently (reviewed in Machado & Macías-Ordóñez 2007). Field experiments have demonstrated that maternal care is crucial for preventing egg predation: entire clutches may be consumed by predators, including ants, crickets and conspecifics, in a few hours when left unprotected (Machado & Oliveira 1998, 2002). In one of these species, *A. longipes,* eggs are also attacked by fungi, especially during the wet and warm season, when nearly 50% of the clutches may be infected. Although fungus-infected eggs do not develop, guarding females do not eat or remove such eggs. Furthermore, field experiments with this species have shown that females are unable to protect their eggs against fungal attack (Machado & Oliveira 1998).

The main goal of this paper is both to identify and quantify the costs and benefits of egg-guarding for female *Acutisoma proximum* (Gonyleptidae: Goniosomatinae), a neotropical harvestman that exhibits maternal care (Ramires & Giaretta 1994). We estimated the fitness of female egg-guarding by comparing its lifetime reproductive success with that of a simulated alternative strategy, the desertion by the mother. The methods used here have been already used to study the adaptiveness of maternal care in the lace bug *Gargaphia solani* (Tallamy & Denno 1982) and in the lynx spider *Peucetia viridans* (Fink 1986). However, and unlike these studies, the present study is the first one to use this experimental approach in the field. Finally, the study also addresses the question on the effects of egg-guarding behavior on female lifespan. We expect female care in *A. proximum* to have a positive and crucial effect on egg survival, but a detrimental effect on female fecundity and lifespan since parental individuals are prevented to forage and are more exposed to predators, parasites, and climatic harshness.

Materials and methods

Study site

The study was conducted in a dense ombrophyle Atlantic Forest fragment at the Intervales State Park, southern São Paulo state, south-eastern Brazil. The annual rainfall in the region ranges from 2,000 to 3,000 mm and the mean annual temperature ranges from 12°C to 20°C. There is a well marked seasonality, with a wet and warm period lasting from October to March and a dry and cold period from April to September, when frosts are common. Both experiments and naturalistic observations were done on the vegetation flanking the river that follows the Caçadinha track (24°14′ S; 48°04′ W; 800 m alt.). The river is approximately 5 m wide at the location and is flanked by abundant vegetation that sometimes partially covers the river's bed. Two transects spaced by 50 m were established alongside this river: one with 200 m length (named T1) and another one with 500 m length (named T2).

Reproductive biology of A. proximum

The reproduction of *Acutisoma proximum* has been briefly studied by Ramires & Giaretta (1994) in Paranapiacaba, São Paulo state, south-eastern Brazil. The authors found that *A. proximum* females lay their eggs among gaps in granitic stones on river margins and remain guarding eggs and first instar nymphs. During the guarding period, females were always seen on the clutches, frequently monitoring their eggs using the first and second pairs of legs and pedipalps. The authors suggested that the maternal egg-guarding behavior in *A. proximum* may protect the clutches from infection attacks by pathogenic micro-organisms or parasites and also reduce egg and young predation.

Previous unpublished data indicates that females from Intervales population lay their eggs primarily on the underside of leaves (81.4% of clutches found on leaves against 16.6% found on rocky surfaces, n = 350), using mainly the vegetation hanging above the river (Figure 1a). Following oviposition, females guard their eggs until they hatch nearly 30 days later, remaining with nymphs until these disperse, usually seven days after hatching. Contrary to other goniosomatine species previously studied (review in Machado 2002), a female *A. proximum* does not stay close to the offspring all the time (see Figure 1b). Guarding females stay on average 73.3% (range 9 - 100%) of the time above their eggs or young (Figure 1b) and the frequency of female attendance is marginally correlated with monthly temperature taken *in loco* ($r_s = 0.461$; n = 17; $P = 0.062$). This relationship probably occurs because harvestmen are very sensitive to low temperatures (Santos 2007), and during the coldest hours of the day some guarding females are commonly found sheltered amongst the moss that grows on tree trunks close (from 0.1 to 1.0 m) to their clutches.

The Intervales population shows a marked seasonal reproduction, with females concentrating their egg-laying activities exclusively from September to April. Although there are no data on the lifespan of *A. proximum*, adults possibly live at least two years, as recorded for other goniosomatine species (Gnaspini 1995). With this relatively long lifespan, females are capable of surviving as adults for up to three reproductive seasons, then potentially producing multiple clutches during their lifetime (see Results).

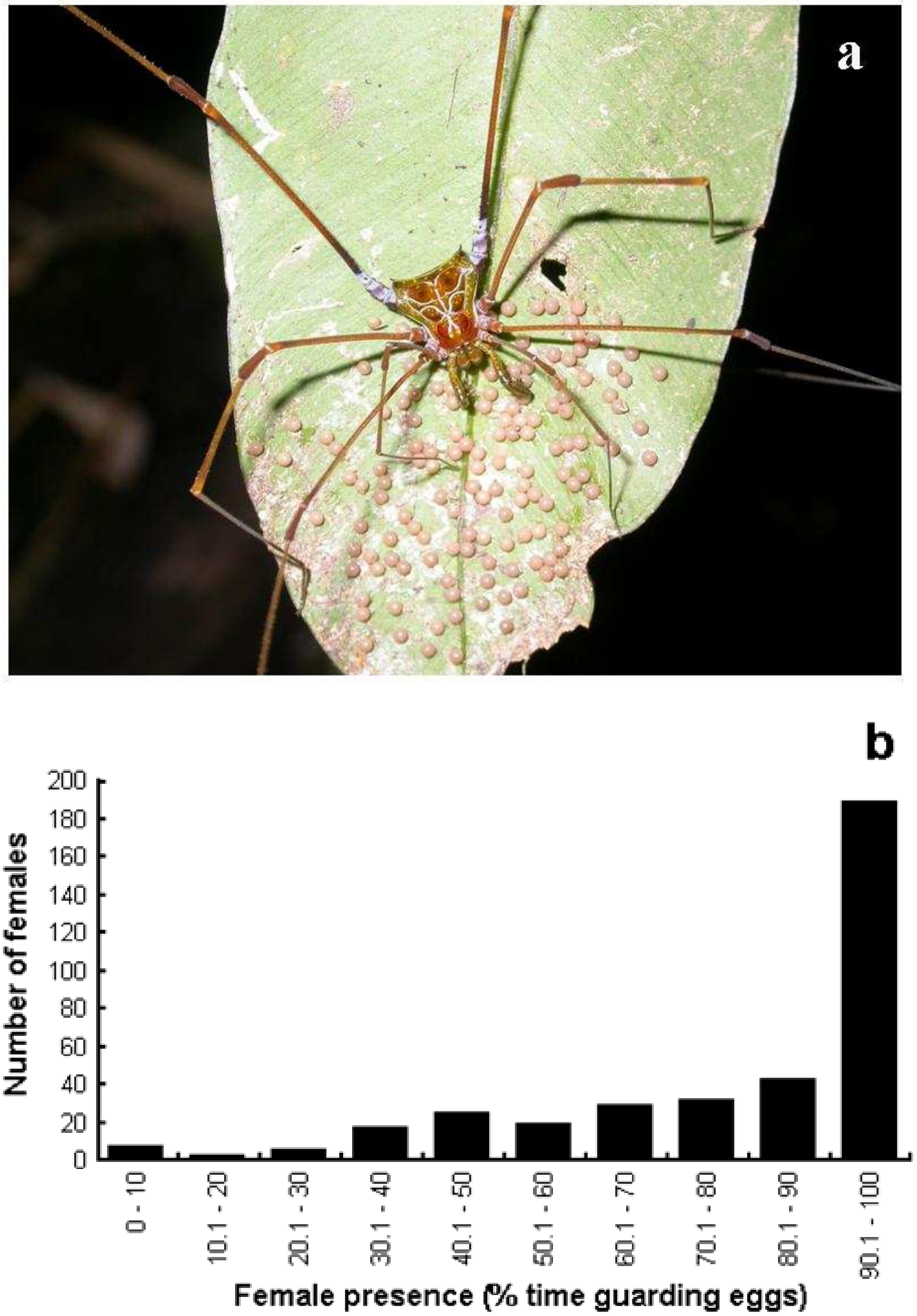

Figure 1: (a) Female *Acutisoma proximum* guarding her eggs on the underside of a leaf. (b) The majority of the 363 guarding females monitored in the field spent more than 90% of their time with their clutches. However, the frequency of female presence on their clutches showed great variation, and some females did not spend more than 10% of their time guarding the offspring.

Benefits of egg-guarding

To quantify the effectiveness of female egg-guarding against egg predators and parasites, a field experiment was conducted using 40 guarding females found on T2 during February 2004. Whenever a clutch with eggs in an early stage of embryonic development was found, the number of eggs was counted and the guarding female was randomly allocated to one of two experimental groups. In the treatment group, females were removed from their clutches and maintained alive in captivity, while their eggs were left unattended in the field for 14 days (cf. Machado & Oliveira 1998, 2002). At the end of the experiment, the experimental females were set free on the exact locations previously occupied by their clutches. In the control group, females were individually marked with enamel paint (Gnaspini 1996, Machado & Oliveira 1998) and then released again on their original clutches. During this period, all clutches were inspected four times a day (01:00, 07:00, 13:00 and 19:00 h) as an attempt to identify egg predators. Whenever predators were encountered consuming eggs, the event was observed along with a continuous record of the predator's behavior. Pictures of predators were also taken for latter identification, to avoid the disturbing the predator. The number of eggs on each clutch was counted on the days 1, 4, 8 and 14, and the number of eggs was compared between experimental groups through a Friedman's ANOVA.

Costs of egg-guarding

To quantify the costs of female egg-guarding behavior, an almost 2-year capture-mark-recapture study was conducted on T1. A set of 144 females found guarding recently laid eggs on the first reproductive season of our study, between September 2003 and April 2004, were used. All of these females were measured for the dorsal scute length and width and individually marked with enamel paint. This marking procedure has been previously used in other studies with harvestmen and has the advantages of not affecting egg-laying or egg-guarding behavior (Machado & Oliveira 1998), and the paint remains over two years (Gnaspini 1996). After marking, females were than randomly allocated to one of two experimental groups. In the first experimental group ("no care"), the clutches were removed and the guarding females were released on the location previously occupied by the eggs. This procedure was done five days after each experimental female was found in order to ensure that they had already completed their oviposition by the time of our manipulation (in *A. proximum* all eggs are laid within three days, B.A. Buzatto unpub. data). In the second group ("care"), females were released on their intact clutches. In both groups, the number of eggs per clutch was counted before the experimental manipulation.

The reproductive activity of experimental females of both groups were followed for two years (comprising the reproductive seasons of 2003-2004 and 2004-2005) in which T1 was inspected three times a day (from 08.30 to 12.00 h, from 14.00 to 17.30 h and from 20.30 to 00.00h) during four consecutive days per month. Whenever a female from the "no care" group was found guarding a new clutch, the eggs were removed in the same way described before. Females that were never found guarding new clutches on T1 were considered incapable of reproducing again (cf. Stegmann & Linsenmair 2002). This procedure underestimates the number of females reproducing more than once and gives us a conservative estimation of iteroparity in the species. It is important to notice, however, that this bias occurs in both experimental groups.

Modelling survival and recapture rates

To assess whether the egg-guarding behavior influences the survival of females, the survival (ϕ) and recapture (p) rates were modelled using a statistical modelling approach (Lebreton et al. 1992), applying program MARK (White & Burnham 1999). The capture-recapture history data used in this analysis were obtained from 55 females assigned to the "care" group and 55 assigned to the "no care" group between January and March 2004 and followed monthly from September 2004 to May 2005. To test the effect of the experimental group on the survival and recapture rates of females, the following models were fitted to the capture-recapture history data (notation within parentheses): 1) with no effects (.); 2) with experimental group effects (group); 3) with temperature effects (temperature) and 4) with temperature and experimental group effects (temperature + group). Therefore, the set of candidate models formulated included a total of 16 models.

We assessed the fit of the global model, which is the fully parameterized model {ϕ (temperature + group) p (temperature + group)}, to the data using the median $\hat{c}$ approach implemented in program MARK (White & Burnham 1999). This approach is based on the assumption that the best estimate of the overdispersion parameter of the global model, $\hat{c}$, is the value for which the observed deviance $\hat{c}$ (i.e., the model deviance divided by the model degrees of freedom) falls at the median of the distribution of all possible deviance $\hat{c}$ simulated under the hypothesis that a given value of c is the true value. Data are simulated and replicated for a range of c values and a deviance $\hat{c}$ is estimated for each of the simulated data sets. A logistic regression between the c values and the simulated deviance $\hat{c}$ values is then performed to estimate the value of c for which the observed deviance $\hat{c}$ falls at the median of the distribution of all simulated deviance $\hat{c}$. The global model is considered to fit the data if the estimated value of c is between 1 and 3 (Lebreton et al. 1992). We simulated data for 10 evenly spaced values of *c* ranging from 1 to 3, with 100 replications for each value.

To select the most parsimonious model to describe the survival and recapture rates of the experimental groups, we used the bias-corrected version of the Akaike Information Criterion, $\mathrm{AIC_c}$ (Burnham & Anderson 1998), which is computed as

$$\mathrm{AIC_c} = -2(\ln L) + 2k\left(\frac{n}{n-k-1}\right), \qquad \text{eqn 1}$$

where $\ln L$ is the natural logarithm of the likelihood function evaluated at the maximum likelihood estimate for a given model, *k* is the number of parameters and *n* is the sample size (Burnham & Anderson 1998, Johnson & Omland 2004). The model with the lowest $\mathrm{AIC_c}$ value was selected as the most parsimonious model to describe the survival and recapture rates of female *A. proximum*.

Results

Benefits of egg-guarding

There was a reduction in egg number in the clutches that were left unattended experimentally (Figure 2a), with 12 out of the initial 20 clutches in this group disappearing entirely during the 14-day experiment. In the control group, however, there was no significant reduction in the number of eggs in the clutches and only one of them was attacked and suffered high predation (96.1%). In this only case, the female was missing since the second day of the experiment, suggesting that she naturally deserted or was preyed on. The difference in egg survival between experimental groups was first detected eight days after female removal, and such difference increased over time (Figure 2b).

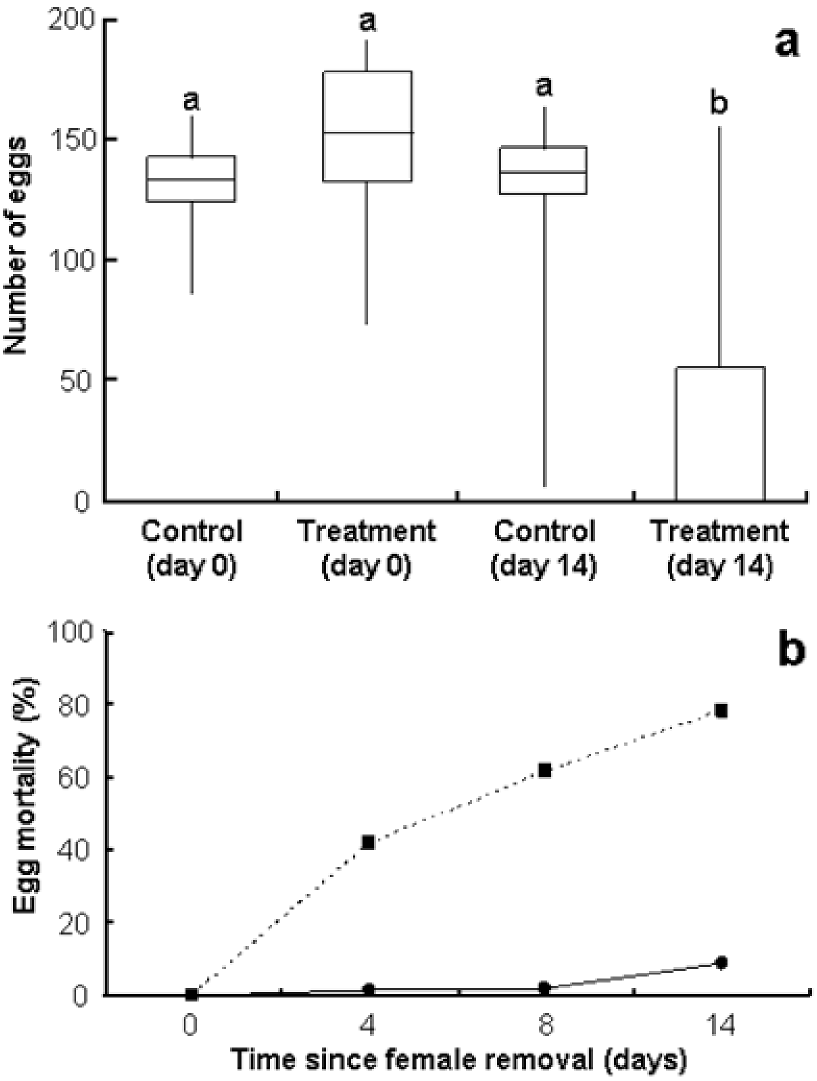

Figure 2: Predation on *Acutisoma proximum* eggs during the experiment concerning the benefits of egg-guarding. (a) There was no difference in the initial number of eggs between experimental groups, or between the initial and final number of eggs in the control group. However, there was a significant difference between the initial and final number of eggs in the treatment group and in the final number of eggs between groups (Friedman ANOVA = 28.36, df = 3, $p < 0.001$). The horizontal lines represent the median, the boxes represent the upper and lower quartiles, and the vertical lines represent the range (minimum and maximum values in each sample). Different letters above the box-plots indicate significant difference with $p < 0.05$. (b) Mean percentage of consumed eggs on both experimental groups along the experiment. Treatment group = dashed line; control group = solid line.

We recorded 45 predation events, all of them on unprotected clutches. The main source of egg mortality was cannibalism, both by conspecific males (n = 12 clutches) and females (n = 4). Once, we observed a guarding female next to her clutch repelling a conspecific female through an attack using pedipalps and chelicerae. Other predators of *A. proximum* eggs were: other harvestmen, such as the gonyleptids *Discocyrtus* sp. (n = 1), *Iporangaia pustulosa* (n = 4) and *Promitobates ornatus* (n = 9); the spider *Osoriella rubella* (n = 7, Figure 3a); an unidentified jumping spider (n = 2); the scolopendrid centipede *Otostigmus* sp. (n = 1); a katydid nymph and the wasp *Agelaia multipicta* (n = 4, Figure 3b).

Fecundity costs of egg guarding

Between September 2003 and April 2005, we found a total of 144 females guarding eggs on T1 and half of them were assigned to the "care" group (n = 72) whereas the other half were assigned to the "no care" group (n = 72). From January 2004 to April 2005, we found 35 females reproducing for a second time, 13 of them belonging to the "care" group and 22 to the "no care" group. Six females from the "no care" group produced a third clutch after the experimental removal of their second one and, remarkably, three months after the experimental removal of the third clutch, one female was capable to produce a fourth batch containing 62 eggs (Table 1). This frequency of egg production was significantly different between experimental groups (G-test = 10.67; df = 3; *P* = 0.014) and there was no difference on the body size measures of females that produced a single clutch and females that reproduced more than once (Table 2). Additionally, the time between two consecutive reproductive events in the "no care" group was shorter than in the "care" group (Mann-Whitney test, U = 118.50; *P* = 0.057; Table 1). Finally, females of the "no care" group laid more eggs than did females of the "care" group over the study period (t = 2.26; df = 142; *P* = 0.013; Table 1).

Table 1. Quantitative features associated with female fecundity obtained in the field experiment on costs of egg-guarding in *Acutisoma proximum*. For categories related to "number of eggs", the mean ± SD is shown; for the category related to the interval between clutches, the median is shown with the range in parentheses.

Reproductive parameters	**"Care" group** (n = 72)	**"No care" group** (n = 72)
Females that produced two clutches	13	22
Females that produced three clutches	0	6
Females that produced four clutches	0	1
Eggs in the 1st clutch	124.53 ± 28.9 (n = 72)	127.08 ± 30.0 (n = 72)
Eggs in the 2nd clutch	107.00 ± 35.4 (n = 13)	110.05 ± 32.8 (n = 22)
Eggs in the 3rd clutch	-	106.50 ± 31.5 (n = 6)
Eggs in the 4th clutch	-	62 (n = 1)
Lifetime fecundity (nº of eggs laid)	143.85 ± 51.9 (n = 72)	170.44 ± 85.4 (n = 72)`
Time between 1st and 2nd clutch (months)	4 (2 – 13, n = 13)	2 (1 – 11, n = 22)
Time between 2nd and 3rd clutch (months)	-	2.5 (1 – 10, n = 6)
Time between 3rd and 4th clutch (months)	-	3 (n = 1)

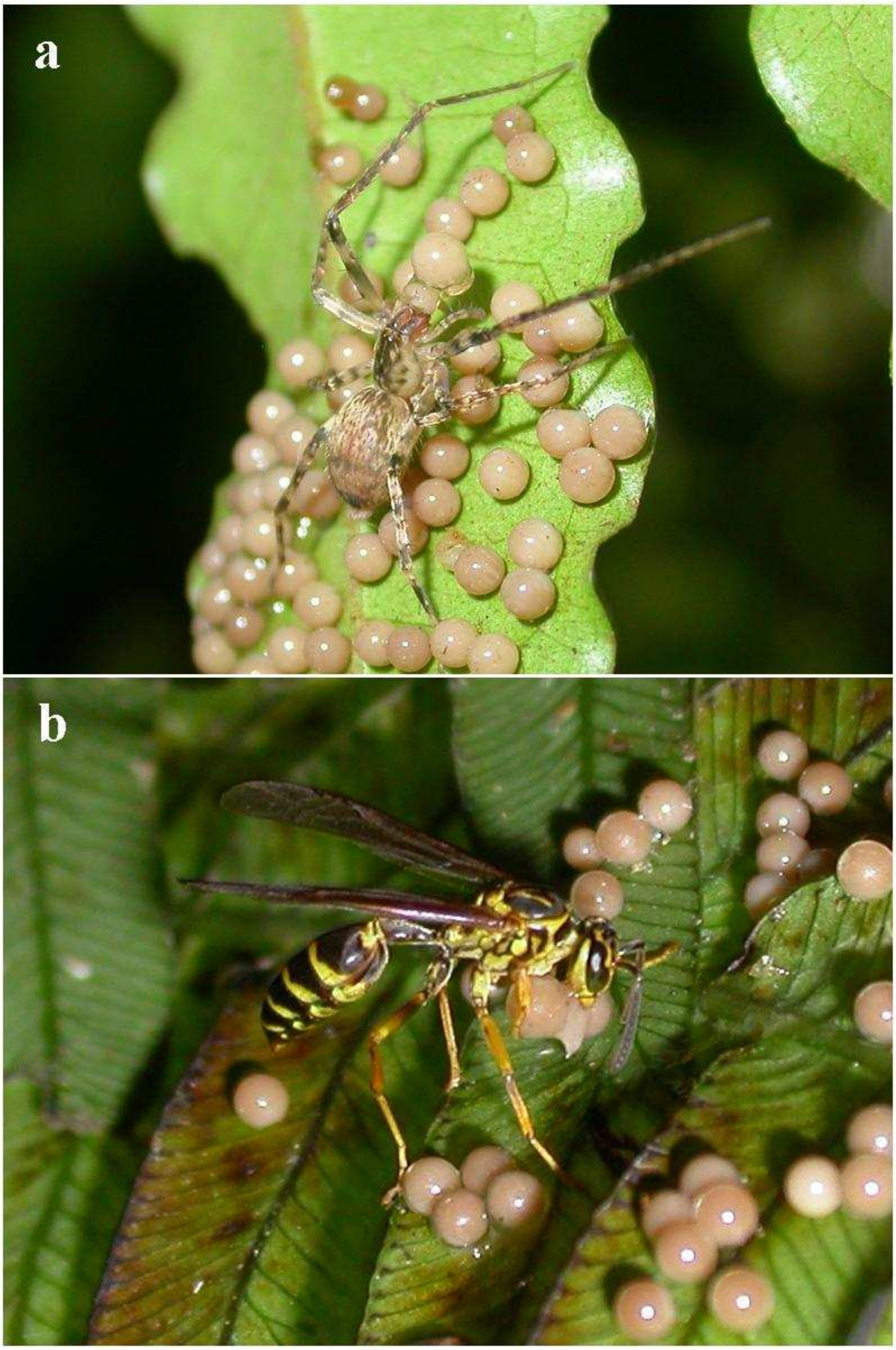

Figure 3: Two of the major predators of *Acutisoma proximum* eggs. (A) The spider *Osoriella rubella* (Anyphaenidae) and (B) the wasp *Agelaia multipicta* (Vespidae).

Table 2. Comparison of two body size features of *Acutisoma proximum* females that produced one or more clutches in the "care" group (females that guarded eggs) and in the "no care" group (females experimentally prevented from egg-guarding).

Size features	Females that produced a single clutch	Females that produced more than one clutch	Statistics
"CARE" GROUP			
Dorsal scute length	7.58 ± 0.23 (n = 59)	7.54 ± 0.33 (n = 13)	t = 0.451; P = 0.330
Dorsal scute width	8.30 ± 0.36 (n = 59)	8.25 ± 0.39 (n = 13)	U = 311.0; P = 0.289
"NO CARE" GROUP			
Dorsal scute length	7.55 ± 0.25 (n = 50)	7.57 ± 0.34 (n = 22)	U = 491.0; P = 0.471
Dorsal scute width	8.36 ± 0.39 (n = 50)	8.24 ± 0.39 (n = 22)	t = 0.945; P = 0.175

Survival costs of egg guarding

The global model, {ϕ (temperature + group) p (temperature + group)}, fitted well to the data since the overdispersion parameter was close to 1 ($\hat{c}$ = 1.13 ± 0.02 *SE*), as estimated by the median $\hat{c}$ approach. The most parsimonious model among those in the set of candidate models did not include the effect of the experimental group and temperature on the survival rate of female *A. proximum* (Table 3). As for recapture rates, the most parsimonious model included only the effect of temperature (Table 3). The monthly survival rate estimate obtained by the best model, {ϕ (.) p (temperature)}, was 0.857 ± 0.022 for females in both experimental groups. Although we cannot determine directly from this analysis whether the estimated survival rate was biased by emigration out of the sampled area, we have quantitative evidence that this source of bias in our estimates may affect both experimental groups in a similar way. The frequency of females that were captured only once was similar between the "care" (70.8%) and "no care"

(65.3%) group (chi-square test, $X^2 = 0.511$; $P = 0.475$). Additionally, the total displacement of females captured more than once in each experimental group did not differ significantly (mean ± SD, "care" = 9.0 ± 11.3 m; "no care" = 10.9 ± 16.9 m; t = 0.08; df = 44; $P = 0.939$).

Table 3. Summary of the model selection statistics for models describing survival and recapture rates of *Acutisoma proximum* females. Models are ranked by increasing order of their AIC_c values, which is the bias-corrected modified Akaike Information Criterion. Δ_i is the difference between the AIC_c value of model i and the AIC_c value of the most parsimonious model, w_i is the Akaike weight of model i, K is the number of estimable parameters in the model and Dev is the deviance. The best model is indicated in bold.

Model	**AIC_c**	**ΔAIC_c**	**w_i**	**K**	**Dev**
$\{\phi_{(.)}\ p_{(\text{temperature})}\}$	**271.09**	**0.00**	**0.28**	**3**	**73.82**
$\{\phi_{(.)}\ p_{(\text{temperature + group})}\}$	272.73	1.64	0.12	4	73.34
$\{\phi_{(\text{group})}\ p_{(\text{temperature})}\}$	272.88	1.79	0.12	4	73.49
$\{\phi_{(\text{temperature})}\ p_{(\text{temperature})}\}$	273.36	2.27	0.09	4	73.98
$\{\phi_{(.)}\ p_{(.)}\}$	273.58	2.49	0.08	2	78.40
$\{\phi_{(\text{temperature})}\ p_{(\text{temperature + group})}\}$	274.42	3.33	0.05	5	72.89
$\{\phi_{(\text{group})}\ p_{(\text{temperature + group})}\}$	274.84	3.75	0.04	5	73.31
$\{\phi_{(.)}\ p_{(\text{group})}\}$	275.10	4.01	0.04	3	77.84
$\{\phi_{(\text{group})}\ p_{(.)}\}$	275.34	4.25	0.03	3	78.07
$\{\phi_{(\text{temperature})}\ p_{(.)}\}$	275.36	4.27	0.03	3	78.10
$\{\phi_{(\text{temperature + group})}\ p_{(\text{temperature})}\}$	275.37	4.28	0.03	5	73.84
$\{\phi_{(\text{temperature})}\ p_{(\text{group})}\}$	276.41	5.32	0.02	4	77.03
$\{\phi_{(\text{temperature + group})}\ p_{(\text{temperature + group})}\}$	276.51	5.42	0.02	6	72.80
$\{\phi_{(\text{group})}\ p_{(\text{group})}\}$	277.21	6.12	0.01	4	77.82
$\{\phi_{(\text{temperature + group})}\ p_{(.)}\}$	277.35	6.25	0.01	4	77.96
$\{\phi_{(\text{temperature + group})}\ p_{(\text{group})}\}$	278.47	7.38	0.01	5	76.94

Discussion

Benefits of egg-guarding

Maternal care is widespread among terrestrial arthropods and the benefits of this behavior may include feeding facilitation or provisioning as well as protection of the offspring against biotic (such as predators, pathogens and parasites) and abiotic (such as temperature, dehydration and flooding) factors (references in Clutton-Brock 1991). Among harvestmen, there is no evidence that females can benefit their offspring by enhancing the feeding efficiency of the nymphs, or that parents are able to actively protect offspring against environmental stress, such as dehydration (Machado & Oliveira 1998). Nevertheless, field experiments with two neotropical species, the cavernicolous *Acutisoma longipes* and the bromelicolous *Bourguyia hamata*, have demonstrated that maternal care is crucial for preventing egg predation (Machado & Oliveira 1998, 2002). In our female removal experiment, the majority of clutches left unattended were entirely consumed by predators within a few days, demonstrating the importance of female presence preventing egg predation in *A. proximum*.

The main detected sources of egg mortality were conspecifics (especially males) and other harvestman species, which together totaled almost 65% of observed predation events. Contrary to what was found for the neotropical harvestman *B. hamata* (Machado & Oliveira 2002), another maternal species that also reproduces on vegetation, ants were not the most important egg predators. Furthermore, our results indicated several novel egg predators, such as centipedes, wasps and katydids. The latter two were capable of causing great egg mortality on short periods of time (sometimes less than 30 min). Predatory wasps, for instance, attacked the same clutch repeatedly, always leaving with groups of two to three eggs (Figure 3b). These predators kept returning until all eggs of the clutch were taken, probably to provision of the wasps nests. It is also worth mentioning that predation events took place both during day and night, suggesting that unattended clutches are constantly under high predation risk.

Given that predation on unattended eggs seems high, the benefit of guarding is certainly enormous in *A. proximum*. However, to estimate the net benefit of this behavior it is also crucial to calculate the cost of guarding. Additionally, both the costs and the benefits must be measured in terms of lifetime reproductive success, rather than the success of a single brood. In the following sections we discuss the costs of egg-guarding behavior in this study species and then we integrate costs and benefits in order to quantify the lifetime output of two hypothetical alternatives: female care and female desertion.

Costs of egg-guarding

The reproductive costs associated with maternal care can be classified into two major categories: (a) ecological costs, which are related to an increased exposure to predation while searching for oviposition sites, laying or guarding the offspring and (b) the physiological costs, related to the allocation of resources to egg production and other parental activities rather than to individual growth or somatic maintenance (Clutton-Brock 1991). In relation to ecological costs, our results show that female survival in *A. proximum* was not influenced by egg-guarding behavior, given that the most parsimonious model describing female survival did not include the effect of the experimental group ("care" *vs.* "no care") on the survival rate of females (Table 3). Our study is the first to assess the effects of egg-guarding behavior on the survival of a female arthropod by modelling survival and recapture rates of marked individuals monitored in the field. Our findings contrast with those obtained by Tallamy & Denno (1982) for the hemipteran *Gargaphia solani*, in which females that guarded their brood produced fewer clutches, but survived longer than females that had been experimentally prevented from guarding eggs. However, Tallamy & Denno's study was conducted with captive individuals, which may have not faced ecological hazards and predation risk. On the other hand, our study was conducted in the field and the ecological costs of maternal care in *A. proximum*, if any, may be regarded as minimal. There are at least two non-excluding reasons for that: (1) all known predators of Goniosomatinae harvestmen, including opossums, wandering spiders, and toads (Cokendolpher & Mitov 2007), are active at night, when females of both experimental groups are found exposed on the vegetation, and (2) females of the "care" group temporarily abandon their clutches during the coldest hours of the day and shelter in places where they get protection from climatic harshness (see section "Reproductive biology of *A. proximum*"), which possibly attenuate their physiological stress.

Concerning the physiological costs of reproduction in *A. proximum*, females are known to invest a considerable amount of energy to produce large and nutritionally rich eggs, which may reach 2.0 mm in diameter each (Ramires & Giaretta 1994). In other Goniosomatinae species, the total volume of a single clutch may correspond to 50% of the female body volume (Machado & Macías-Ordóñez 2007). After oviposition, maternal care reduces foraging activities and probably decreases the intake of energy to produce additional eggs in *A. proximum* females, a critical issue in iteroparous species (Tallamy & Brown 1999). In fact, our experimental manipulations in the field indicate that egg-guarding behavior reduces the probability of females to oviposit again. When considering exclusively the probability of producing new clutches, females of the "no care" group were much more likely to do so than were females of the "care" group (40.3% *vs.* 18.1%, respectively; chi-square test, $X^2 = 15.02$; $P < 0.001$). Additionally, the mean fecundity of the females in the "no care" group during the study was nearly 18% higher than that of females of the "care" group. These results provide additional support to a pattern previously described for hemipterans (Tallamy & Denno 1982, Zink 2003) and spiders (Fink 1986, Gundermann et al. 1997) in which future reproduction is negatively affected by present reproductive investment.

Females of the great majority of maternal harvestman seem limited to a single oviposition event per breeding season or even during their entire lives (Machado & Macías-Ordóñez 2007). This is also the case of *A. proximum*, in which nearly 82% of the females that were allowed to care for the offspring produced only one clutch during two years. If environmental constraints, such as food and/or nest site shortage, reduce the success of subsequent ovipositions during a breeding season, the fecundity-related costs of maternal care are minimized, regardless of whether the female lives to produce another clutch in the following breeding season (Tallamy & Brown 1999). Females constrained to produce only one clutch in a given breeding season could increase their lifetime reproductive success by guarding the eggs that they have already laid, especially in environments with high egg and juvenile predation, as seems to be case in *A. proximum*.

Combining costs and benefits

Once the costs and benefits of egg-guarding were identified and quantified, we used a simple equation that quantifies the lifetime fitness (W) of female *A. proximum*. This W refers to the lifetime number of eggs produced that survive at least 2/3 of the embryonic development, and not overall reproductive success since we do not have information about nymph mortality. In this way, W is calculated as follow:

$$W = S\left(N_1 + \sum_{k=2}^{n} N_k P_k\right), \qquad \text{eqn 2}$$

where S is the mean survival of eggs for the first 14 days after oviposition (empirically estimated in the field experiment described in session "BENEFITS OF EGG-GUARDING"), N_k is the mean number of eggs per batch produced in reproductive event k, and P_k is the probability of having the reproductive event k (P_k was empirically estimated in the field experiment described in sessions "COSTS OF EGG-GUARDING", based on the assumption that the probability of capturing a reproductive female is 1). P_k is comprised of two probability terms:

$$P_k = \phi^t \times d_k, \qquad \text{eqn 3}$$

where ϕ is the monthly survival rate obtained from the best model, exponent t is the median number of months elapsed between two reproductive events (see values in Table 4), and d_k is the probability of producing the k-th clutch given that the female survived from reproductive event k–1 to k. We can rearrange equation 3 to compute d_k as

$$d_k = {P_k}/{\phi_k^t}\,. \qquad \text{eqn 4}$$

Solving equation 4 for values of P_k and ϕ^t (see values in Table 4), we find that females that choose to desert clutches have a slightly greater probability of producing a second clutch (0.417) than do females that choose to care for the offspring (0.372). Moreover, the probability of producing additional clutches in females of the "no care" group decreases over time (Table 4). The parameter d_k has never been estimated in any previous field study that we are aware of, which makes our work the first attempt to investigate the effect of maternal care on the physiological ability of producing new clutches. Our approach disentangles the ecological costs of egg-guarding that would affect survival, and suggests that maternal egg-guarding also constrains fecundity through physiological costs of spending the whole guarding period (until 37 days) without actively foraging. The low food intake during this period probably prevents females from producing eggs in the next months.

Table 4. Variables used to estimate the fitness (W) of guarding and deserting females of *Acutisoma proximum*. Unless otherwise stated, the values are presented as means. See text for equations and explanation on the meaning of each variable.

Term	**Explanation**	**"Care" group**	**"No care" group**
S	Egg survival (assayed for 14 days)	0.911	0.222
N_k	Number of eggs in the clutch k	124.5 ($k = 1$)	127.1 ($k = 1$)
		107.0 ($k = 2$)	110.0 ($k = 2$)
		-	106.5 ($k = 3$)
		-	62 ($k = 4$)
P_k	Probability of having the reproductive event number k	0.181 ($k = 2$)	0.306 ($k = 2$)
		0 ($k = 3$)	0.273 ($k = 3$)
		0 ($k = 4$)	0.167 ($k = 4$)
	Monthly survival probability	0.857	0.857
t	Median number of months elapsed between two reproductive events	4 months	2 months
d_k	Probability of producing the k^{th} clutch	0.336 ($k = 2$)	0.417 ($k = 2$)
			0.372 ($k = 3$)
			0.227 ($k = 4$)
W	Lifetime fitness	125.12	33.46

To assess the net benefit of egg-guarding behavior, we used equation 1 to contrast the mean fitness of guarding females with that of females that were experimentally prevented from guarding. This approach was modified from Fink (1986), with the considerable advantage that in the present study we were able to quantify female fecundity and estimate their lifespan under more natural conditions, without caging females. Solving equation 1 for the values of S, N_k and P_k estimated for both experimental groups ("care" and "no care", see values in Table 4) we demonstrate that the hypothetical female decision to desert causes a reduction of 73.3% in lifetime fitness (33.46 surviving eggs produced by females of the "no care" group against 125.12 surviving eggs produced by females of the "care" group).

Although guarding behavior diminishes the females' future fecundity, the great improvement in egg survival more than compensates the costs of care in *A. proximum*. Among arthropods with the capacity to enhance the survival of their young, maternal care will only evolve when the benefits of such behavior exceed the costs (Clutton-Brock 1991). This can occur either when the benefits of care are extraordinarily large in relation to moderate costs, or when the costs are exceptionally low in relation to moderate benefits. The first option seems to be the case not only for *A. proximum* but also for all the other maternal arthropods studied so far.

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CONCLUSÃO GERAL

"If you want to do evil, science provides the most powerful weapons to do evil; but equally, if you want to do good, science puts into your hands the most powerful tools to do so. The trick is to want the right things, then science will provide you with the most effective methods of achieving them."

Richard Dawkins

"There is a theory which states that if ever anyone discovers exactly what the Universe is for and why it is here, it will instantly disappear and be replaced by something even more bizarre and inexplicable. There is another theory which states that this has already happened."

Douglas Adams

De acordo com o estudo de Emlen & Oring (1977) sobre a evolução de sistemas de acasalamento, a distribuição dos recursos reprodutivos utilizados pelas fêmeas influencia a distribuição das mesmas que, por sua vez, influenciam a distribuição dos machos e o tipo de sistema de acasalamento em uma dada espécie. A descrição detalhada do sistema de acasalamento do opilião *Acutisoma proximum* no Capítulo 1 ilustra de forma clara como o comportamento reprodutivo das fêmeas e a sua utilização de recursos reprodutivos influencia todo o sistema de acasalamento da espécie. Fêmeas de *A. proximum* depositam seus ovos na face inferior de folhas na vegetação que margeia pequenos riachos no local de estudo. Essas fêmeas exibem uma preferência por algumas das espécies de plantas, que utilizam como sítio de oviposição. Depois de depositarem seus ovos, elas permanecem cuidando da prole por até 37 dias. No começo da estação reprodutiva, machos territoriais lutam entre si pela posse de territórios na vegetação, que serão visitados por fêmeas receptivas em busca de um local para reprodução. O fato de fêmeas exibirem uma preferência por algumas das espécies de planta presentes no ambiente possibilita que os machos se posicionem precisamente onde as fêmeas chegarão para se reproduzir e, dessa forma, defendam locais na vegetação que serão especialmente atrativos para as fêmeas. Esse é um caso típico de poliginia por defesa de recurso, no qual os machos estão monopolizando as fêmeas através da defesa de um recurso reprodutivo importante para elas (Shuster & Wade 2003).

Alguns meses mais tarde, na mesma estação reprodutiva, os machos de *A. proximum* reduzem suas atividades de patrulha para os pequenos trechos de seus territórios onde as fêmeas depositaram os ovos e passam a gastar boa parte do tempo guardando cada fêmea individualmente em seqüência. Nessa etapa, todas as fêmeas já chegaram aos territórios e passa a ser mais vantajoso para os machos defendê-las até que elas terminem de depositar seus ovos. A partir desse momento, o sistema de acasalamento passa a se parecer mais com uma poliginia por defesa de fêmeas (Shuster & Wade 2003) e os machos seguem copulando com as fêmeas de seus haréns até que todas tenham terminado o processo de oviposição. O opilião *A. proximum* é o primeiro aracnídeo não-acarino que apresenta uma mudança em seu sistema de acasalamento ao longo da estação reprodutiva. Dessa forma, os resultados descritos no Capítulo 1 podem ser considerados como uma fonte de dados filogeneticamente independentes para se testar hipóteses sobre a evolução e o funcionamento de sistemas de acasalamento poligínicos.

O Capítulo 1 também provê dados sobre o cuidado paternal ocasional que machos territoriais de *A. proximum* fornecem à prole de fêmeas que desertam dentro de seus territórios. Esses dados nos permitem compreender melhor a evolução do cuidado paternal exclusivo em diversas linhagens dentro da ordem Opiliones, que é a única dentre os aracnídeos a compreender espécies paternais (Machado & Macías-Ordóñez 2007). É provável que a defesa de haréns ou territórios onde os ovos são incubados minimize os custos do cuidado paternal e predisponha os machos de opilião a defenderem os ovos que se encontram em seus territórios (Williams 1975). Assim, o comportamento de guarda de ovos por parte dos machos de opiliões provavelmente evoluiu de espécies ancestrais maternais que possuíam um sistema de acasalamento poligínico baseado na defesa de fêmeas ou territórios.

Após a compreensão do sistema de acasalamento de *A. proximum* fornecida pelo Capítulo 1, o Capítulo 2 descreveu a existência de duas morfologias alternativas associadas a distintas estratégias de acasalamento em machos de *A. proximum*. Os machos de *A. proximum* se dividem em dois morfos distintos, de acordo com a relação alométrica do comprimento da perna II, que é também mais longa nos machos do que nas fêmeas.

Além disso, a alometria estática das pernas II dos machos indica que as morfologias dos morfos de machos não são extremos de uma variação contínua, mas representam planos de corpo distintos que resultam de distintos programas genéticos de desenvolvimento presentes na espécie (Eberhard 1980). Apesar desses programas genéticos distintos, a diferença na morfologia dos dois morfos não é muito conspícua. Ao invés disso, o dimorfismo entre machos de *A. proximum* só se manifesta no comprimento do segundo par de pernas, e mesmo assim só é detectável ao observador quando um grande conjunto de machos é medido e a relação entre o comprimento do segundo par de pernas e o tamanho do corpo são analisados. Esse tipo de dimorfismo sutil entre machos se encaixa na definição de dimorfismo intra-sexual críptico (Cook & Bean 2006).

Os diferentes morfos de machos de *A. proximum* também diferem em seu comportamento reprodutivo. Machos com pernas II longas defendem territórios na vegetação, onde fêmeas (de uma a seis) depositam seus ovos. Após a cópula, esses machos guardam suas parceiras por até dois dias, repelindo qualquer outro macho que se aproxime. Após o período de guarda de parceira, machos territoriais continuam copulando repetidamente com as fêmeas por até 14 dias, até elas terminarem de depositar seus ovos. Quando dois machos territoriais se encontram, eles se engajam em longas brigas ritualizadas, nas quais se posicionam frontalmente e se golpeiam mutuamente com as longas pernas II. Machos com pernas II curtas nunca defendem territórios ou brigam. Ao contrário, esses machos se deslocam entre os territórios dos machos grandes, invadindo-os e copulando com as fêmeas que estão dentro deles. Durante uma invasão, machos pequenos geralmente copulam com as fêmeas ali presentes, mas ocasionalmente acabam sendo detectados e repelidos pelos donos dos territórios. Esse trabalho é o primeiro a descrever, com dados comportamentais e morfológicos, a existência de estratégias alternativas de acasalamento na ordem Opiliones.

Após a descrição básica do sistema de acasalamento de *A. proximum* no Capítulo 1 e o enfoque nas duas estratégias alternativas de acasalamento em machos no Capítulo 2, o Capítulo 3 foi dedicado à compreensão da biologia reprodutiva das fêmeas da espécie. Nesse capítulo, os custos e benefícios da guarda de ovos por fêmeas foram investigados de maneira extensiva, com manipulações experimentais e métodos objetivos de se estimar a fecundidade e sobrevivência das fêmeas. Em primeiro lugar, em um experimento de remoção de fêmeas guardiãs, os ovos deixados desprotegidos em condições naturais sobreviveram 75,6% menos aos ataques de predadores do que os ovos protegidos pelas fêmeas guardiãs. Esse experimento revelou a importância da presença materna como mecanismo de proteção contra predação. Em segundo lugar, foi realizado um outro experimento em que as desovas de metade das fêmeas foram experimentalmente removidas e, em seguida, o sucesso reprodutivo dessas fêmeas foi monitorado por dois anos. Os resultados desse experimento indicam que as fêmeas que foram experimentalmente impedidas de cuidar da prole produziram novas desovas mais frequentemente e tiveram um sucesso reprodutivo ao longo da vida 18% maior que o obtido pelas fêmeas que foram deixadas cuidando de sua prole.

Com relação à sobrevivência das fêmeas, o estudo de captura-marcação-recaptura não demonstrou nenhuma diferença entre as taxas de sobrevivência de fêmeas que foram impedidas de cuidar da prole e fêmeas que cuidaram de sua prole. Finalmente, uma equação concebida para dissociar os custos do cuidado da prole em termos de sobrevivência e em termos de fecundidade demonstrou que fêmeas experimentalmente impedidas de cuidar da prole possuem uma probabilidade maior de produzir uma nova desova do que fêmeas que cuidaram da prole, independente da probabilidade que ambas possuem de viver o suficiente para isso. Essa abordagem adotada no Capítulo 3 é inédita, pois isola os custos ecológicos do cuidado maternal, como o aumento no risco de predação, e nos possibilita concluir que o cuidado maternal da prole também limita a fecundidade através de custos fisiológicos. Esses custos podem emergir do fato de que fêmeas guardiãs suprimem suas atividades de forrageamento quase que completamente durante o período de guarda da prole. O estudo também demonstra que, pesando os custos e benefícios do comportamento de guarda de ovos, uma estratégia de abandono da prole implicaria em uma redução média de 73,3% no sucesso reprodutivo total das fêmeas. Portanto, apesar dos custos que a guarda de ovos representa para a fecundidade das fêmeas, o cuidado maternal ainda assim aumenta o seu sucesso reprodutivo, já que a proteção contra predadores fornecida pela guarda de ovos compensa os custos deste comportamento.

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