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Seleção sexual na aranha urbana *Hasarius adansoni* (Araneae: Salticidae)

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Sumário

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Introdução geral

Darwin (1871) foi o primeiro a sugerir que as diferenças entre machos e fêmeas, incluindo ornamentos e comportamentos de corte, não favoreciam diretamente a sobrevivência dos indivíduos (como predito pela sua teoria de seleção natural) e que, em geral, tais fenótipos são encontrados em machos. Ele sugeriu que esses fenótipos seriam usados por machos para atrair fêmeas ou competir com outros machos por acesso às fêmeas. Darwin também observou que fêmeas normalmente podem escolher com qual macho se acasalarão baseando-se nesses fenótipos distintos.

Enquanto a teoria de seleção sexual de Darwin se tornou muito bem aceita, a razão pela qual geralmente apenas os machos competem por fêmeas, enquanto estas aparentemente não competem por parceiros permaneceu desconhecida. Aproximadamente 70 anos mais tarde, Bateman (1948) publicou seu clássico trabalho sobre seleção sexual em *Drosophila melanogaster*, mostrando que a variância no sucesso reprodutivo dos machos é muitas vezes maior que no sucesso reprodutivo das fêmeas. Esse padrão é normalmente apresentado como evidência de que a seleção sexual advém de competição entre machos por acesso às fêmeas. Outra importante conclusão do trabalho de Bateman foi que o sucesso reprodutivo dos machos aumenta com o número de cópulas, enquanto que o sucesso reprodutivo das fêmeas permanece virtualmente invariante, independentemente da quantidade de cópulas sucedendo à primeira. Esse padrão foi interpretado como resultado da maior quantidade de gametas produzidos por machos. Isso explicaria o padrão geral observado na natureza, onde machos tentam atrair e copular com o máximo de fêmeas possível, enquanto estas escolhem apenas poucos deles para se acasalar.

Apesar de sua importância, o estudo de Bateman não responde uma das perguntas centrais da área de seleção sexual: porque as fêmeas escolhem um fenótipo específico em seus parceiros? A hipótese mais simples prediz que fêmeas possuem uma preferência inata por certas características. Por exemplo, nas aves *Taeniopygia guttata* e *Peophila acuticauda* (Aves: Estrildidae), os machos não possuem cristas. No entanto, se cristas artificiais forem colocadas em machos de ambas as espécies, as fêmeas desenvolvem uma forte preferência por tais machos (Burley & Symanski 1998). Além disso, cristas brancas são preferidas a cristas de outras cores. Esses resultados sugerem que as fêmeas dessas duas espécies possuem uma preferência inata por machos com cristas, apesar de tais ornamentos não existirem nessas espécies na natureza. Se um mutante nascer com uma grande crista branca, terá uma vantagem imediata em relação a outros machos, ainda que este ornamento não seja um indicador honesto de qualidade ou condição do animal.

De acordo com Fisher (1930), quando tais preferências inatas ocorrem, a maior parte das fêmeas irá copular com os machos que exibem a característica preferida. Os filhos de tais acasalamentos serão beneficiados por herdarem a característica buscada pelas fêmeas da população, enquanto as filhas herdarão a preferência de suas mães. Isso daria origem a uma cascata de eventos evolutivos, a qual Fisher (1930) chamou de *run-away selection*. Modelos matemáticos pioneiros demonstraram que, uma vez que a maioria das fêmeas de uma população exibe uma preferência, o processo de seleção *run-away* pode iniciar-se e apenas irá parar quando os custos para os machos de portar um caráter sexual secundário exagerado, causado pela seleção natural, for maior que o ganho reprodutivo causado pela seleção sexual (Lande 1981; Kirkpatrick 1982). Existem evidências consideráveis de que preferências inatas podem ocorrer em aves (Hunt 1997; Madden & Tunner 2003), peixes (Basolo 1995; Rodd et al. 2002; MacLaren 2006) e anfíbios (Ryan & Rand 1990), mas aracnídeos foram menos estudados nesse contexto, quando comparados a outros taxa (Huber 2005).

Uma hipótese proposta mais tarde por Zahavi (1975) sugere que fenótipos que restringem o movimento (e.g., caudas longas em muitas aves) ou aumentam a conspicuidade (e.g., colorações brilhantes e *displays* sexuais) resultam em uma desvantagem em termos de seleção natural, e apenas animais com carga genética de maior qualidade poderiam arcar com o custo de tal desvantagem e ainda conseguir escapar de predadores. As fêmeas, portanto, se beneficiariam escolhendo tais machos porque seus filhotes iriam herdar os atributos dos pais, o que possibilitaria maior capacidade de escape de predadores.

Os trabalhos de Fisher (1930) e Zahavi (1975) deram origem à atual dicotomia nos estudos de seleção sexual. Quando se tenta explicar a origem de caracteres sexuais secundários e das preferências de fêmeas por tais caracteres, as hipóteses tendem a se separar em duas grandes linhas: as hipóteses "fisherianas" não admitem um ganho imediato para fêmeas que copulam com machos com algum fenótipo específico, e suas preferências são ditas não adaptativas. Por outro lado, as hipóteses "zahavianas" admitem que fêmeas e suas proles possuem algum ganho imediato por meio da escolha da fêmea, e tais escolhas são ditas adaptativas.

Entre as hipóteses "fisherianas", há aquelas predizendo viés sensorial por parte das fêmeas (Endler & Basolo 1998; Holland & Rice 1998). De acordo com essas hipóteses, os caracteres sexuais secundários dos machos exploram um viés sensorial pré-existente das fêmeas, o que as faz mais atraídas por algum ornamento por meio da estimulação de seu sistema nervoso. Por exemplo, os sapos *Engystomops pustulosus* evoluíram um chamado de acasalamento que estimula os órgãos do ouvido interno das fêmeas mais que outros chamados (Ryan & Rand 1990). Machos do caranguejo *Uca beebei* constroem pilares de lama para atrair as fêmeas. Tais pilares são semelhantes aos refúgios utilizados pelas fêmeas para proteção e esta é a provável razão pela qual as fêmeas são atraídas por eles (Endler & Basolo 1998).

Dentre as hipóteses “zahavianas”, além da mais tradicional hipótese da desvantagem (Zahavi 1975), existe a hipótese da desvantagem de imunocompetência (Folstad & Karter 1992) e a hipótese do parasitismo (Hamilton & Zuk 1982). Conjuntamente, essas hipóteses são normalmente chamadas de “bons genes” e predizem que uma fêmea irá se beneficiar ao acasalar com um macho que possua uma melhor qualidade genética, já que sua prole também possuirá tais genes. Essas hipóteses também foram testadas em uma variedade de taxa, incluindo vertebrados e invertebrados (Lenington 1983; Gilburn & Day 1994; Moore 1994; Jia & Greendfield 1997; Weatherhead et al. 1997; Krokene et al. 1998; Strohbach et al. 1998; Lesna & Sabelis 1999; Roulin et al. 2000; Willis & Poulin 2000; Ditchkoff et al. 2001; Doty & Welch 2001; Landry et al. 2001; Watt, et al. 2001; Kozielska et al. 2004; Friedl & Klump 2005; Wedekind et al. 2008; Eizaguirre et al. 2009; Ilmonen et al. 2009) e parecem ser mais marcantes em aves (Møller & Alatalo 1999).

Adicionalmente, alguns modelos predizem que a escolha das fêmeas é adaptativa, mas os benefícios advindos dessa escolha não são genéticos (Rosenqvist & Johansson 1995; Saetre et al. 1995; Forsgren et al. 1996; Fedorka & Mousseau 2002; Hadfield et al. 2006; revisado por Møller & Jennions 2001). Essas hipóteses, normalmente chamadas de hipóteses do “benefício direto”, predizem que as fêmeas escolheriam um par que possua maior capacidade para prover recursos para seus filhotes (Hoelzer 1989). Embora benefícios diretos possam ser mais importantes para espécies com cuidado paternal, eles também podem se aplicar a outras espécies. Alguns autores propuseram que fêmeas podem sofrer com insuficiência espermática de machos (Wedell et al. 2002), então a quantidade de esperma que um macho tem para oferecer pode ser uma característica importante. Por exemplo, no peixe *Oryzias latipes*, o cortejo dos machos é um indicador honesto da fertilidade daquele indivíduo e pode ser utilizado pelas fêmeas durante a escolha de parceiros (Weir & Grant 2010).

Outra faceta menos explorada da teoria de seleção sexual é a possibilidade de machos também escolherem suas parceiras. Embora a ideia de que machos podem possuir certas preferências não seja nova (Trivers 1972), apenas recentemente estudos empíricos começaram a testar esta hipótese. Hoje já existem evidências de preferências sexuais por parte de machos em muitos grupos, incluindo aves (Amundsen 2000), peixes (Amundsen & Forsgren 2001; Baldauf et al. 2013) e aranhas (Riechert & Singer 1995; Bukowski & Christenson 1997; Bukowski et al. 2001; Hoefler 2007; Morse 2010; Senteská & Pekar 2013; MacLeod & Andrade 2014).

A maioria dos estudos na área de seleção sexual trata de preferências sexuais gerais, encontradas a nível populacional ou no nível de espécie. No entanto, muitos estudos recentes apontam que diferenças individuais ocorrem em diversos comportamentos e são comuns em diversos *taxa* (Sih et al. 2004a Sih et al. 2004b). O comportamento sexual, contudo foi menos estudado quanto à possibilidade de diferenças individuais (Schuett et al. 2010), e pouco se sabe sobre a variedade intraespecífica de preferências por diferentes parceiros.

As aranhas papa-moscas (Salticidae) são um grupo diverso de invertebrados com mais 5.950 espécies listadas no World Spider Catalog (http://www.wsc.nmbe.ch). Estes animais são bem conhecidos por suas cortes complexas, incluindo elementos visuais e vibracionais tanto por parte de machos como por parte das fêmeas (Richman 1982; Richman & Jackson 1992). Muitas espécies possuem coloração chamativa e dimorfismo sexual (Levi & Levi 1990), o que faz delas um bom modelo para estudos de comportamento sexual e escolha de parceiros.

A aranha *Hasarius adansoni* é muito comum em áreas urbanas dos trópicos e é facilmente encontrada em prédios e muros das cidades (Levi & Levi 1990). Esta espécie é sexualmente dimórfica, sendo que os machos adultos podem ser diferenciados das fêmeas por sua coloração negra e manchas brancas nos pedipalpos (Levi & Levi 1990; Figura 1). Os machos realizam um *display* sexual, aproximando-se da fêmea com o primeiro e segundo par de pernas abertos. Se a fêmea estiver receptiva, ela curva suas pernas e permanece imóvel até que o macho monte sobre ela (capítulo 1 desta tese). Os dados deste estudo indicam que as fêmeas apenas aceitam certo número de cópulas de um macho específico. Após chegar a este número, o macho ainda corteja e se aproxima, mas a fêmea foge ou ataca seu parceiro. Embora muito comum, especialmente na região neotropical, *H. adansoni* foi alvo de apenas um estudo comportamental até hoje. Cloudsley-Thompson (1949), descreveu brevemente o comportamento de corte da espécie, no entanto suas observações são anedóticas, com baixo tamanho amostral, em situação não controlada e sem registro de ovos férteis.

Tendo em vista a falta de estudos comportamentais em *H. adansoni*, o objetivo geral deste estudo é analisar o processo de seleção sexual nessa espécie. Especificamente, pretende-se descrever o comportamento sexual da espécie, testar as preferências gerais de machos e fêmeas no processo de escolha de parceiros, além de verificar a existência de uma possível variação individual destas preferências. Também pretende-se avaliar se o sistema de seleção sexual da espécie opera prevalentemente de uma maneira zahaviana, fisheriana e/ou por meio de benefícios diretos.

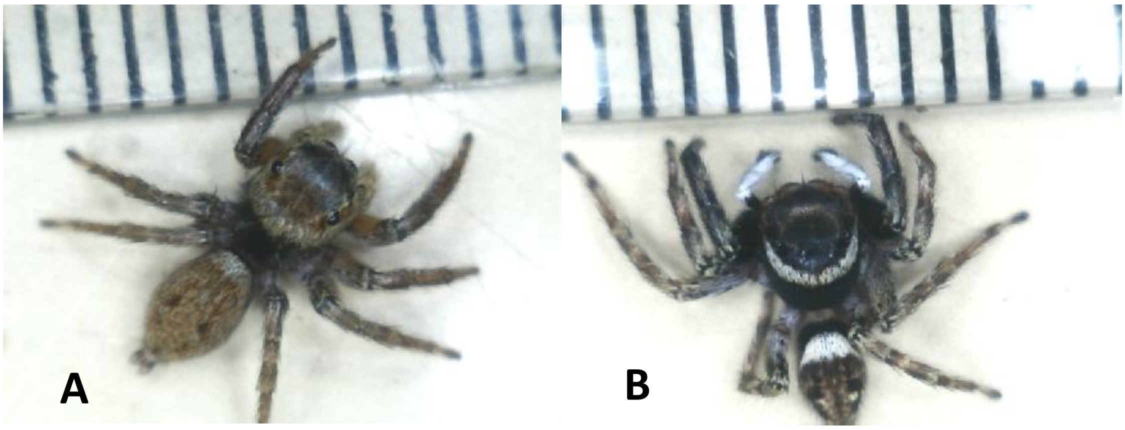

Figura 1- Dimorfismo sexual em *Hasarius adansoni*. A- Fêmea. B- Macho. A escala na imagem corresponde a 1mm.

Referências bibliográficas

Amundsen, T. 2000. Why are females birds ornamented? *Trends in Ecology and Evolution*, 15: 149-155.

Amundsen, T., Forsgren, E. 2001. Male mate choice selects for female coloration. *Proceedings of the National Academy of Science*, 98, 13155-13160.

Baldauf, S., Engqvist, L., Ottenheym, T., Bakker, T. C. M., Thünken, T. 2013. Sex-specific conditional mating preference in a cichlid fish: implications for sexual conflict. *Behavioral Ecology and Sociobiology*, 67, 1179-1186.

Basolo, A. L. 1995. Phylogenetic evidence for the role of a pre-existing bias in sexual selection. *Proceedings of the Royal Society of Londom B*, 259, 307-311.

Bateman, A. J. 1948. Intra-sexual selection in *Drosophila*. *Heredity*, 2, 349-368.

Bukowski, T. C., Christeton, T. E. 1997. Determinants of sperm release and storage in a spiny orbweaving spider. *Animal Behaviour*, 53, 381-395.

Burley, N. T., Symanski, R. 1998. "A taste for the beautiful": Latent aesthetic mate preferences for white crests in two species of Australian grassfinches. *The American Naturalist*, 152, 792-802.

Cloudsley-Thompson, J. L. 1949. Notes on Arachnida. 12. Mating habits of *Hasarius adansoni*. *Enthomologist Monthly Magazine*. 85, 261-262.

Darwin, C. R. 1871. *The descent of man, and selection in relation to sex*. London: Murray. Vol.1.

Ditchkoff, S. S., Lochmiller, R. L., Masters, R. E., Hoofer, S. R., Bussche, R. A. V. D. 2001. Major-histocompatibility-complex-associated variation in secondary sexual traits of white-tailed deer (*Odocoileus virginianus*): evidence for good genes advertisement. *Evolution*, 55, 616-625.

Doty, G. V & Welch, A. M. 2001. Advertisement call duration indicates good genes for offspring feeding rate in gray tree frogs (*Hyla versicolor*). *Behavioral Ecology and Sociobiology*. 49, 150– 156.

Eizaguirre, C., Yeates, S. E., Lenz, T. L., Kable, M., Milinski, M. 2009. MHC-based mate choice combines good genes and maintenance of MHC polymorphism. *Molecular Ecology*, 18, 3316-3329.

Endler, J. & A., Basolo, A. L. 1998. Sensory ecology, receiver bias and sexual selection. *Trends in Ecology and Evolution*, 13, 415-420.

Fedorka, M. K. & Mousseau, T. A. 2002. Material and genetic benefits of female multiple mating and polyandry. *Animal Behaviour*, 63, 1-7

Fisher, R. A. 1930. *The genetical theory of natural selection*. Oxford: Clarendon.

Folstad, I., Karter, A. J. 1992. Parasites, bright males, and the immunocompetence handicap. *The American Naturalist*, 139, 603-622.

Forsgren, E., Karlsson, A., Kvarnemo, C. 1996. Female sand gobies gain direct benefits by choosing males with eggs in their nests. *Behavioral Ecology and Sociobiology*, 39, 91-96.

Friedl, T. W., Klump, G. M. 2005. Sexual selection in the lek-breeding European treefrog: body size, chorus attendance, random mating and good genes. *Animal Behaviour,* 70, 1141-1154.

Gilburn, A. S. & Day, T. H. 1994. Evolution of female choice in seaweed flies : Fisherian and good genes mechanisms operate in different populations. *Proceedings of the Royal Society B*, 255, 159-165.

Hoefler, D. C. 2007. Male mate choice and size-assortative pairing in a jumping spider, *Phidippus clarus*. *Animal Behaviour*, 73, 943-954.

Hadfield, J. D., Burgess, M. D., Lord, A., Phillimore, A. B., Clegg, S. M., Owens, I. P. F. 2006. Direct versus indirect sexual selection: genetic basis of colour, size and recruitment in a wild bird. *Proceedings of the Royal Society of Londom B,* 273, 1347-1353.

Hamilton, W. D., Zuk, M. 1982. Heritable true fitness and bright birds: a role for parasites? *Science*, 218, 384-387.

Hoelzer, G. A. 1989. The good parent process of sexual selection. *Animal Behavior*, 38, 1067-1078.

Holland, B., Rrice, W. R. 1998. Perspective: Chase-away selection: Antagonistic seduction versus resistance. *Evolution*, 52, 1-7.

Huber, B. A. 2005. Sexual selection research on spiders: progress and biases. *Biological Reviews of the Cambridge Philosophical Society*, 80, 363–85.

Hunt, S., Cuthill, I. C., Swaddle, J. P., Bennet, A. T. D. 1997. Ultraviolet vision and band-colour preferences in female zebra finches, *Taeniopygia guttata*. *Animal Behaviour*, 54, 1383-1392.

Ilmonen, P., Stundner, G., Thoß, M., Penn, D. J. 2009. Females prefer the scent of outbred males : good-genes-as-heterozygosity? *BMC Evolutionary Biology* 10, 1–10.

Jia, F. & Greenfield, M. D. 1997. When are good genes good ? Variable outcomes of female choice in wax moths. *Proceedings of the Royal Society of Londom B,* 264, 1057-1063.

Kirkpatrick, M. 1982. Sexual selection and the evolution of female choice. *Evolution*, 36, 1-12.

Kozielska, M., Krzeminska, A., Radwan, J., Krzemin, A. 2004. Good genes and the maternal effects of polyandry on offspring reproductive success in the bulb mite. *Proceedings of the Royal Society of Londom B,* 271, 165-170.

Krokene, C., Rigstad, K., Dale, M., Lifjeld, J. T. 1998. The function of extrapair paternity in the blue tits and great tits: good genes or fertility insurance? *Behavioral Ecology*, 9, 649-656.

Lande, R. 1981. Models of speciation by sexual selection on polygenetic traits. *Proceedings of the National Academy of Sciences,* 78, 3721-3752.

Landry, C., Garant, D., Duchesne, P., Bernatchez, L. 2001. “Good genes as heterozygosity”: the major histocompatibility complex and mate choice in Atlantic salmon (*Salmo salar*). *Proceedings of the Royal Society of London B*, 268, 1279–85.

Lenington, S. 1983. Social preference for partners carrying 'good genes' in wild house mice. *Animal Behaviour*, 31, 325-333.

Lesna, I. & Sabelis, M. W. 1999. Diet-dependent female choice for males with “good genes” in a soil predatory mite. *Nature,* 401, 581–584.

Levi, H. W., Levi, L. R. 1990. *A golden guide: spiders and their kin. New York*: Golden Press.

MacLaren, D. R., Rowland, W. J. 2006. Female preference for male lateral projection area in the shortfin molly, *Poecilia mexicana*: Evidence for a pre-existing bias in sexual selection. *Ethology*, 112, 678–690.

MacLeod, E. C., Andrade, M. C. B. 2014. Strong, convergent male mate choice along two preference axes in field populations of black widow spiders. *Animal Behaviour*, 89, 163-169.

Madden, J. R., Tanner, K. 2003. Preferences for coloured bower decorations can be explained in a nonsexual context. *Animal Behaviour*, 65, 1077-1083.

Møller, A. P., Alatalo, R. V. 1999. Good-genes effects in sexual selection. *Proceedings of the Royal Society of Londom B,* 266, 85-91.

Møller, A. P., Jennions, M. D. 2001. How important are direct benefits of sexual selection? *Naturwissenschaften*, 88, 401-415.

Moore, A. J. 1994. Genetic evidence for the “good genes” process of sexual selection. *Behavioral Ecology and Sociobiology*, 35, 235–241.

Morse, D, H. 2010. Male mate choice and female response in relation to mating status and time since mating. *Behavioral Ecology*, 21, 250-256.

Rodd, F. H., Hughes, K. A, Grether, G. F., Baril, C. T. 2002. A possible non-sexual origin of mate preference: are male guppies mimicking fruit? *Proceedings of the Royal Society of London B*, 269, 475–81.

Rosenqvist, G. & Johansson, K. 1995. Male avoidance of parasitized females explained by direct benefits in a pipefish. *Animal Behaviour,* 49, 1039-1045.

Roulin, A., Jungi, T. W., Pfister, H., Dijkstra, C., Hedi, P. 2000. Female barn owls (*Tyto alba*) advertise good genes *Proceedings of the Royal Society of London B*, 267, 937-941.

Richman, D. B.1982. Epigamic display in jumping spiders (Araneae, Salticidae) and its use in systematics. *Journal of Arachnology*, 10, 47-67.

Richman, D. B. Jackson, R, R. 1992. A review of the ethology of jumping spiders (Araneae, Salticidae). *Bulletin of the British Arachnology Society*, 9, 33-37.

Riechert, S., Singer, F. D. 1995. Investigation of potential male mate choice in a monogamous spider. *Animal Behaviour*, 49, 715:723.

Ryan, M., Rand, S. A. 1990. The sensory basis of sexual selection for complex calls in the túngara frog, *Physalaemus pustulosus* (sexual selection for sensory exploration). *Evolution*, 44, 305-314.

Saetre, G. P., Fossness, T., Slagsvold, T. 1995. Food provisioning in the pied flycatcher: Do females gain direct benefits from choosing bright-coloured males? *Journal of Animal Ecology*, 64, 21-30.

Schuett, W., Tregenza, T., Dall, S. R. X. 2010. Sexual selection and animal personality. *Biological Reviews*, 85, 217-246.

Senteská, L, Pekar, S. 2013. Mate with the young, kill the old: reversed sexual cannibalism and male mate choice in the spider *Micaria sociabilis* (Aaraneae: Gnaphosidae). *Behavioral Ecology and Sociobiology*, 67, 1131-1139.

Sih, A., Bell, A., Johnson, C. 2004a. Behavioral syndromes: an ecological and evolutionary overview. *Trends in Ecology and Evolution*, 19, 372-378.

Sih, A. Bell, A., Johnson, C., Ziemba, R. E. 2004b. Behavioral syndromes: an integrative overview. *The Quarterly Review of Biology*, 79, 241-277.

Strohbach, S., Curio, E., Bathen, A., Epplen, J. T., Lubujhn, T. 1998. Extrapair paternity in the great tit (*Parus major*): a test of the "good genes" hypothesis. *Behavioral Ecology*, 9, 388-396.

Trivers, R.L. (1972). Parental investment and sexual selection. In: Sexual Selection and the Descent of Man (B. Campbell, ed.), pp. 136–179. Chicago, Aldine.

Watt, P. J., Shohet, A. J., Renshaw, K. 2001. Female choice for good genes and sex-biased broods in guppies. *Journal of Fish Biology*, 59, 843-850.

Weatherhead, P. J., Dufour, K. W., Lougheed, S. C., Eckert, C. G. 1997. A test of the good-genes-as-heterozygosity hypothesis using red-winged blackbirds. *Behavioral Ecology.* 10, 619– 625.

Wedekind, C., Evanno, G., Urbach, D., Jacob, A. A., Müller, R. 2008. "Good-genes" and "compatible-genes" effects in an Alpine whitefish and the information content of breeding tubercles over the course of the spawning season. *Genetica*, 132, 199–208.

Wedell, N., Gage, M. J. G., Parker, G. A. 2002. Sperm competition, male prudence and sperm-limited females. *Trends in Ecology and Evolution,* 17, 313-320.

Weir, L. L., Grant, J. W. A. 2010. Courtship rate signals fertility in an externally fertilizing fish. *Biology Letters,* 6, 727-731.

Willis, C. & Poulin, R. 2000. Preference of female rats for the odours of non-parasitised males : the smell of good genes. *Folia Parasitologica,* 47, 6–10.

World Spider Catalog. 2017. Dsiponível em: < http://www.wsc.nmbe.ch/statistics/>. Acesso em:13 de Jun de 2017.

Zahavi, A. 1975. Mate selection-A selection for a handicap. *Journal of Theoretical Biology*, 53, 205-214.

CAPÍTULO 1- DESCRIPTION OF THE REPRODUCTIVE BEHAVIOR OF THE JUMPING SPIDER *Hasarius adansoni* (ARANEAE: SALTICIDAE)

Este capítulo foi submetido para publicação na revista Journal of Arachnology, portanto está formatado de acordo com as normas da revista.

Abstract. Jumping spiders perform multi-modal displays during courtship and thus are considered important models for the study of sexual selection and mate choice. However, studies focus on only a few genera of jumping spiders. Here we describe for the first time the breeding behavior of the tropical jumping spider *Hasarius adansoni*. We collected individuals in the field and reared them in the laboratory until adulthood. Then, we took male body measurements, paired couples in mating trials, and collected subsequent egg batches and spiderlings. We confirmed the presence of a multi-modal display with visual and vibratory signals (tremulations) by the males. Females responded with their own tremulations and occasionally a receptive posture. Otherwise, they avoided mating by attacking or running away from the male. Multiple matings were common and females laid numerous batches of eggs while enclosed in silk cocoons. Number of young per batch decreased during laying bouts. No measured male morphological attributes were important for male mating success. Future studies should focus on other morphological and behavioral measurements to understand how mate choice process functions in this species.

Keywords: Multi-modal display, mate choice, fecundity, courtship, mating success, sexual selection, urban environment.

Salticid spiders have excellent vision (Levi & Levi 1990; Hill & Richman 2009) and most of their behaviors are visually guided (Richman & Jackson 1992). Courting behavior is no exception, and males from this family are known for performing extravagant visual and vibratory displays to attract females (e.g., Jackson & Macnab 1989; Hill & Richman 2009; Girard et al. 2011). In many species females respond to male courtship with their own display behaviors (Levi & Levi 1990). Recent work has shown that jumping spiders also produce vibratory (seismic) signals, and these are often complex and coordinated with visual displays (Foelix 2011; Elias et al. 2012). For these reasons, salticids are important models for studies of evolution of communication, including hypotheses about signal elaboration, multi-modal signals, and signal function across diverse habitats. Moreover, male sexual displays in salticids are important in speciation and can be key characters for taxonomic classification (Richman 1982; Masta & Maddison 2002). Richman (1982) presents a comprehensive description of the displays of species across genera, information that is critical for salticid systematics. However, as it is the case for many spider families, behavioral data are available for relatively few species, and there are entire genera with little or no information available. In salticids, behavioral studies are concentrated in the genera *Habronattus* and *Phidippus*, focusing mostly on breeding behavior (e.g., Sivalinghem 2010; Elias et al. 2012). This bias hampers studies of the evolutionary history of the family as a whole and precludes broad comparative analyses of signal evolution.

Here, we describe the mating behavior and sexual signals of *Hasarius adansoni* Audouin, 1826, a salticid that is common in urban environments throughout the tropics (Levi & Levi 1990). Despite its widespread distribution, (Levi & Levi 1990), this species has been the subject of only one behavioral study to date. Cloudsley-Thompson (1949) provided some descriptive notes about *H. adansoni* sexual behavior, including courtship displays, copulation and egg laying. However, this was based on a very small sample size, largely anecdotal observations, and since no viable eggs were produced it is unclear whether matings were successful. However, Cloudsley-Thompson's description suggests *H. adansoni* males produce visual signals, and this is also suggested by their sexually dimorphic coloration; while females are cryptic brown, males are black with conspicuous white patches on their palps (Levi & Levi 1990). Thus, the objective of this study is to describe in detail this species´ courtship displays, copulation and egg laying behaviors. Specimens of both sexes of *H. adansoni* are deposited in the arachnid collection of the Universidade de Brasília (UnB), Laboratório de Aracnídeos, collection number 4304.

METHODS

Rearing. -A total of 94 animals were used in mating experiments. We captured *H. adansoni* juveniles before their last instar on urban walls and buildings in Brasília, Brazil and brought them to the Laboratório de Comportamento Animal in Universidade de Brasília (UnB; 15°45'47.4"S, 47°52'14.3"W) where mating trials were conducted. One pair of spiders was transported to the University of Toronto Scarborough (43°47'1.47"N, 79°11'15.66"W) where vibratory signals were recorded. All animals were kept in glass containers measuring 9cm X 4.5cm in natural photoperiod and room temperature. Animals were fed every four to seven days. In each feeding episode individuals were given 10 to 15 adult *Drosophila* spp. and one *Gryllus* cricket nymph. We also fed the spiders on the day before conducting the breeding experiment described below.

Vibratory signals.-Since substrate-borne vibratory signals are common in salticid spiders (Foelix 2011), we used one pair of spiders to determine whether such signals occur for this species. The pair was placed together in a cylindrical mating arena

(11cm diameter and ~25cm height) on a turntable. The mating arena walls were made of transparent plastic and the arena substrate was made of stretched nylon, enabling easy vibration measurement. This arena has been previously shown to transmit salticid courtship signals (Elias et al. 2003). Laser Doppler vibrometry (LDV, PDV100 portable laser vibrometer, Polytec, Tustin CA, USA) was used to detect the occurrence of substrate vibrations during the pair's interactions. Three small pieces of reflective, lightweight tape (~1mm) were placed near the center of the nylon-covered turntable and used as measurement points for the laser. Laser output was fed through a speaker to allow real-time audio monitoring of vibratory signals. Simultaneously, the pair was filmed using a digital high-speed camera (500 frames s-1; PCI 1000; RedLake Motionscope, San Diego, CA, USA) while the spiders were illuminated with a Frezzi Minifill light. For this exploratory analysis, we monitored the high speed video while listening to the LDV output to determine candidate body movements that might generate vibratory signals (e.g., Elias et al. 2012).

Mating trials.-A total of 47 mating trials were recorded on digital video during the experiments, and males and females were used only once. For the remainder of the mating experiments the arena consisted of a square acrylic container (13cm x 13cm x 4cm) with two opaque dividers that allowed two spiders to be held simultaneously without visual contact. The container also had niches in the four corners where spiders could avoid each other. For every trial, one male and one female were held inside the arena but kept apart by the opaque dividers, which were simultaneously opened to start the experiment after a 1-h acclimatization period (Fig. 1). Each pair was videotaped for 3h (Kodak Zx1 Pocket Video Camera) and all experiments were conducted under a natural light-simulating lamp (Arcadia Bird lamp. Model FB 36).

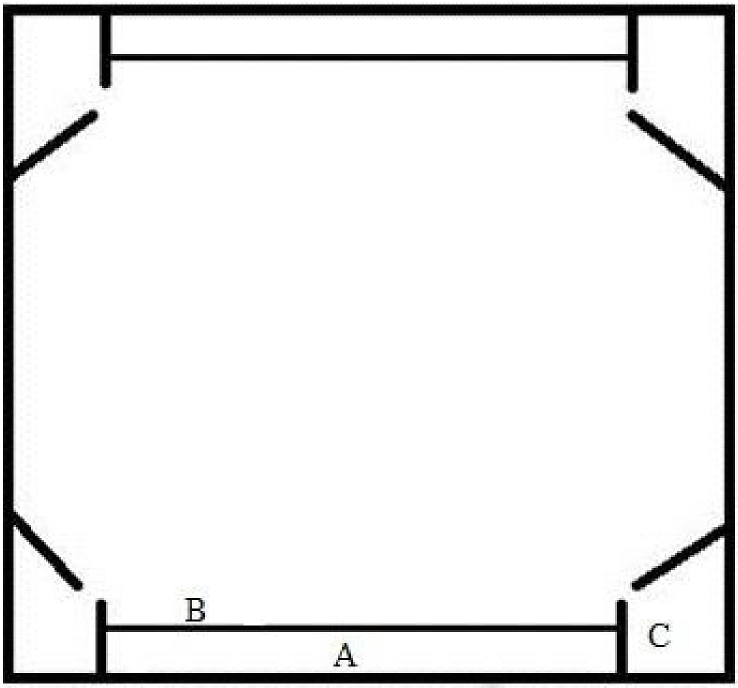

Figure 1- Upper view of the mating arena used for mating experimentos of *Hasarius adansoni*. A: Compartment to hold spiders; B: Opaque doors that can be opened; C: Refuge.

The videos were then analyzed to develop an ethogram of the three stages of breeding for both males and females: (i) pre-copulation display and response; (ii) copulation behavior; and (iii) post-copulation behavior (i.e., egg-laying behavior). Below we describe the behavioral repertoires, time spent in each of these phases and number of eggs and young produced by *H. adansoni*.

Measurements.-Before every trial, males were anesthetized and weighted to the nearest 0.001g. After every trial, males were measured, and then sacrificed and their palps and front legs (used in the courtship, see below) removed for measurement. Anesthesia was done by putting males inside a refrigerator (approximately 4°C) for a couple minutes until they stopped moving. Sacrifice was done similarly, but males were let inside the refrigerator overnight. Carapace width was used as a measure of animal size. Palps and legs were photographed and with ImageJ, we measured leg length, white patch area and percentage of palp covered with the white patch area. To summarize male morphology, cephalothorax width, legs length, male mass, white patch area and percentage of white patch cover were entered in a Principal Component Analysis (PCA). We assessed whether any morphological traits predicted mating success, using regression analysis including mating success as response variable, along with male morphology (as predicted by the PCA) and male condition as predictive variables. We considered any variable indicating that a male is particularly attractive to a female as an indicative of mating success (e.g., occurrence of copulations, total copulation time, see below). Total time of copulations was used as response variable in non-parametric correlation analysis, since these data were highly overdispersed. We assessed if number of copulations predicted probability of laying viable eggs through a linear model with binomial error distribution. Similar models were created to assess the influence of male size, white percentage of white patch cover and male condition on probability of copulation. The influence of male size, white patch cover and condition on number of copulations were assessed through linear models with negative binomial error distributions, to correct for excessive data variation. We also assessed if number of spiderlings changed over subsequent batch of eggs and this was done through a mixed-model, entering number of young as response variable, egg batch number as a predictor and female identity as a random factor. Male condition was calculated as the residuals of the regression between male weight and male cephalothorax width, as proposed by Jakob et al. (1996). Results are presented as mean ± standard deviation.

RESULTS

Mating trials.-We had nine trials in which animals did not see each other, and were considered unsuccessful and thus excluded from further analysis. Among our successful trials, 60.5% resulted in copulations. Among those that did not result in copulations, only four were because males did not attempt copulation and one was because the female cannibalized the male. The other 10 were because females did not accept males (see description below).

When the male orients and moves towards the female, he typically spreads the first pair of legs and his palps (33/38 successful trials). Given the location of the white patches, this would reveal them to a female oriented towards him. The male then walks towards the female in a zig-zag fashion. Here, the female may respond in two ways: (i) facilitate palp insertion by curling her legs close to her abdomen and staying motionless, or (ii) avoid palp insertion, by running away or attacking the male. If the first option happens (23/38 successful trials), the male can approach and mount the female, and she then exposes the side of her abdomen and this facilitates palp insertion. Palps are not inserted simultaneously, thus each insertion was counted as a separate copulation. Mean palp insertion duration was 22.96 ± 14.86 s. Pairs that copulated did so an average of 5.82 times (min = 1; max = 18). Multiple copulations were separated by a period of other behaviors, such as wandering around the arena, self-grooming, and many times, spiders lost visual contact with each other. Usually, males continued courting and mounting the female multiple times until she stopped adopting the receptive posture. Once this happened, females frequently adopted the second possible response to courtship (i.e., attacking or running away from the male). Cannibalism of the male by the female was extremely rare, and was observed only once in our 47 trials.

Vibratory signals.-We confirmed the presence of substrate-borne vibrations during courtship from both male and female. These appeared to be primarily tremulations, a type of substrate-borne vibration signal in which a part of the spider’s body vibrates but does not touch the substrate. The energy of such vibrations, however, is transferred to the substrate by the spider's legs and allows communication (Uhl & Elias 2011).

In this exploratory trial, when the male started moving towards the female, he used tremulation of the abdomen to create vibrations that were detected by the LDV and probably also by the female. Once in the receptive posture (i.e., legs curled), the female started her own tremulation as the male approached.

Egg laying.-An average of 36.25 ± 29.92 days after mating, females build a silk cocoon and stay enclosed for an average of 21.21 ± 12.1 days while laying eggs. Usually, after the female leaves the cocoon, the young molt for the first time and only then do they disperse. Among the females that mated, 69.5% (n = 23) laid viable eggs. Considering just the females that copulated, the number of copulations did not predict the likelihood of laying viable eggs (Binomial Model: $\beta = 0.054$; $P = 0.567$).

Mated females laid between zero and nine batches of eggs (mean = 3.13) after mating. The number of young per batch of eggs varied from zero (eggs failed to hatch) to 41. The number of young per batch decreased over the laying bout for each female (Fig. 2; $\beta = -1.87$; $p < 0.01$).

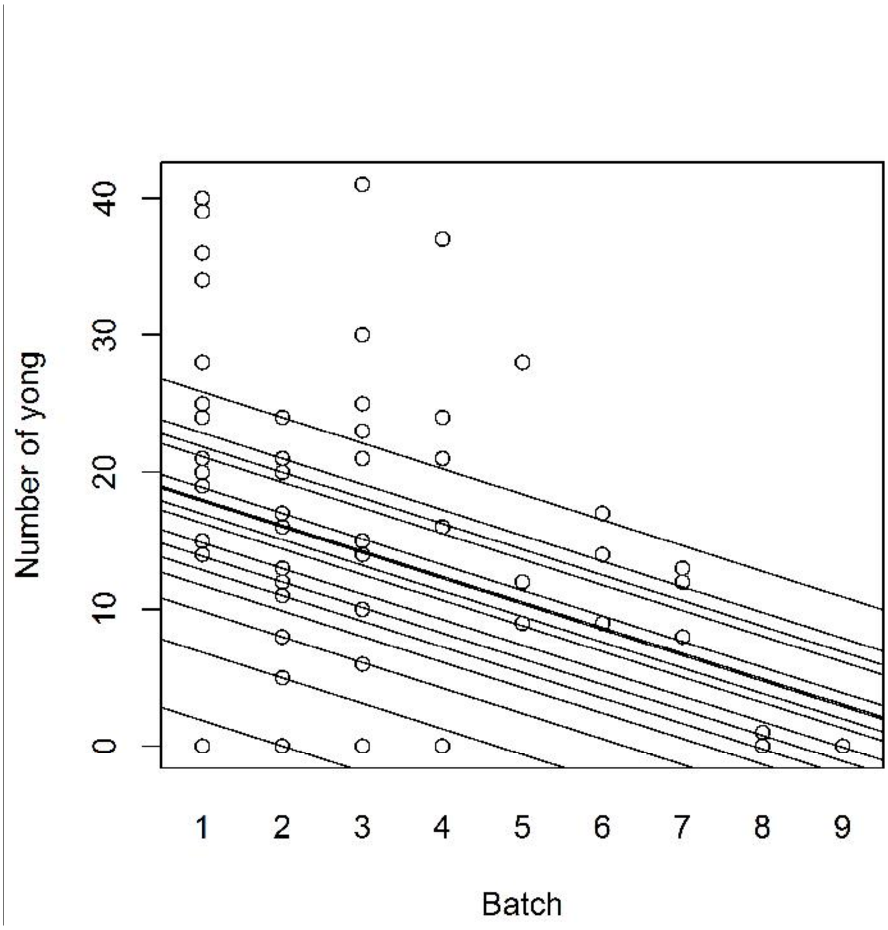

Figure 2- Number of young per batch of eggs and number of batches laid by females *Hasarius adansoni*. Each line represents a different female. Slopes and intercepts were calculated with a mixed model, entering number of young as a response variable, batch of eggs as predictor variable and female identity as a random factor.

Morphology and mating success.-The first principal component of the PCA for male morphology explained 63.9% of the total variance in the traits measured and was highly correlated with legs, cephalothorax width, and mass; and moderately correlated to white patch area. The second principal component explained another 23.28% of the variance and was highly correlated to percentage of white patch cover and also moderately correlated to white patch area (Table 1). This shows that the variance in white patch area is partly associated to both body size and partly to percentage of cover. Thus, we used the first principal component as a measure of body size and white patch size and the raw values of percentage of white patch cover in subsequent regression models.

Table 1- Correlations (r) between raw variables of male *Hasarius adansoni* size and weight measurements and the six components from the PCA. White patch area corresponds to the area of the patch present in male pedipalps. Percentage of white patch cover corresponds to the proportion of the total pedipal area covered by the white patch.

	Principal Components					
Variables	PC1	PC2	PC3	PC4	PC5	PC6
Weight	0.93	-0.14	0.04	0.13	0.3	0.04
Cephalotorax width	0.89	-0.12	0.22	0.31	-0.18	-0.03
Lenght of keg 1	0.93	-0.20	-0.20	-0.16	0.003	-0.15
Lenght of leg 2	0.93	-0.15	-0.21	-0.13	-0.12	0.14
White patch area	0.62	0.66	0.31	-0.27	-0.00	0.004
% of white patch cover	0.17	0.93	-0.26	0.20	0.002	-0.01

Among the females that copulated, number of copulations was not predicted by male size or percentage of white patch cover (Negative Binomial Model; PC1: β = -0.29, p= 0.18; white cover: β = 1.44; *P* = 0.58). Among these females, number of copulations also did not correlate with male condition (Negative Binomial Model; Condition: β = 56.52; p= 0.43).

The probability of copulation was not predicted by male size or percentage of patch cover (Binomial Model; PC1: β = 0.47, *P* = 0.26; Percentage of white cover: β = -5.4, *P* = 0.43). Furthermore, male condition and probability of copulation were not correlated (Binomial Model; Condition: β = -82.55, *P* = 0.44).

Total copulation time did not correlate with any of the predictor variables (PC1: Spearman's ρ = 0.15, *P* = 0.47; Percentage of patch cover: Spearman's ρ = -0.15, *P* = 0.45; Condition: Spearman's ρ = 0.11, *P* = 0.52).

DISCUSSION

Jumping spiders produce relatively intricate displays (Richman & Jackson 1992) and our observations show complex, multimodal displays are also a feature of mating in *H. adansoni*, with males tremulating during approach, and females responding with tremulation in turn. We found high levels of prolonged courtship by male *H. adansoni*, and clear receptivity postures among females. Remating was common within pairs that mated. Among breeding females, the first batch of eggs typically had the most offspring, and this number declined with subsequent batches. In every batch, females usually stay enclosed for a while guarding eggs inside a dense silk cocoon, a behavior considered widespread in salticids (Richman & Jackson 1992). Surprisingly, despite a high frequency of mate rejection (11/38 pairings), we could detect no relationships between male body size and condition, or the white patch on male palps and any of our measures of mating success. Notwithstanding these results, it remains clear that this species may be useful to test hypotheses about mating behavior and sexual selection, given the combination of visual and vibrational parameters in the male displays, and the different behavioral and vibrational responses from females.

The features that compose the visual display in *H. adansoni* (i.e., leg spreading, zig-zag walking and palp spreading) have been observed in other salticid species (Richman 1982), and in *H. adansoni* by Cloudsley-Thompson (1949). Similarly, substrate-borne vibrations have also been observed during courtship in many Salticidae, although the type of vibrations and repertory size vary substantially (Elias et al. 2003, 2005, 2010, 2012; Sivalinghen et al. 2010; Girard et al. 2011). Such conspicuous traits and displays usually play a role in sexual selection and mate choice (Andersson 1994). Both visual (Huber 2005; Uhl & Elias 2011), and vibratory displays (Elias et al. 2004, 2005, 2006, 2010; Sivalinghen et al. 2010) are used by female jumping spiders to assess potential males for mating during courtship. These display characteristics typically convey male condition, which may influence brood survival and success (Uhl & Elias 2011). For another salticid species, *Habronattus pyrrithrix*, male coloration is related to diet (Taylor et al. 2011), and possibly to female choice. Seismic signals are also important in female choice in the same genus (Elias et al. 2004, 2005). In *Phiddippus*, another well studied genus, vibration is also important for female mate choice (Sivalinghem 2010). In contrast to these results, in *H. adansoni*, no morphological character we measured, nor the white patch area or percent of white coverage were related to female response. However, we found that *H. adansoni* also exhibits seismic signals that might be important in sexual selection, but these have not yet been explored. Moreover, although white patch area does not predict female choice, it is possible that colorimetric variables, such as reflectance in different wave lengths, play a role in sexual selection. Finally, for such multi-modal signals, it may be a combination of traits that is critical for female preference (see Girard et al 2015).

Most of the pairs that failed to copulate did so because of female rejection. Remating, as observed here, has been reported in other jumping spiders (Jackson & Macnab 1989a; Jackson & Macnab 1989b). Females usually determine the end of remating by not accepting further attempts by a particular male. Long copulation durations have been suggested as a strategy of mate guarding in other spiders. Since monogamy is rare in spiders (Schneider & Andrade 2011), and first sperm priority is common (Huber 2005), males may try to prolong copulations (Huber 2005; see also Drengsgaard & Toft 1999), which may partly explain the high copulation rates observed in this study, if multiple mating is a way to prolong the whole process of copulation. In the field, males and females have territories with very little overlap (personal observation), which may select for both sexes to engage in copulation multiple times and for long durations if the encounter rates are low in natural populations.

This is the first study to describe the sexual behavior of *H. adansoni* in detail and, as expected for a jumping spider, male courtship was complex and involved multimodal features. Morphological traits did not predict male mating success, and future work should focus on the vibratory display and reflectance of the white patch to fully understand female mate choice in this species.

LITERATURE CITED

Andersson, M. 1994. Sexual Selection. New Jersey, Princeton University Press.

Cloudsley-Thompson, J.L. 1949. Notes on Arachnida. 12. Mating habits of *Hasarius adansoni*. Entomologist Monthly Magazine 85: 261-262.

Drengsgaard, I.L. & S. Toft. 1999. Sperm competition in a nuptial feeding spider, *Pisauria mirabilis*. Behaviour 136: 877-897.

Elias, D.O., A.C. Mason, W.P. Maddison & R.R. Hoy. 2003. Seismic signals in a courting male jumping spider (Araneae: Salticidae). The Journal of Experimental Biology 206: 4029-4039.

Elias, O.D., A.C. Mason & R.R. Hoy. 2004. The effect of substrate on the efficacy of seismic courtship signal transmission in the jumping spider *Habronattus dossenus* (Araneae: Salticidae). Journal of Experimental Biology 207: 4105-4110.

Elias, O.D., E.A. Hebets, R.R. Hoy & A.C. Mason. 2005. Seismic signals are crucial for male mating success in a visual specialist jumping spider (Araneae: Salticidae). Animal Behaviour 69; 931-938.

Elias, O.D., E.A. Hebets & R.R. Hoy. 2006. Female preference for complex/novel signals in a spider. Behavioral Ecology 17: 765-771.

Elias, O.D., S. Sivalinghem, A.C. Mason, M.C.B. Andrade & M. M. Kasumovic. 2010. Vibratory communication in the jumping spider *Phidippus clarus*: Substrate-borne courtship signals are important for male mating success. Ethology 116: 990 -998.

Elias, D.O., W.P. Maddison, C. Peckmezian, M.B. Girard & A. Mason. 2012. Orchestrating the score: complex multimodal courtship in the *Habronattus coecatus* group of *Haborattus* jumping spiders (Araneae: Salticidae). Biological Journal of the Linnean Society 105: 552-547.

Foelix, R.F. 2001. Biology of Spiders. 3rd ed. New York. Oxford University Press.

Girard, M.B. & M.M. Kasumovic, D.O. Elias. 2011. Multi-model courtship in the peacock spider, *Maratus volans* (O.P.-Cambridge, 1874). PLoS One 6: 1-10.

Hill, D.E. & D.B. Richman. 2009. The evolution of jumping spiders (Araneae: Slaticidae): a review. Pekhamia 75: 1-7

Huber, B.A. 2005. Sexual selection research on spiders: progress and biases. Biological Reviews of the Cambridge Philosophical Society 80: 363-385.

Jakob, M.E., S.D. Marshall & G.W. Uetz, 1996. Estimating fitness: A comparison of body condition indeces. Oikos 77 61-67.

Jackson, R.R. & A.M. Macnab. 1989a. Display behaviour of *Corythalia canosa* , an ant-eating jumping spider (Araneae: Salticidae) from Florida. New Zealand Journal of Zoology 16: 169-183.

Jackson, R.R. & A.M. Macnab. 1989b. Display, mating, and predatory behaviour of the jumping spider *Plexippus paykulli* (Araneae: Salticidae). New Zealand Journal of Zoology 16: 151-168.

Levi, H.W. & L.R. Levi. 1990. A Golden Guide: Spiders and Their Kin. New York, Golden Press.

Masta, S.E. & W.P. Maddinson. 2002. Sexual selection driving diversification in jumping spiders. Proceedings of the National Academy of Sciences of the United States of America 99: 4442-4447.

Richman, D.B.1982. Epigamic display in jumping spiders (Araneae, Salticidae) and its use in systematics. Journal of Arachnology 10: 47-67.

Richman, D.B. & R.R. Jackson. 1992. A review of the ethology of jumping spiders (Araneae, Salticidae). Bulletin of the British Arachnology Society 9: 33-37.

Schneider, J. & M. Andrade. 2011. Mating behaviour and sexual selection. Pp. 215-274. *In* Spider Behavior: Flexibility and Versatility. (M. E. Herberstein, ed.). New York, Cambridge University Press.

Sivalinghem, S.M.M. Kasumovic, A.C. Manson, C.B. Andrade & D.O. Elias. 2010. Vibratory communication in the jumping spider *Phidippus clarus*: polyandry, male courtship signals, and mating success. Behavioral Ecology 21: 1308-1314.

Taylor, L.A., D.L. Clark & K.J. McGraw. 2011. Condition dependence of male display coloration in a jumping spider (*Habronattus pyrrithrix*). Behavioral Ecology and Sociobiology 65: 1113-1146.

Uhl, G. & D.O. Elias. 2011 Communication. Pp. 127-189. 2011. Spider Behavior:

Flexibility and Versatility (E. M. Herberstein, ed.). New York. Cambridge University Press.

CAPÍTULO 2-SEXUAL PREFERENCES AND THEIR BENEFITS IN THE JUMPING SPIDER *Hasarius adansoni*

Abstract

Sexual selection predicts that individuals will choose their partners to enhance their own reproductive output. Sexual selection and mate choice have been shown to be ubiquitous in animal taxa and may occur in both males and females. We assessed the sexual selection process in both sexes of the tropical jumping spider *Hasarius adansoni*. We measured mate choosiness in males and females and determined how adult quality influences offspring quality and survival. For males, we also estimated the number of sperm cells per microliter of sperm for both, unused and used palps. Number of sperm cells in the unused palp was used as a measure of the amount of resources available for usage (i.e. amount of sperm available) and the difference between the number of sperm cells in the unused and used palps was a measure of the amount of resources deposited in a particular female. We found no evidence of mate choice in either sex, nor any relationship between adult quality and offspring quality or survival. For males, we found strong evidence that the amount of resources invested in a particular female is a function of the amount of sperm the male had available for usage, and this is likely explained by the fact that males invest less in reproduction. Different individual preference functions may explain the lack of a population level preference for females. In this case, individual females would differ as to which male is preferred and/or to what extent he is preferred.

Key words: Mate choice, preference functions, sexual preference, Salticidae

Introduction

Sexual selection models predict that animals should not mate at random (Andersson 1994). As the sex that usually invests more in reproduction, females are usually referred to as the ‘choosy sex’ and, according to a number of theoretical models, are expected to mate non-randomly with males that will enhance their reproductive output (Bateman 1948; Andersson 1982). However, the mechanisms by which choice affects fitness may vary and are usually divided into direct and indirect benefits. Direct benefits are non-genetic benefits and include male parental care (Hoelzer 1989); higher male fecundity (Dewsbury 1982; Wedell et al. 2002); and better territory, gift or nest quality (Andersson & Iwasa 1996). Indirect benefits are genetic benefits that may enhance offspring fitness, by ensuring higher offspring attractiveness (‘sexy sons’; Fisher 1930); higher predation escaping capacity (Zahavi 1975); lower parasite contamination (Hamilton & Zuk 1982); or higher offspring immunocompetence (Folstad & Karter 1992). The amount of evidence supporting non-random mating by females is remarkable and encompasses virtually all main taxonomic groups (Andersson 1982; Kirkpatrick 1982; Møller & Alatalo 1999; Schantz et al. 1999; Candolin 2003; Cotton et al. 2006; Kokko et al. 2006; Ronald et al. 2012).

Empirical studies focusing on male choosiness, on the other hand, are much less common. It has only been recently that this topic has been more intensively examined, resulting in growing evidence that male choosiness occurs in certain contexts. Male choosiness has been found in birds (e.g.: Jones et al. 2001, reviewed by Amundsen 2000); fishes (Amundsen & Forsgren 2001; Baldauf et al. 2013) and spiders (Riechert & Singer 1995; Bukowski & Christenson 1997; Bukowski et al. 2001; Hoefler 2007; Morse 2010; Senteská & Pekar 2013; MacLeod & Andrade 2014). Although pioneer models predicted that male choosiness should be more important when paternal care is high (Trivers 1972), many modern models and empirical research predict male choosiness in other circumstances. Males are expected to be choosy when females vary in quality, when investment in reproduction (e.g, secondary sexual traits) is high and when females are encountered simultaneously (Edward & Chapman 2011; Barry & Kokko 2010; Nandy et al. 2012). In spiders, males usually select females based on size, age or sexual history (i.e.: virgin versus mated females), since those characters are related to female fecundity (Riechert & Singer 1995; Bukowski & Christenson 1997; Bukowski et al. 2001; Elgar et al. 2003; Gaskett et al. 2004; Hoefler 2007; Morse 2010; Senteská & Pekar 2013; MacLeod & Andrade 2014). In cannibalistic species, males may also prefer females that yield less risk of being cannibalized (e.g.: Pruitt & Riechert 2009).

Jumping spiders (Salticidae) are good models to test many sexual selection hypotheses. Males are typically bright colored and perform extravagant displays to attract females, which may respond with their own behaviors (Richman 1982; Levi & Levi 1990; Richman & Jackson 1992). Such characteristics, along with facility in capturing and maintaining these animals in the laboratory allow the development of mate choice experiments in controlled environments to assess different aspects of sexual selection and mate choice. The jumping spider *Hasarius adansoni* is a tropical species, commonly found in urban areas of South America (Levi & Levi 1999). Males are black with a conspicuous white patch area on their palps, exhibited to females during displays. Sexual displays consist of approaching the female while spreading the first pair of legs, showing the white patch area on the palps and simultaneously performing abdominal vibrations. If receptive, females will curl their legs, stay motionless and vibrate their abdomens (this thesis). Although common in the tropical region, behavioral studies of

H. adansoni are extremely rare. There is only one study concerning reproduction in this species, and it is mostly descriptive (Cloudsley-Thompson 1949).

The objective of this study was to assess the mate choice process in *H. adansoni*. Specifically, we asked if choosiness occur in the process of mating, exercised by either males or females, and tested this in controlled mating experiments. Since males *H. adansoni* invest in reproduction, through secondary sexual characters and courtship displays, which have been shown to be costly in spiders (Edward & Chapman 2011; Kotiaho 2001), we predicted that males would show some degree of mate choice. We also tested if offspring quality was related to any adult quality-indicator trait in both, males and females. This is the first time mate choice is explicitly tested in this tropical spider.

Methods

Capture and handling

Animals were captured in the urban environment around the city of Brasília, in central Brazil (-15° 46' 47" N; -47° 55' 47" W). Spiders were brought to the Laboratório de Comportamento Animal at the Universidade de Brasília main campus. Spiders were maintained in glass vials (9cm X 4.5cm) with a piece of wet cotton, and were fed with 15 adult *Drosophila* spp. and one *Gryllus* sp. cricket nymph every four to seven days. A pilot study showed that non-virgin females may decrease their acceptance to courting males, thus we only used virgin females in the mating trials. We were able to distinguish between adult and non-adult females by the presence of a visible epigynum, assessed under a stereomicroscope. Females captured as adults were not considered virgins and were discarded. Males and females captured as non-adults were reared until adulthood and experiments were conducted only with adult, sexually mature animals.

Morphological measurements

Before every mating trial, animals were put inside a small plastic vial which was in contact with ice. This anesthetized spiders within a few minutes and allowed us to weight them to the nearest 0.001g with a precision balance and also to photograph animals with a digital stereomicroscope to have their carapace width measured to the nearest 0.001mm with the ImageJ software. After this procedure, animals were moving normally and feeding within few minutes. We considered that quality of the spiders can be assessed through their size and weight, through a variable we call condition. Individual condition was estimated using the residuals of a regression of animal weight on animal size (Jakob et al. 1996). In spiders, well-fed animals are usually larger, heavier and in better condition, and this affects their ability to court and fight over mates (e.g.: Elgar & Fahey 1996; Kasumovic & Andrade 2006; Hoefler et al. 2008; Kasumovic et al 2009).

After mating trials, males were sacrificed to allow access to their palps. Palps were also photographed and palp and the white patch size area were measured (to the nearest 0.001mm^2), allowing an estimate of the percentage of the palp area covered by the white patch. White patch area was correlated with body size (R=0.38; p=0.039) and with percentage of white patch cover (R=0,49; p=0.005). However, percentage of white patch cover was not related to body size (R=-0.18; p=0.35). This shows that percentage of white patch cover is a measure of white patch size controlled for body size. Then, we used male size and percentage of patch cover in subsequent models. All palp measurements were taken using the ImageJ software.

Sperm counting

Males also had the amount of sperm in the palps counted. For this, males were sacrificed, their palps detached and then preserved in 100% ethanol in ependorff tubes until the day of counting. For counting, palps were crushed with plastic pestles in a mixture of 75 μL of spider salin and triton-X detergent. Spider saline consisted of a mixture of 3.26g of NaCl; 0.13g of KCl; 0.30g of $CaCl_2 + 2H_2O$; 0.26g of $MgCl_2 + 6H_2O$ in 250ml of distilled water. Tris-HCl was used to raise the pH of spider saline to an ideal value of 8.2. Once crushed inside this mixture, palps were centrifuged at 4000 RPM for 10 min, three times. After every centrifuge trial (total of three for each palp), we vortexed palps for 30s. After this procedure, we took 10μL from the ependorff solution and placed on a hemocytometer for counting. We only counted sperm cells located in the 64 large squares of the hemocytometer. The number of sperm cells per μL of sperm was calculated using the standard formula for counting cells in the hemocytometer. In our case:

$$x = {}^{(75 \times C)}/_{0.4}$$

in which x is the number of sperm cells per microliter of sperm and C is the number of cells counted.

Spider sperm cells usually clump together (Herberstein et al. 2011), so a useful method for counting sperm cells should allow separation of cells. We rarely found any sperm clumps, and when that occurred, sperm cell number was low enough to ensure easy counting. A more detailed description of the methodology we used is available in Gable & Uhl (2013) and in Snow & Andrade (2004).

Mating trials

Mating trials took place in a mating arena, which consisted of an acrylic square box, measuring (13cm X 13cm X 4cm). The arena had two opaque dividers, which allowed holding two animals at the same time without visual contact.

In each trial, animals were placed inside the arena and separated by the dividers for 1h (acclimatization). After this period, the dividers were opened and the animals were allowed to see and interact with each other for a period of 3h. Each trial had one focal animal and one non-focal animal. We videotaped every trial and, from the videos and further analysis, we extracted variables indicating mating receptivity by the focal animal. When the focal animal was a male, the receptivity variables included: (i) number of attempted copulations (e.g.: walking towards the female performing courtship and touching her with front legs and palps); (ii) percentage of encounters that led to sexual display and (iii) the amount of sperm transferred to the female (see below). When the focal animal was a female, the variables collected were: (i) number of copulations; (ii) total copulation time; (iii) number of unreceptive behaviors (i.e. attacking the male, running away from the male or not adopting receptive posture when in front of a displaying male) and (iv) percentage of copulation attempts by the male that were unsuccessful. These variables were chosen based on our observations indicating that, in *H. adansoni*, no copulations occur if the male does not court; but females control the total number of copulations.

Whenever the focal animal was a male, we anesthetized males before the trial with ice and cut off one of his palps (hereafter: unused palp). Unused palps were cut off at the base of the bulb, so the white patch, used in courtship and possibly sexually selected, was intact. After the trial, males were sacrificed and we cut off the other palp (used palp). Since sexual plugs do not happen in this species, we had full access to used palps, which were kept with the male. The difference between the concentration of sperm in the unused and used palps was considered a measure of the amount of sperm transferred to the female´s reproductive tract. We also counted the sperm in both palps of 33 random males without performing mating trials. This was done to assess any asymmetry on the number of sperm between right and left palps.

The amount of sperm between two palps of the same male that has not been part of any copulation experiment was correlated (R=0.6; p=0.0002; N=33). Moreover, there was no difference in the amount of sperm in both palps (paired t-test: t=0.13; p=0.89). This shows that there is no asymmetry between the two palps and ensures that the difference of sperm between palps can be used as a measure of sperm deposited in females.

Before each trial, the arena was cleaned with soap, water and alcohol to remove any hormone traits that might have been left behind by previous animals. Since sexual ornaments of some jumping spiders reflect light in the ultraviolet spectrum (e.g.: Bulbert et al. 2015), we conducted experiments under a natural light simulating lamp (Arcadia Bird lamp. Model FB 36). Trials in which it was clear that animals did not see each other were excluded from analysis.

Offspring quality

After mating trials, females were kept in the same conditions as described above until they produced eggs. The number of egg sacs per female was counted, and after hatching and leaving the egg sac, the number of offspring per egg sac was also counted. After spiderlings emerged from the egg sac, they were kept individually in small glass vials (~5cm X 1.5cm). Vials were empty and with no wet cotton, since such excessive moisture usually killed young spiders in pilot studies. Half of each brood was left unfed inside the vials until they died, providing us with the data to conduct survival analysis (Cox-Proportional Hazard Model, described below), and the other half was used in a feeding performance trial (see below).

Since newly dispersed jumping spiders usually rely on their own preying ability to survive (Richman & Jackson 1992), we measured feeding performance as another young quality indicator. We created a protocol to measure feeding performance in young jumping spiders, using a model prey small enough to be easily captured by newly born jumping spiders. Feeding performance trials consisted of placing individual spiderlings in a petri dish with a single live springtail (Collembola) as a model prey. We replaced the springtail if it died before being captured by the spiderling. Since static prey does not attract the spider, we kept the springtails moving by touching them with a paintbrush every time they stopped moving. We also kept the spider inside the petri dish by gently pushing it back inside with a paintbrush every time it tried to escape. Such protocol usually ended with the springtail being captured and eaten by the spider. Each feeding trial was videotaped and the following variables were extracted from the videos as measurements of feeding performance: (i) latency to start moving towards prey, once oriented towards it; and (ii) speed while approaching prey (in mm/s). Our three springtail populations were obtained from an independent seller in Toronto, Canada.

Statistical analyses

Principal component analyses (PCA) were used to reduce data dimensionality. Female preference variables (i.e.: number of copulations; total copulation time; unreceptive behaviors and percentage of rejections) and offspring feeding performance variables (i.e.: latency to move towards prey, speed) along with egg sac number (to control for any early versus late brood effect) were reduced by PCA analysis. The PCA for male mate choice could not effectively reduce the dimensionality of the data, so we ran one separate model for each response variable.

The PCA of female mate choice resulted in a first principal component heavily and positively loaded on number of copulations and total copulation time; and negatively loaded on percentage of copulation attempts that failed. The second component loaded heavily only on unreceptive behaviors. Together, both components explained 86% of total variance (Table 1). The PCA with offspring feeding performance variables yielded a first principal component heavily and positively loaded with brood number and heavily and negatively loaded with speed. The second component only loaded heavily (and positively) with latency to start moving towards prey (Table 2). The two principal components explained a total of 78% of total variance.

General linear models were used to test mate choice, with variables of choice as response variables and quality variables of partners as predictors. For female mate choice, we used the first principal component as response variable in one model and the number of unreceptive behaviors in another model. Predictors were male weight, condition and percentage of white patch cover. For males mate choice, we ran one separate model for each response variable: number of copulation attempts, percentage of visualizations that led to courtship and amount of sperm transferred. For all models, predictors were female size and condition. Amount of sperm in the unused palp was used as a covariate in all models for male choice, to control for the amount of sperm cells available for usage before mating.

A Cox proportional hazard model was used to perform a survival analysis of young. The model uses brood number as a fixed effect and brood identity nested in female identity as random factors. Female Cox proportional hazard coefficients were then regressed on males and females quality variables to assess the influence of adult quality on young survival.

Adult quality, as measured by weight, condition and percentage of white patch cover were also inserted in linear models, with young quality, as measured by the two first axes of the PCA, with response variables, to test for any effect of adult quality on young predatory performance. We also regressed number of young produced against adult quality, using a quasipoisson model to correct for excessive variation in the response variable.

Results

Female mate choice

Female copulation acceptance was not related to male weight, condition or percentage of white patch cover (GLM: β_{weight}=154.98, p=0.15; $\beta_{Condition}$=-44.13, p=0.81; β_{patch}=-0.86, p=0.85, N=11). Similarly, female unreceptive behaviors were unrelated to male weight or condition, and only weakly affected by percentage of white patch cover (GLM: β_{weight}=437.83, p=0.48; $\beta_{Condition}$=1968.50, p=0.13; β_{patch}=-0.73, p=0.053, N=11). A closer evaluation of the percentage of white patch cover revealed no further relationships with unreceptive female behaviors. (Spearman rank correlation: ρ=-0.27; p=0.42, N=11).

Table 1- Component loadings of a principal component analysis on variables used to measure female choice in *Hasarius adansoni.*

Variable	PC1	PC2	PC3	PC4
Number of copulations	0.92	0.32	0.16	0.18
Total copulation time	0.91	0.36	0.06	-0.18
Unreceptive behaviors*	-0.32	0.84	-0.44	0.02
Percentage of rejections	-0.64	0.55	0.54	-0.01

*Running away from the male or attacking him

Male mate choice

Neither the number of copulation attempts nor the percentage of encounters that led to courtship had any relationship with female quality indicators (number of copulation attempts GLM: β_{size}=-25.64, p=0.16; $\beta_{Condition}$=577.09, p=0.17; β_{Sperm}=-0.003; p=0.28; N=19; Percentage of visualizations leading to courtship: β_{size}=-0.46, p=0.17; $\beta_{Condition}$=3.77, p=0.6; β_{Sperm}=0.000008; p=0.85; N=19). The amount of sperm transferred to the female, however, was associated with the amount of sperm in the unused palp (β_{size}=-0.001, p=0.37; $\beta_{Condition}$=-0.0002, p=0.50; β_{Sperm}=0.51; p=0.028; N=20; Figure 1; Table 3). For 17 out of 33 males, we found more leftover sperm in the used palp than in the unused palp. Since we calculated the amount of sperm transferred to the female as the sperm in the unused palp minus the sperm in the used palp, 17 males had a negative value for amount of sperm transferred, which does not make biological sense. Since the amount of sperm is an estimated value, it is possible that those males actually did not transfer sperm at all, or transferred a very small amount of sperm. We thus ran another analysis treating those negative values as zeros, and regressing it against the amount of sperm available for usage (i.e.: sperm in the unused palp). Our results from that analysis were similar to the ones considering negative values of sperm transfer (Spearman's rank correlation: ρ=0.59; p=0.0008; N=27). These results show that the amount of resources (as measured by the amount of sperm) a male transfers to a particular female is determined by the amount of resources he has available before mating.

Table 2- Component loadings of a principal component analysis on *Hasarius adansoni* young hunting performance. Egg sac number was included to control for any effect of brood number and hunting performance

	Components		
Variables	PC1	PC2	PC3
Latency to move towards prey	0.28	0.96	-0.06
Speed moving towards prey	-0.79	0.22	0.57
Egg sac number	0.8	-0.12	0.58

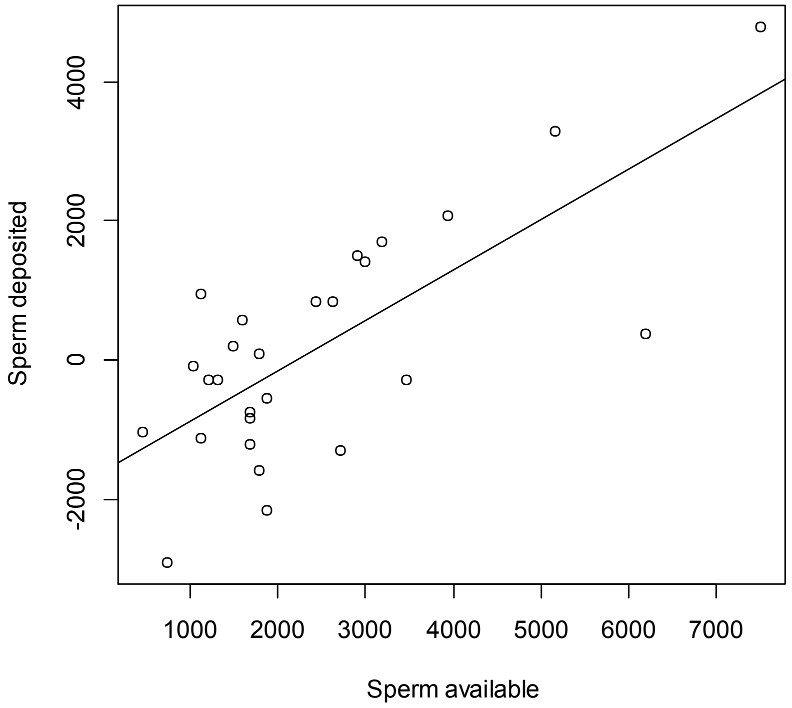

Figure 1- Amount of sperm cells transferred to a female as a function of the amount of sperm cells available for usage by male *Hasarius adansoni*

Offspring quality

There was no relationship between paternal condition, percentage of white patch cover or amount of sperm available with the total number of spiderlings produced by the female (Quasipoisson model: $\beta_{\text{female condition}}$=-0.22, p=0.99 ; $\beta_{\text{male condition}}$=-2.8, p=0.97; β_{patch}=-1.5 , p=0.65; β_{sperm}=-0.0001; p =0.38; N=14).

Table 3- Variables influencing the amount of sperm that *Hasarius adansoni* transfers to the female reproductive tract.

Fixed effects	β (±SE)	p
Female size	0.001 (0.003)	0.37
Female condition	0.0002 (0.0003)	0.5
Male sperm available	0.51	0.02

Although the first principal component of offspring quality (which loaded positively with egg sac number and negatively with animals' speed) correlated positively with female condition, this relationship was no longer significant after the removal of one single outlier. This outlier removal yielded a non-significant model with an AIC value 8.6 units lower than the previews model ($\beta_{\text{female size}}$=0.50; p =0.51; $\beta_{\text{female condition}}$=-10.51; p =0.37, AIC=29.40; N=14). Every other relationship between adult quality and first and second principal components yielded non-significant results (PC1: $\beta_{\text{male size}}$=-1.32; p =0.14; $\beta_{\text{male condition}}$=-30.64; p =0.67; PC2: $\beta_{\text{female size}}$=0.49; p =0.34; $\beta_{\text{female condition}}$=6.08; p =0.14, $\beta_{\text{male size}}$=-0.05; p =0.91; $\beta_{\text{male condition}}$=24.12; p =0.55).

We found that adult quality did not influence offspring survival. The Cox proportional hazard mixed-model random female coefficients were not related to female condition, male condition or percentage of white patch cover ($\beta_{\text{female condition}}$=13.89; p=0.397; $\beta_{\text{male condition}}$=97.64; p=0.208; β_{patch}=0.21; p=0.92).

Discussion

We found no evidence of adult quality directly affecting offspring quality, as measured by predation capacity or survivorship. This is in line with the lack of mate choice criteria that we found. For males, we were able to demonstrate that animals invest as much effort (as measured by the amount of sperm transferred to the female) as they have available, regardless of partner quality. Because secondary sexual characters, such as the white patch area in the palps of *H. adansoni* males, are thought to have evolved under sexual selection (Andersson 1994), the lack of mate choice found in this study is unexpected, at least for females, as they are usually the choosy sex. One possible explanation is the presence of individual preference functions that might be driven by individual female genotypes. In what follows, we will discuss these results, and propose a putative explanation for the lack of female preference and mate choice.

Many species exhibit variable individual preferences for a specific partner phenotype, instead of a population level preference, although individual preferences can be expressed in conjunction with population level preferences (e.g, Forstmeier & Birkhead 2004). One common reason for individual preferences is differences in the quality of the choosy sex. In many species, including many spiders, males and females mate assortatively, thus, a poor quality animal possibly would not be particularly choosy and could accept poor quality animals as partners, or could actively select low quality partners (Bel-Venner et al. 2008; Hoefler et al., 2009; Baldauf et al., 2013; reviewed by Cotton et al. 2006). Additionally, what constitutes a high quality partner can also vary with individual genotypes. In many taxa individuals appear to choose their partners in ways that enhance offspring heterozygosity (Tregenza & Wedell 2000; Landry et al. 2001). Different environments may also select for distinct individual preference functions. For instance, in high predation risk environments, it might be advantageous to copulate with small males that forage less and are less easily detected by predators (if such characters are heritable). In a low level predation environment, it would be beneficial to do the opposite (e.g.: Jia & Greenfield 1997; Doty & Welch 2001). If such individual preference functions are expressed at the level of choice, a population general trend in choice would not be detectable, even if individual choice levels were high.

In a parallel study with *H. adansoni*, we were able to show that females express a strong individual sexual preference for male size, and such preference cannot be detected at the general population level. Although that study only assessed male absolute size, similar preference functions are theoretically possible for other traits assessed here, like white patch size or male condition. If and how such individual preferences influence offspring quality is currently unknown. It is possible that females choose their partners in order to enhance offspring genetic quality (e.g.: heterozygosity; Tregenza & Wedell 2000; Landry et al. 2001) or offspring adaptation to current environment (e.g.: Jia & Greenfield 1997; Doty & Welch 2001). This would make difficult to assess any population level preference, since preference functions would be different for each individual and possibly neutral at the population level.

Some recent research proposes that male mate choice is usually constraint to evolve in cases where males find females sequentially. The uncertainty of weather to find or not a future mate would select males to invest high in the present copulation partner. On the other hand, if many females are encountered at once, males would have the opportunity to choose among all potential partners present (Edward & Chapman 2012; Barry & Kokko 2010). In our field sessions we observed that both males and females seem to be territorials, with the same individuals being found in subsequent days in the same territory. This probably means that the frequency of encounters between two individuals is low, and males probably find females sequentially and this could explain the lack of male choosiness.

It is important to note that there are two possibly important features in mate choice that we were unable to measure. *Hasarius adansoni* males vibrate their abdomen during courtship, and this variable was not assessed in the current study. This is a common behavior in the Salticidae family and has been reported to be important in sexual selection (Elias et al. 2003; Elias et al. 2004; Elias et al. 2005a; Elias et al. 2006; Elias et al. 2010; Elias et al. 2012; Hoefler 2007; Sivalinghem et al. 2010). Additionally, although white patch size was not important for mate choice and could not predict offspring quality, there is a possibility that colorimetric measures might be important, especially considering that some jumping spider ornaments reflect ultra-violet light (Bulbert et al. 2015). Future studies will focus on these two characters as possible candidates for sexually selected traits.

In conclusion, we found no evidence of mate choice for males or females; neither any relationship between adult quality and offspring quality. Individual preference functions may explain the lack of population level preferences for males and females. In the specific case of males, in which we found direct evidence that individuals invest proportionally to the amount of resources available, sequential encounters of females may also be related to the lack of preference criteria. Future studies will assess preference for vibratory and color signals in females, which will help explain the sexual selection process in *H. adansoni*.

References

Amundsen, T. 2000. Why are females birds ornamented? *Trends in Ecology and Evolution*, 15: 149-155.

Amundsen, T., Forsgren, E. 2001. Male mate choice selects for female coloration. *Proceedings of the National Academy of Science*, 98, 13155-13160.
Andersson, M. 1982. Female choice selects for extreme tail length in a widowbird. *Nature*, 299, 818-820.

Andersson, M. 1994. Sexual Selection. New Jersey, Princeton University Press.

Baldauf, S., Engqvist, L., Ottenheym, T., Bakker, T. C. M., Thünken, T. 2013. Sex-specific conditional mating preference in a cichlid fish: implications for sexual conflict. *Behavioral Ecology and Sociobiology*, 67, 1179-1186.

Barry, K., L. & Kokko, H. 2010. Male mate choice: why sequential choice can make its evolution difficult. *Animal Behavior*, 80, 163-169.

Bateman, A. J. 1948. Intra-sexual selection in *Drosophila*. *Heredity*, 2, 349-368.

Bel-Venner, M. C., Dray, S., Allainé, D., Menu, F., Venner, S. 2008. Unexpected male choosiness for mates in a spider. *Proceedings of the Royal Society of London B*, 275, 77-82.

Bukowski, T. C., Christeton, T. E. 1997. Determinants of sperm release and storage in a spiny orbweaving spider. *Animal Behaviour*, 53, 381-395.

Bukowski, T. C., Linn, C. D., Christeton, T. E. 2001. Copulation and sperm release in *Gasteracantha cancriformis* (Araneae: Araneidae): differential male behaviour based on female history. *Animal Behaviour*, 62, 887-895.

Bulbert, M. W., O'Hanlon, J. C., Zappettini, S., Zhang, S., Li, D. 2015. Sexually selected UV signals in the tropical ornate jumping spider, Cosmophasis umbratica may incur costs from predation. Ecology and Evolution, 5, 914-920.

Candolin, U. 2003. The use of multiple cues in mate choice. *Biological Reviews*, 78, 575-595.

Cloudsley-Thompson, J. L. 1949. Notes on Arachnida. 12. Mating habits of *Hasarius adansoni*. *Enthomologist Monthly Magazine*. 85, 261-262.

Cotton, S., Fowler, K., Pomiankowski, A. 2004 Do sexual ornaments demonstrate heightened condition-dependent expression as predicted by the handicap hypothesis? *Proceedings of the Royal Society of London B*, 271, 771-783.

Cotton, S., Small, J., Pomiankowski, A. 2006. Sexual selection and condition-dependent mate preferences. *Current Biology*, 16, R755–R765.

Dewsbury, D. A. 1982. Ejaculate cost and male choice, *The American Naturalist*. 119, 601-610.

Doty, G. V & Welch, A. M. 2001. Advertisement call duration indicates good genes for offspring feeding rate in gray tree frogs (*Hyla versicolor*). *Behavioral Ecology and Sociobiology*. 49, 150– 156.

Edward, D. A. & Chapman, T. 2011. The evolution and significance of male mate choice. *Trends in Ecology and Evolution*, 26, 647-654.

Elgar, M. A., Fahey, B. F. 1996. Sexual cannibalism, competition, and size dimorphism in the orb-weaving spider *Nephila plumipes* Latreille (Araneae: Araneoidea). *Behavioral Ecology*, 7, 195-198.

Elgar, M. A., Bruce, M. J., Champion de Crespigny, F. E., Cutler, A. R., Cutler, C. L., Gaskett, A. C., Herberstein, M. E., Ramamurthy, S., Schneider, J. M. 2003. Male mate choice and patterns of paternity in the polyandrous, sexually cannibalistic orb-web spider *Nephila plumpies*. *Australian Journal of Zoology*, 51, 357-365.

Elias, D.O., Mason, A. C., Maddison, W.P., Hoy, R. R.. 2003. Seismic signals in a courting male jumping spider (Araneae: Salticidae). *The Journal of Experimental Biology*, 206, 4029-4039.

Elias, O.D., Mason, A. C., Hoy, R. R.. 2004. The effect of substrate on the efficacy of seismic courtship signal transmission in the jumping spider *Habronattus dossenus* (Araneae: Salticidae). *Journal of Experimental Biology*, 207, 4105-4110.

Elias, O.D., Hebets, E. A., Hoy, R.R., Mason, A. C. 2005. Seismic signals are crucial for male mating success in a visual specialist jumping spider (Araneae: Salticidae). *Animal Behaviour*, 69, 931-938.

Elias, O.D., Hebets, E.A., Hoy, R.R.. 2006. Female preference for complex/novel signals in a spider. *Behavioral Ecology*, 17, 765-771.

Elias, O.D., Sivalinghem, S., Mason, A.C., Andrade, M.C.B., Kasumovic, M. M. 2010. Vibratory communication in the jumping spider *Phidippus clarus*: Substrate-borne courtship signals are important for male mating success. *Ethology*, 116, 990-998.

Elias, D.O., Maddison, W.P., Peckmezian, C., Girard, M.B., Mason, A. 2012. Orchestrating the score: complex multimodal courtship in the *Habronattus coecatus* group of *Haborattus* jumping spiders (Araneae: Salticidae). *Biological Journal of the Linnean Society*, 105, 552-547.

Fisher, R. A. 1930. The genetical theory of natural selection. Clarendon, Oxford University Press.

Folstad, I., Karter, A. J. 1992. Parasites, bright males, and the immunocompetence handicap. *The American Naturalist*. 139, 603-622.

Forstmeier, W., Birkhead, T. R. 2004. Repeatability of mate choice in the zebra finch: consistency within and between females. *Animal Behaviour*, 68, 1017-1028

Gable, E., Uhl, G. 2013. How to Prepare Spider Sperm for Quantification. Arachnology, 16, 109-112.

Gaskett, A. C., Herberstein, M. E., Downes, B. J., Elgar, M. A. 2004. Changes in male mate choice in a sexually cannibalistic orb-web spider (Araneae: Araneidae). *Behaviour*, 141, 1197-1210.

Hamilton, W. D., Zuk, M. 1982. Heritable true fitness and bright birds: a role for parasites? *Science*, 218, 384-387.

Herberstein, M. E., Schneider, J. M., Uhl, G., Michalik, P. 2011. Sperm dynamics in spiders. *Behavior Ecology*, 22, 692-695.

Hoefler, D. C. 2007. Male mate choice and size-assortative pairing in a jumping spider, *Phidippus clarus*. *Animal Behaviour*, 73, 943-954.

Hoefler, C. D., Carlascio, A. L., Persons, M. H., Rypstra, A. L. 2009. Male courtship repeatability and potential indirect genetic benefits in a wolf spider. *Animal Behavior*, 78, 183-188.

Hoelzer, G. A. 1989. The good parent process of sexual selection. *Animal Behavior*, 38, 1067-1078.

Jakob, E. M. 1996. A comparison of body condition indices. *Oikos*, 77, 61-67.

Jia, F. & Greenfield, M. D. 1997. When are good genes good ? Variable outcomes of female choice in wax moths When are good genes good ? Variable outcomes of female choice in wax moths.*Proceedings of the Royal Society of London B* . 264, 1057-1063.

Jones, K. M., Monaghan, P., Nager, R.G. 2001. Male mate choice and female fecundity in zebra finches, *Animal Behaviour*. 62, 1021-1026.

Kirkpatrick, M. 1982. Sexual selection and the evolution of female choice. *Evolution*. 36, 1-1-2.

Kokko, H., Jennions, M. D., Brooks, R. 2006. Unifying and testing models of sexual selection. *The Annual Review of Ecology, Evolution, and Systematics*, 37, 43-66.

Kotiaho, J. 2001. Costs of sexual traits : a mismatch between theoretical considerations and empirical evidence. *Biological Reviews*, 76, 365-376.

Landry, C., Garant, D., Duchesne, P. & Bernatchez, L. 2001. “Good genes as heterozygosity”: the major histocompatibility complex and mate choice in Atlantic salmon (Salmo salar). *Proceedings of the Royal Society of London B*, 268, 1279–85.

Levi, H. W., Levi, L. R. 1990. A Golden Guide: Spiders and Their kin. New York: Golden Press.

MacLeod, E. C., Andrade, M. C. B. 2014. Strong, convergent male mate choice along two preference axes in field populations of black widow spiders. *Animal Behaviour*, 89, 163-169.

Møller, A. P., Alatalo, R. V. 1999. Good-genes effects in sexual selection. *Proceedings of the Royal Society B*. 266, 85-91.

Morse, D, H. 2010. Male mate choice and female response in relation to mating status and time since mating. *Behavioral Ecology*, 21, 250-256.

Pruitt, J. N., Riechert, S. E. 2009. Male mating preference is associated with risk of pre-copulatory cannibalism in a socially polymorphic spider. *Behavioral Ecology and Sociobiology*, 63, 1537-1580

Richman, D. B.1982. Epigamic display in jumping spiders (Araneae, Salticidae) and its use in systematics. *Journal of Arachnology*. 10, 47-67.

Richman, D. B. Jackson, R, R. 1992. A review of the ethology of jumping spiders (Araneae, Salticidae). *Bulleting of the British Arachnology Society*, 9, 33-37.

Riechert, S., Singer, F. D. 1995. Investigation of potential male mate choice in a monogamous spider. *Animal Behaviour*, 49, 715:723.

Ronald, K. L., Fernández-Juricic, E., Lucas, J. R. 2012. Taking the sensory approach: how individual differences in sensory perception can influence mate choice. *Animal Behavior*, 84, 1283-1294.

Schantz, T., Bensch, S., Grahn, M., Hasselquist, D., Wittzel, H. 1999. Good genes, oxidative stress and condition-dependent sexual signals. *Proceedings of The Roayal Society of London B*, 266, 1-12

Senteská, L, Pekar, S. 2013. Mate with the young, kill the old: reversed sexual cannibalism and male mate choice in the spider *Micaria sociabilis* (Aaraneae: Gnaphosidae). *Behavioral Ecology and Sociobiology*, 67, 1131-1139.

Sivalinghem, S., Kasumovic, M.M., Manson A. C., Andrade, C.B., Elias, D. O. 2010. Vibratory communication in the jumping spider *Phidippus clarus*: polyandry, male courtship signals, and mating success. *Behavioral Ecology*, 21, 1308-1314.

Snow, L. S. E., Andrade, M. C. B. 2004. Pattern of sperm transfer in redback spiders: implications for sperm competition and male sacrifice. *Behavioral Ecology*, 15, 785-792.

Tregenza, T., Wedell, N. 2000. Genetic compatibility, mate choice and patterns of parentage: invited review. *Molecular Ecology*, 9, 1013-1027.

Trivers, R.L. (1972). Parental investment and sexual selection. In: Sexual Selection and the Descent of Man (B. Campbell, ed.), pp. 136–179. Chicago, Aldine.

Uhl, G., Elias, D. O. (2011). Communication. In: Spider Behavior: Flexibility and Versatility (E. M. Herberstein, ed.), Pp. 127-189. New York, Cambridge University Press.

Wedell, N., Gage, M. J. G., Parker, G. A. 2002. Sperm competition, male prudence and sperm-limited females. *Trends in Ecology and Evolution*. 17, 313-320.

Zahavi, A. 1975. Mate selection-A selection for the handicap. *Journal of Theoretical Biology*, 53, 205-214.

CAPÍTULO 3- INDIVIDUAL SEXUAL PREFERENCES IN FEMALES OF THE JUMPING SPIDER *Hasarius adansoni*

Abstract

Consistent individual differences in behavior (i.e.: personalities) have been detected in many taxa and include different behaviors. However, sexual behavior is under-represented in this scenario. Here we assess within-individual consistency and between-individual variation for mate choice in the tropical jumping spider *Hasarius adansoni,* by building individual preference functions. Each female was presented to three different males in different days and their acceptance of males was measured. We found that females differ both in their average propensity to engage in sexual behavior as well as in their preference direction. Females that were more sexually responsive preferred larger males while those that were less sexually responsive preferred smaller males. We found no evidence of assortative mating, since females' preferences were not correlated to female quality. We suggest that previous female experiences may be responsible for the highly variable preference functions observed, and this may result in the maintenance of different-sized males in the population by diminishing any directional selection for male size.

Key words: Animal personalities, individual differences, preference functions

Introduction

In recent years, animal behavior studies have veered from a ‘general behavior tendency’ approach, surrounded by non-adaptive individual noise, to an ‘individual behavior tendency’ approach. The idea that individuals consistently differ in the way they perform specific behaviors is now well recognized, and recent research has shown that such differences may be highly adaptive (Dall et al. 2004; Sih et al. 2004a Sih et al. 2004b; Dingemanse & Réale 2005; Bell 2007; Réale et al. 2007; Stamps & Groothuis 2010; Wolf & Wessing 2010). Such different personalities or behavioral types (i.e.: consistent individual differences in behavior) and different behavioral syndromes (i.e.: suites of correlated behaviors across situations; Sih et al. 2004a; Bell 2007) have also been detected in a variety of taxa, including fishes (e.g.: Smith & Blumstein 2010; Conrad et al. 2011; Bierbach et al. 2015), birds (e.g.: Dingemanse et al 2003; Møller 2010; Castilho & Macedo 2016; Zhao et al. 2016), lizards (e.g.: Carter et al. 2010), insects (e.g.: Sih & Watters 2005), and spiders (e.g.: Rabaneda-Bueno et al. 2014; DiRienzo & Montiglio 2016).

Most of the studies to date, however, have focused on a few key behaviors and their syndromes. Aggression, environment exploration and fear response to novel or threatening situations (usually called boldness) are among the most common behaviors assessed in the personalities paradigm (Dingemanse et al. 2003; Carter et al. 2010; Møller 2010; Smith & Blumstein 2010; Rabaneda-Bueno et al. 2014; Castilho & Macedo 2016; Zhao et al. 2016). These studies have documented a variety of ways whereby such behavioral differences may affect fitness, such as distinct habitat use by different personality types (Carter et al. 2010); differences in predation avoidance capacity (Smith & Blumstein 2010); in dispersion patterns (Dingemanse et al. 2003;

Cote et al. 2010; Møller 2010; Fogarty et al. 2011), in aggression behavior rate (Rabaneda-Bueno et al. 2014), and in mating success (Sih & Watters 2005).

However, the influence of consistent differences in behavior has been less assessed in the sexual selection context (Schuett et al. 2010). To date, there is some evidence that females may vary in the propensity to engage in copulations (Godin & Dugatkin 1995), extra-pair copulations (Forstmeier 2007) and in the direction of mate choice. In the zebra finch, *Taeniopygia guttata*, some females prefer males with red beaks while others prefer those with orange beaks. Similarly, some females prefer high song rates while others prefer low song rates, even though song rate is related to average male attractiveness (Forstmeier & Birkhead 2004). Additionally, some studies have shown that both males and females may mate assortatively, with low quality individuals mating with similarly low quality partners and vice-versa (Bel-Venner et al., 2008; Hoefler et al., 2009; Baldauf et al., 2013), and such difference in quality may last for long periods (e.g, Taylor et al. 2011).

Jumping spiders are underrepresented in the literature of behavioral syndrome when compared to sexual cannibalistic species (e.g.: Jonhson 2001, DiRienzo & Montiglio 2016). However, they make excellent models to assess many aspects of behavior, including sexual behavior, since most species perform complex displays (Levi & Levi 1990), which usually imply mate choice, and are easy to capture and maintain in controlled conditions. The tropical jumping spider *Hasarius adansoni* males present sexual courtship display, with visual and vibrational signals, and females perform a receptive posture when accepting a male. Thus, we hypothesized that females should be expressing preference and choosiness at some level.

The objective of this study was to test for the presence of consistent individual differences in sexual behavior of the tropical jumping spider *Hasarius adansoni*.

Specifically, we tested the presence of both within-individual consistency and between-individual variation in mate preference by females *H. adansoni* and asked whether such differences may arise due to assortative mating for individual quality and/or different sexual experiences by the females.

Methods

Study species

Hasarius adansoni is very common in urban areas throughout the tropics, and they are easily found in buildings and city walls (Levi & Levi 1990). The species is sexually dimorphic, and males can be differentiated from females by their black coloration and white patches in the palpi (Levi & Levi 1990). Males perform a courtship display, approaching females with the first pair of legs raised. Females show acceptance of the male by curling their legs and staying motionless until the male mounts her (first chapter of this thesis). Previous experiments indicate that females will only accept a certain number of copulations from a single male. After this point, the male will still court and approach, but the female will either run from or attack the male.

Capture and handling

Individuals of *H. adansoni* were captured year round in the city of Brasília, Brazil, from 2012 to 2016. Individuals were brought to the Laboratório de Comportamento Animal in the Universidade de Brasília main campus where they were kept in individual glass vials, approximately 9cm X 4.5cm in size. Each individual was given a unique code and fed every four to seven days with 10 to 15 adult *Drosophila* spp. and one young *Gryllus* sp. nymph. Adult males are easily distinguished from juveniles by their strong black coloration. Females' life stages were assessed with a stereomicroscope: only adult females have visible and open epigynums. Experiments were only conducted with adult animals.

We measured carapace width (i.e. body size) by photographing individuals with an electronic stereomicroscope and taking measurements with ImageJ software from the photos. A subsample of 23 adult males was used to establish the mean and standard errors of size in the population. We classified males in three body size categories: medium males (between -1SE and +1SE), small males (< -1SE); and large males (> +1SE). We also measured female body size in the same way, and used this as a measurement of female quality.

Males in the large male group were larger than those in the medium group, and the latter were larger than males in the small group (Large: 2.21mm ± 0.12; Medium: 2.11 ± 0.09; Small: 1.89 ± 0.14), and these differences were significant ($F = 29.2$; $p < 0.0001$; $N = 49$). This shows that our categorization correctly divided males into three distinct size groups.

Mating trials

Mating trials took place in a mating arena, which consisted of an acrylic cubic container, measuring 13cm x 13cm x 4cm. The arena contained two opaque dividers, which made it possible to handle two spiders simultaneously, without visual contact. The two dividers could be opened simultaneously to start the mating experiment. Each female was presented to three different-sized males (small, large and medium) in random order and on separate days, to prevent excessive stress and fatigue from handling. Since one copulation may alter the chance of a female copulating again (see above), experiments were interrupted right before mating took place or after one hour, if no copulations took place. Before every trial, the mating arena was cleaned with soap, water and alcohol, to remove any hormone traits. Experiments were conducted under a natural light simulating lamp (Arcadia Bird lamp. Model FB 36) and filmed with a Kodak Zx1 Pocket Video Camera.

From the videos, we recorded the following female behaviors: (i) number of unreceptive behaviors (i.e.: attacking or running away from the male, even if he does not attempt to copulate); (ii) number of rejections (i.e.: number of unsuccessful copulation attempts by the male, even if the female does not attack or clearly runs away); (iii) percentage of copulation attempts by the male that were unsuccessful; and (iv) the presence of copulations (coded as 1 or 0). Trials in which males did not court females at least once, were excluded from further analysis.

Statistical Analysis

A principal component analysis (PCA) was performed to reduce the dimensionality of female receptiveness variables. Thus, the results from the PCA were used as a measure of female preference for a given male (see Results).

To measure within-individual consistency and between-individual variation we used the reaction norms approach (Dingemanse et al. 2009). Briefly, such approach considers a behavioral trait (e.g.: sexual response) as a dependent *y* variable, and an environmental gradient (e.g.: different sizes of a sexual partner) as an explanatory *x* variable. Additional explanatory variables can be included as fixed effects, if one wishes to control for that variable (e.g.: animal's age, sex or health). Measuring the same individuals in different environmental gradients (i.e.: different levels of the *x* variable), allows the addition of individual identity as a random factor. Finally, one can centralize the *x* variable by its mean, so the different intercepts of the model can represent different levels of behavior in a typical (mean) environment. Different slopes indicate different levels of individual plasticity among the environmental gradient of interest.

With such an approach, it is easy and intuitive to measure the two main aspects of individual differences in behavior: an intercept relative to a particular individual represents its behavioral type (between-individual variation), and a slope relative to a particular individual represents its plasticity (within-individual consistency; Dingemanse et al. 2009). Testing for the significance of both, equality of intercepts and equality of slopes, allows one to infer about how behaviors vary within and between individuals of a population.

We built preference functions of female *H. adansoni* using the reaction norms approach described above. Female sexual receptivity was included as a response variable, male size centralized by its mean was included as a fixed continuous variable and female identity was included as a random factor.

To test for difference in preference functions due to female quality, we regressed female size with the values of her intercept and slope of the reaction norm model. Since such relationships appeared to be non-linear, we performed General Additive Models (GAM) with the local weighted linear regression (LOWESS) smoothing technique as described in Zuur et al. (2009).

All analyses were performed in R (R Core Team 2014) using the package nlme and nlme4 to build reaction norms and the package gam to build GAMs.

Results

Data reduction

The first principal component explained 63.6% of the total variance of female acceptance, and was strongly and negatively correlated with number of unreceptive behaviors, number of rejections and percentage of rejections. The first component was also moderately and positively correlated with the presence of copulations. The second component explained another 20.6% of the variance, making up 84.2% of total variance, and was moderately and negatively correlated with presence of copulations; and only weakly related to other variables (Table 1). Since the first component did not explain a large part of total variance, and the second component was highly influenced only by presence of copulations, we used the first principal component in one model, and the raw values of presence of copulations in a separate model.

Table 1- Correlations between principal components of a Principal Component Analysis (PCA) and four response variables (unreceptive behaviors*, rejections, percentage of rejections and presence of copulations**) collected from females *Hasarius adaonsoni* presented to different sized males

Variables	PC1	PC2	PC3	PC4
Unreceptive behaviors*	-0.82	-0.40	0.32	-0.24
Rejections	-0.87	-0.37	-0.08	0.31
% of Rejections	-0.82	0.25	-0.49	-0.15
Presence of copulations**	0.65	-0.68	-0.32	-0.08

* Attacking the male or running away from the male
** Coded as 1 or 0

Individual preferences

The first model, using the first component as a measure of female acceptance, had no significant general effect of male size on female acceptance behavior ($\beta = -0.09$; $p = 0.89$, N=46). This shows that, overall, females are not choosing males by their size. However, significant differences in slopes (LR = 13.20; $p = 0.0014$) shows that females differed in how much they prefer different sized males (Figure 1). Slopes and intercepts were highly correlated ($R = 0.9$) showing that females with higher average sexual response (i.e.: have higher preference for an average male) also preferred larger males.

On the other hand, females with lower average sexual response preferred smaller males. Also, the absolute values of slopes of high slope females were similar to the absolute values of slopes of low slope females, indicating that females with lower average behavioral response are not just accepting males at the same rate. Rather, they actually prefer small males over the large ones.

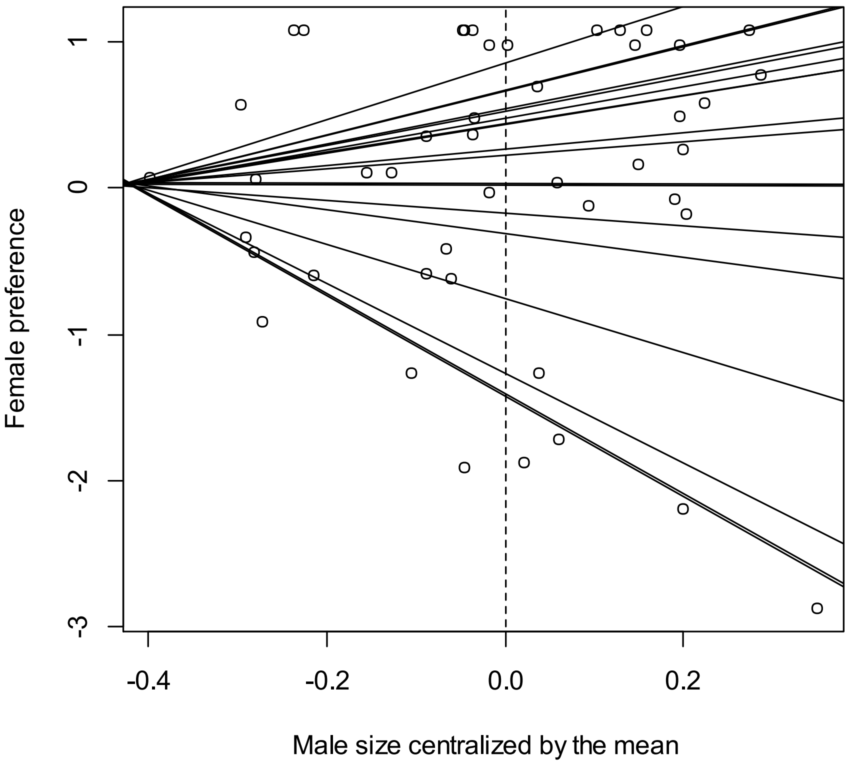

Figure 1- Preference functions for individual females of *Hasarius adansoni* as a function of male size. Male size is centralized by its mean, so the intercept (dotted line) represents the preference of the females for an averaged sized male.

Assortative mating for size

Contrary to our expectations, GAMs did not reveal any relationship between female size and females' intercept (F = 1.5; p = 0.24, N=14) or females' slope (F =1.5 ;

p = 0.25, N=14) (Figure 2). This shows that the female preference functions cannot be predicted by female quality measured as body size.

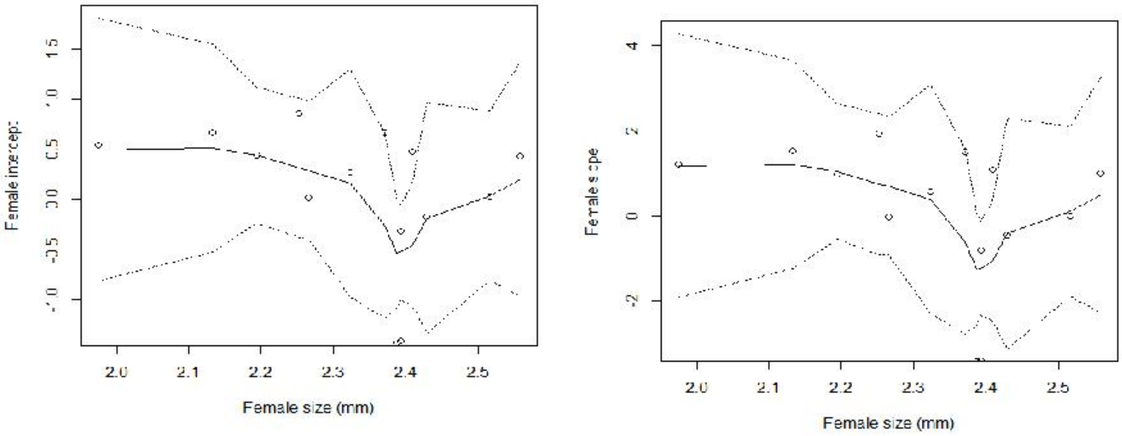

Figure 2- Lack of relationship between *Hasarius adansoni* females' size and females' slopes and intercepts of their preference functions for male sizes. The dotted lines represent the 95% confidence interval. Smoothers were calculated via the local weighted linear regression (LOWESS) of General Additive Models (GAMs)

For the behavioral binomial reaction norm using presence of copulation as the response variable, we included female size as a fixed effect in the model to ensure random effects normality. Again, the correlation between intercept and slopes was positive and high (R = 0.9), but the effect of slopes was non-significant ($\chi^2 = 4.2$; p = 0.12), while the effect of intercept was significant ($\chi^2 = 4.9$; p = 0.02). Thus, we further analyzed the model with random intercepts only. Similar to our first reaction norm model, we had no significant effect of male or female size in the probability of copulation (male size: $\beta = 2.6$; p = 0.3; female size: $\beta = -13.9$; p = 0.11, N = 38). This shows that, overall, male size does not affect probability of copulation, but females differ intrinsically in the probability of copulation with different sized males. Although the difference in slopes was non-significant, the high correlation between slopes and intercepts shows a similar pattern as in our first model: females that are more likely to copulate choose large males over small ones. And females that are less likely to copulate choose small males over big ones.

Previews sexual experience and mate choice

To test the effect of previous experience on female preference functions, we also tested the effect of male presentation order on the intercepts and slopes of female general preference (first model). We found that females presented first to larger males showed no difference in intercepts or slopes when compared to females first presented to small males ($F_{slope} = 0.67$; $p = 0.42$ $N = 14$; $F_{Intercept} = 0.66$; $p = 0.43$, $N = 14$). This shows that different sexual experiences are not dictating individual preference for females.

Discussion

We found that although individual female *H. adansoni* choose males of different sizes when mating, and this is not reflected in a general population level pattern of preference. Females differ intrinsically in their preference functions and those that are less sexually responsive to an average male choose small males over large ones. On the other hand, females that are more sexually responsive to an average male choose large males over small ones. However, these individual differences in behavior could not be predicted by which male the females encounter first or female quality as measured by female size.

Different personalities for mate choice have been detected before, but the reasons why animals differ in their preference may vary. One of the most common reasons for different mating preferences is differences in the female´s state. Females in a poor state are expected to choose males in a similar poor state in many species

(Reviewd by Cotton et al. 2006). Since neither female average sexual responsiveness or female preference direction was predicted by female size (which is a function of weight, unpublished data), we believe this does not explain the highly variable preference functions observed here.

What constitutes a valuable mate may change with environment, which could explain the individual variation in mate preference found in this and other studies. For example, in the wax moth (*Achroia grisella*), females copulating with more attractive males had faster-growing and heavier offspring when food was abundant and temperature was close to an optimum (Jia & Greenfield 1997). However, when food availability was reduced and temperatures changed from optimum, females mating with less attractive males were the ones producing offspring with such beneficial capacities. Similarly, in the gray tree frog (*Hyla versicolor*), females copulating with more attractive males had offspring that ate at higher rates. Although this might be beneficial in terms of offspring growth, it can be costly if predators are abundant, since the young will have to leave their shelters to feed and this increases predation risk (Doty & Welch 2001). Many taxa also choose mates based on their own genotype, seeking for mates with more compatible genes to enhance offspring heterozygosity (Tregenza & Wedell 2000; Landry et al. 2001). All such examples may help explain why individual preference functions evolve and are maintained in different taxa, including *H. adansoni*.

Although we did not find an effect of first male encountered by the female on female preference function, it is possible that first encounters have a more important effect in shaping future mate choice when happening early in life (Dukas 2005). Since not all of our females were captured before the last instar, we cannot completely rule out the possibility that males encountered earlier in life had an effect on adult mate preference. Besides, many other experiences throughout an individual's life may generate and maintain individual differences in behavior (Stamps & Groothuis 2010). There is considerable evidence that both males and females may copy other individuals' sexual preferences (Swaddle et al. 2005; Place et al. 2010; Bierbach et al. 2015). This might be more important in younger individuals, with limited experience in mate choice; or in individuals less capable of assessing a partner's quality as a mate (Schuett et al. 2010). Animals may also change their sexual behavior in many ways as a function of sexual experience (Dukas 2005; Fowler-Finn & Rodríguez 2012; Santangelo 2015), with younger, non-experienced animals exhibiting different, and possibly less optimal, sexual preferences than older, more experienced animals. Since adult *H adansoni* appear to be territorial (personal observation), we believe that coespecific encounters are probably rare. It would be possible, however, that young individuals with no stablished territories encounter adult individuals while wondering. Thus, if such copying strategies occur, it should be dependent on encounters happening during the juvenile phase, more specifically, before the establishment of a territory.

One possible experience that might influence *H. adansoni* female preference functions is predation risk. For instance, in the swordtail *Xiphophorus helleri*, females prefer males with long tails. After being presented to a predator model, however, females preferred males with short tails (Johnson & Basolo 2003). Possibly, long tails attract predators, thus, in a high predation risk environment, females should benefit by having sons with short tails, which causes them to change the direction of preference. In *H. adansoni*, males have a secondary sexual trait in the form of a white patch on their palpi. This ornamental trait is partly related to male size (this thesis) and could be used by predators as a cue to detect potential prey, as in many other taxa (Alatalo et al. 1991; Godin & McDonough 2003; Lindström et al. 2006; Hernandez-Jimmenez & Rios-Cardenas 2012). Possibly, animals used in our study experienced different predatory regimes before being brought to the laboratory, and this could explain the fact that many females actually preferred small rather than medium or large males.

Recent research, including the present study, shows that mate preference is not a static phenotype guided only by an individual´s genotype. Instead, it is a flexible behavior that may vary with environment, the choosy sex genotype, and previous experiences; and partner-derived benefits are also expected to be flexible in the same way (Candolin 2003). Empiricists seeking to describe sexual preferences in a species should be cautious, as it is probably common for a population to display no sexual preferences at all, while preferences may be appreciated only at the individual level, as we found for female *H. adansoni* with respect to male size. These results can also explain the maintenance of different phenotypes (e.g.: different male sizes) in the chosen sex population.

In conclusion, we found that female *H. adansoni* vary intrinsically in both average sexual responsiveness to males and in the direction of their preference for male size. Our study contributes to the growing, but still underrepresented, body of research indicating that mate choice and preference may be a flexible individual feature, not a static feature of the population.

References

Alatalo, R. V., Höglund, J., Lundberg, A. 1991. Lekking in the black grouse-a test of male viability. *Nature*, 325, 155-156.

Baldauf, S. A., Engqvist, L., Ottenheym, T., Bakker, T. C. M., Thünken, T. 2013. Sex-specific conditional mating preferences in a cichlid fish: implications for sexual conflict. *Behavioral Ecology and Sociobiology*, 67, 1179-1186.

Bell, A. M. 2007. Future directions in behavioural syndromes research. *Proceedings of the Royal Society of London B*, 274, 755-761.

Bel-Venner, M. C., Dray, S., Allainé, D., Menu, F., Venner, S. 2008. Unexpected male choosiness for mates in a spider. *Proceedings of the Royal Society of London B*, 275, 77-82.

Bierbach, D., Sommer-Trembo, C., Hanisch, J., Wolf, M., Plath, M. 2015. Personality affects mate choice: bolder males show stronger audience effects under high competition. *Behavioral Ecology*, 26, 1314-1325.

Candolin, U. 2003. The use of multiple cues in mate choice. *Behavioral Ecology*, 78, 575-595.

Carter, A. J., Goldizen, A. W., Tromp, S. A. 2010. Agamas exhibit behavioral syndromes: bolder males bask and feed more but may suffer higher predation. *Behavioral Ecology*, 21, 655-661.

Castilho, L. B., Macedo, R. H. F. 2016. Behavioral types and syndromes in the neotropical passerine blue-black grassquit (*Volatinia jacarina*). *Oecologia Australis*, 20, 28-36.

Conrad, J. L., Weinersmith, K. L., Brodin, T., Saltz, J. B., Sih, A. 2011. Behavioural syndromes in fishes: a review with implications for ecology and fisheries management. *Journal of Fish Biology*, 78, 395-435.

Cote, J., Clobert, J., Brodin, T., Fogarty, S., Sih, A. 2010. Personality-dependent dispersal: characterization, ontogeny and consequences for spatially structured populations. *Philosophical Transactions of the Royal Society of London B*, 365, 4065-4076.

Cotton, S., Small, J., Pmiankowski, A. 2006. Sexual selection and condition-dependent mate preferences. *Current Biology*, 16, R755-R765.

Dall, S. E. X., Houston, A. I., McNamara, M. 2004. The behavioural ecology of personality: consistent individual differences from an adaptive perspective. *Ecology Letters*, 7, 734-739.

Dingemanse, N. J., Both, C., van Noordwijk, A. J., Rutten, A. L., Drent, P. J. 2003. *Proceedings of the Royal Society of London B* 270, 741-747.

Dingemanse, N. J., Kazem, A. J. N., Réale, D., Wright, J. 2009. Behavioural reaction norms: animal personality meets individual plasticity. *Trends in Ecology and Evolution*, 25, 81-89.

Dingemanse, N. J., Réale, D. 2005. Natural selection and animal personality. *Behaviour*, 142, 1165-1190.

DiRienzo, N., Montiglio, P. 2016. Linking consistent individual differences in web structure and behavior in black widow spiders. *Behavioral Ecology*, 27, 1424-1431.

Doty, G. V., Welch, A. M. 2001. Advertisement call duration indicates good genes for offspring feeding rate in gray tree frogs (*Hyla versicolor*). *Behavioral Ecology and Sociobiology*, 49, 150-156.

Dukas, R. 2005. Learning affects mate choice in female fruit flies. *Behavioral Ecology*, 16, 800-804.

Fogarty, S., Cote, J., Sih, A. 2011. Social personality polymorphism and the spread of invasive species: a model. *The American Naturalist*, 177, 273-287.

Forstmeier, W. 2007. Do individual females differ intrinsically in their propensity to engage in extra-pair copulations? *PLoS ONE*, 2, e952.

Forstmeier, W., Birkhead, T. R. 2004. Repeatability of mate choice in the zebra finch: consistency within and between females. *Animal Behaviour*, 68, 1017-1028

Fowler-Finn, K. D., Rodríguez, R. L. 2012. Experience-mediated plasticity in mate preferences: mating assurance in a variable environment. *Evolution*, 66, 459-568.

Godin, J., Dugatkin, L. A. 1995. Variability and repeatability of female mating preference in the guppy. *Animal Behaviour*, 49, 1427-1433.

Godin, J. J., McDonough, H. E. 2003. Predator preference for brightly colored males in the guppy: a viability cost for a sexually selected trait. *Behavioral Ecology*, 14, 194-200.

Hernandez-Jimenez, A., Rios-Cardenas, O. 2012. Natural versus sexual selection: predation risk in relation to body size and sexual ornaments in the green swordtail. *Animal Behaviour*, 84, 1051-1059.

Hoefler, C. D., Carlascio, A. L., Persons, M. H., Rypstra, A. L. 2009. Male courtship repeatability and potential indirect genetic benefits in a wolf spider. *Animal Behaviour*, 78, 183-188.

Johnson, J. B., Basolo, A. L. 2003. Predator exposure alters female mate choice in the green swordtail. *Behavioral Ecology*, 14, 619-625.

Johnson, J. C. 2001. Sexual cannibalism in fishing spiders (*Dolomedes triton*): an evaluation of two explanations for female aggression towards potential mates. *Animal Behaviour*, 61, 905-914.

Jia, F., Greenfield, M. 1997. When are good genes good? Variable outcomes of female choice in wax moths. *Proceedings of the Royal Society of London B*, 264, 1057-1063.

Landry, C., Garant, D., Duchesne, P., Bernatchez, L. 2001. 'Good genes as heterozygosity': the major histocompatibility complex and mate choice in Atlantic salmon (*Salmo salar*). *Proceedings of the Royal Society of London B*, 268, 1279-1285.

Levi, H. W., Levi, L. R. 1990. A Golden Guide: Spiders and Their kin. New York: Golden Press.

Lindström, L., Ahtiainen, J. J., Mappes, J., Kotiaho, J. S., Lyytinen, A., Alatalo, R. V. 2006. Negatively condition dependent predation cost of a positively condition dependent sexual signalling. *Journal of Evolutionary Biology*, 19, 649-656.

Møller, A. P. 2010. Interspecific variation in fear responses predicts urbanization in birds. *Behavioral Ecology*, 21, 365-371.

Place, S. S., Todd, P. M., Penke, L., Asendorpf, J. B. 2010. Humans show mate copying after observing real mate choices. *Evolution and Human Behavior*, 31, 320-325.

Santangelo, N. 2015. Female breeding experience affects parental care strategies of both parents in a monogamous cichlid fish. *Animal Behaviour*, 104, 31-37.

Rabaneda-Bueno, R., Aguado, S., Fernández-Montraveta, C., Moya-Laraño, J. 2014. Does female personality determine mate choice through sexual cannibalism? *Ethology*, 120, 238-248.

R Core Team. 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Réale, D., Reader, S. M., Sol, D., McDougall, P. T., Dingemanse, N, J. 2007. Integrating animal temperament within ecology and evolution. *Biological Reviews*, 82, 291-318.

Schuett, W., Tregenza, T., Dall, S. R. X. 2010. Sexual selection and animal personality. *Biological Reviews*, 85, 217-246.

Sih, A., Bell, A., Johnson, C. 2004a. Behavioral syndromes: an ecological and evolutionary overview. *Trends in Ecology and Evolution*, 19, 372-378.

Sih, A. Bell, A., Johnson, C., Ziemba, R. E. 2004b. Behavioral syndromes: an integrative overview. *The Quarterly Review of Biology*, 79, 241-277.

Sih, A., Watters, J. V. 2005. The mix matters: behavioural types and group dynamics in water striders. *Behaviour*, 142, 1417-1431.

Smith, B. R., Blumstein, D. T. 2010. Behavioral types as predictors of survival in Trinidadian guppies (*Poecilia reticulata*). *Behavioral Ecology*, 21, 919-926.

Stamps, J. A., Groothuis, G. G. T. 2010. Developmental perspectives on personality: implications for ecological and evolutionary studies of individual differences. *Philosophical Transactions of the Royal Society of London B*, 365, 4029-4041.

Swaddle, J. P. Cathey, M. G., Correll, M., Hodkinson, B. P. 2005. Socially transmitted mate preferences in a monogamous bird: a non-genetic mechanism of sexual selection. *Proceedings of the Royal Society of London B*, 272, 1053-1058.

Taylor, L. A., Clark, D. L., McGraw, K. J. 2011. Condition dependence of male display coloration in a jumping spider (*Habronattus pyrrithrix*). *Behavioral Ecology and Sociobiology*, 65, 1113-1146.

Tregenza, T., Wedell, N. 2000. Genetic compatibility, mate choice and patterns of parentage: invited review. *Molecular Ecology*, 9, 1013-1027.

Wolf, M., Weissing, F. J. 2010. An explanatory framework for adaptive personality differences. *Philosophical Transactions of the Royal Society of London B*, 365, 3959-3968.

Zhao, Q., Hu, Y., Liu, P., Chen, L., Sun, Y. 2016. Nest site choice: a potential pathway linking personality and reproductive success. *Animal Behaviour*, 118, 97-103.

Zuur, A., F., Ieno, E., N., Walker, N., J., Saveliev, A., A., Smith, G., M. 2009. Things are not always linear; additive modelling. In: Mixed effects models and extensions in ecology with R. New York: Springer.

CAPÍTULO 4-TESTING THE DIFFERENTIAL COST ASSUMPTION OF THE HANDICAP HYPOTHESIS IN MALES OF THE JUMPING SPIDER *Hasarius adansoni*

Abstract

The handicap hypothesis of sexual selection theory predicts that more elaborate males will attract more predators, but at the same time, these will be more able to deal with that cost by better escaping attacks (i.e.: differential cost). Although widely accepted, the handicap hypothesis as it was originally proposed has seldom been appropriately tested, especially concerning the differential cost assumption. Here we tested this assumption using the tropical jumping spider *Hasarius adansoni* as a model. Animals were captured in the field and maintained in the laboratory with a diet of *Drosophila* sp. and *Gryllus* sp. Experimental trials consisted of touching the spiders with a paintbrush, simulating a predatory attack, and measuring their escape capacity. Measurements of escape capacity were then correlated with size of the male palpi white patches, a secondary sexual trait used during courtship. Spiders with larger white patches did not have a better escaping capacity than spiders with smaller white patches. We conclude that this trait did not evolve under the handicap hypothesis, although we cannot exclude such a possibility relative to other sexually selected traits.

Key words: Predation, escaping capacity, mate choice, sexual selection, male ornament

Introduction

Sexual selection theory predicts that animals will be choosy with respect to what partners to copulate with, and that choosiness should be related to some aspect of quality exhibited by partners that has the potential to enhance reproductive success (Andersson 1994). There are a number of hypotheses in the scope of sexual selection theory that attempt to explain the gains of the choosy sex and what constitutes quality in the chosen sex. The simplest hypothesis is that females have innate preferences for certain characteristics. According to Fisher (1930), when such innate preferences occur, the majority of females will copulate with males exhibiting the preferred character. Many other more complex hypotheses exist, predicting that secondary sexual characters (i.e.: characters exhibited to females during courtship) and sexual displays are costly (Kotiaho 2001), and then, only high quality males would be able to bear that cost, and females would benefit from that quality. The definition of cost, however, may vary from one hypothesis to another. Cost of sexual traits has been described as higher predation risk (Zahavi 1975), reduction in immunocompetence (Folstad & Karter, 1992), higher parasite load (Hamilton & Zuk, 1982) and higher energy expenditure (reviewed by Kotiaho 2001).

The handicap hypothesis, proposed by Zahavi (1975), predicts that male secondary sexual traits and displays are conspicuous and may attract predators, thus representing a cost in the form of higher predation risk. If predation risk correlates monotonically with character size, such cost will also increase with character size. Thus, only males with a higher capacity for escaping predation should be able to bear the cost of a large-sized trait. This difference in capacity of bearing a trait is termed the differential cost (Grose 2011), and the concept predicts that a unit increase in a sexual character will have a lower increase in cost for a high quality male (i.e.: a male with high escaping capacity) than it will for a low quality male. Zahavi (1975) proposed that escaping capacity is genetically determined and that females copulating with high quality males will benefit by having offspring genetically more capable of escaping predators.

Although widely accepted as part of the sexual selection process in animal behavior, the handicap hypothesis has seldom been appropriately tested (Kotiaho 2001, Grose 2011). For validation of the handicap hypothesis as it was originally proposed, two conditions must be met: (i) more extravagant secondary sexual traits must lead to a higher predation risk; and (ii) more highly ornamented individuals should escape predators more efficiently (i.e.: exhibit a lower cost for bearing such traits). Although the first condition is very intuitive, the latter one might seem extremely counter intuitive. Although the current literature accepts such condition almost unrestrictedly (since the publication of the handicap hypothesis), one could argue that the more logical scenario would be for individuals bearing a larger trait to have more difficulty in rapidly escaping a threat.

There are studies testing the effect of courtship on survival (e.g.: Kotiaho 2000) and energy expenditure (reviewed by Kotiaho 2001), but few studies have tested Zahavi´s (1975) original hypothesis, which explicitly called for differences in predation rates. There is considerable evidence that predators use secondary sexual traits as cues to hunt prey (Lindström et al. 2006; Alatalo et al. 1991; Hernandez-Jimmenez & Rios-Cardenas 2012; Godin & McDonough 2003) and some studies have also found that more extravagant secondary sexual traits enhance predation risk (Papeschi & Dessi-Fulgheri 2003; Godin & McDonough 2003; Hernandez-Jimenez & Rios-Cardenas 2012). However, fewer studies have addressed the relationship between extravagance of ornamentation and predation avoidance capacity, and these usually address predator avoidance as recapture data, which could be confused with migration or death from other causes (Alatalo et al. 1991; Petrie 1994; Papeschi & Dessi-Fulgheri 2003).

To test the handicap hypothesis, it is necessary to conduct experiments in which the ability to escape a threat are directly measured and correlated with extravagance of ornamentation. Only a few studies were able to test this condition, but conclusions have not always converged. The barn swallow, *Hirundo rustica*, and the wolf spider *Pardosa milvina* both conform to the predictions of the handicap hypothesis (Møller & Nielsen 1997; Hoefler et al. 2008, respectively). Barn swallow males have elongated tails and were more predated by sparrow hawks (*Accipiter nisus*) than were females, which do not have tail elongation. However, males captured by sparrow hawks had shorter tails than those still alive at the end of the season. The spider *P. milvina* manipulated to be in good condition performed drumming courtship at higher rates and attracted more predatory attacks from another spider, *Hogna helluo*, but escaped those attacks at higher rates. On the other hand, studies show that the fish *Girardinichthys multiradiatus* and the spider *Hygrolycosa rubrofasciata* do not conform to the differential cost condition of the hypothesis (Garcia et al. 1994; Lindström et al. 2006 respectively). Males of the fish *G. multiradiatus* and the spider *H. rubrofasciata* both attract females and predators due to their exaggerated body parts and courtship. However, more extravagant males were not better at escaping predatory attacks, thus, the differential costs prediction does not apply to these two species.

The objective of this study was to test the most counter intuitive condition of the handicap hypothesis as it was originally proposed, using a tropical jumping spider as a model and correlating extravagance of a secondary sexual character with predation escaping capacity. Jumping spider males usually perform multimodal extravagant displays to attract females and many species are also visually extravagant and exhibit a variety of secondary sexual traits (Levi & Levi 1990). Such characteristics, along with the ease of maintaining these animals in controlled conditions make them good models for the study of many hypotheses within general scope of sexual selection, including the hypothesis that more ornamented individuals escape predators better.

Methods

Study Species

Hasarius adansoni is very common in urban areas throughout the tropics, and easily found in buildings and city walls (Levi & Levi, 1990). The species is sexually dimorphic, and males can be differentiated from females by their black coloration and extravagant white patches on the palpi (Levi & Levi, 1990). Males perform a courtship display, approaching females with the first pair of legs raised so that their white patches are clearly visible (this thesis). According to the handicap hypothesis, extravagant secondary sexual characters are sexually selected and should be a honest indicator of predation escaping capacity. Since the white patches in *H. adansoni* males' palpi are extravagant and are shown to females during courtship, such trait should predict males' escaping capacity if the species follow the handicap hypothesis. However, if other sexual selection models are at work instead, such character could be related to other genetic traits, potential of giving direct benefits (Møller & Jennions 2001) or could not be related to any particular male quality at all (Fisher 1930).

Although there are no natural history studies concerning *H. adansoni*, there are a number of possible visually guided predators that could select males according to the handicap hypothesis. First, other salticid, specially from species bigger than *H. adansoni* could function as visually guided natural predators. *Menemerus bivittatus* is the most common jumping spider occurring in the same locations as *H. adansoni* (personal observation). Among vertebrates, *Tropidurus* lizards were commonly found in the same habitats as *H. adansoni*. The most common species was *Tropidurus itambere*, which is known for eating small spiders, specially when young (Sluys 1991).

Collection and housing of animals

Animals were collected in the city of Brasília, Brazil (15° 46′ 48″ S, 47° 55′ 45″ W), and maintained in the Animal Behavior Laboratory in the Universidade de Brasília. Animals were kept inside glass vials (9cm X 4.5cm) with a wet cotton inside to maintain moisture and maintained on a diet of *Drosophila* sp. and young crickets *Gryllus* sp. until the day of experimental trials. An average of 62 ± 42 days separated the capture of animals in the field and the laboratory experiments. The majority of animals were captured as sub adults and raised to adulthood in the laboratory as part of another experiment. Some others were captured as adults in the field.

Experimental procedures

Escape ability trials consisted of letting the spiders rest inside a petri dish placed in the middle of a running track made of cardboard with approximate dimensions of 32cm X 20cm X 10cm. There were two lines drawn inside the running track, 0.6cm apart, which functioned as a scale in the videos. After 30 min of resting, the petri dish was removed, and the spider was touched with a paint brush (simulating a predatory threat) and its response was videotaped. The spider usually ran away from the threat by one single sprint and then stopped moving. Whenever the spider escaped the running track, the trial was discarded and restarted from the beginning (resting period). The videos were analyzed and the spider´s distance and speed were estimated and considered as its escaping capacity. To achieve this, we used the frame from the video where the spider started moving away from the paintbrush and then the frame where the spider stopped moving and inserted both prints in the ImageJ software. The distance between the spider cephalothorax from one print to another was considered as the distance travelled by the animal and was calculated with the scale we had on the running track. By subtracting the video timing in both prints, we could determine how many seconds the animal took to travel that distance, thus calculating speed. A subset of 70 males went through the trials two times sequentially. That is, as soon as the trial was over, they rested inside a petri dish again for 30 min and had their escaping capacity measured again as described above.

Repeatability of distance traveled and speed was calculated from this subsample to ensure we had reliable individual measurements. Speed and distance repeatabilities were calculated with ANOVA tables. Male speed in predation escaping was repeatable ($R = 0.33$, $p = 0.019$, $N=70$), however, distance traveled was not ($R = 0.161$, $p = 0.164$, $N=70$). Since we had some unsuccessful trials due to male escaping the running track, we had to perform several trials with some males to achieve the desired number of successful ones (i.e.: one for general analysis or two to perform repeatability analysis). To assess the influence of the number of trials on repeatability measures, we ran Linear Mixed Models (LMMs) with the number of trials needed to achieve two successful trials as a covariate and speed or distance traveled as response variables, including male identity as a random factor. This allowed us to obtain values of within individual variance and between individual variance (thus enabling the calculation of repeatability), controlling for the number of times a spider escaped the running track (Nakagawa & Schielzeth 2010). Calculating the repeatabilities in this way, however, did not change results qualitatively. This shows that, regardless of the number of trials a male went through, speed was a repeatable variable and distance traveled was not.

Results of the repeatability analysis show that speed is a reliable individual behavioral measurement, but distance traveled is not. Since distance and speed were not correlated (R = 0.156, p = 0.195, N=70) we discarded distance traveled from further analysis and used speed as a predation escaping capacity measure.

Measuring morphological characters

After every trial, males were weighted to the nearest 0.001g with a precision balance. After that, they were euthanized by refrigerating them overnight at 4 °C. Their palps were then removed with scalpel and forceps and photographed with the male´s body. Some males were also part of another experiment and had one bulb cut off before collecting data for this experiment. In those cases, we only measured the remaining intact palp. Males that had one palp cut off did not have different average speed than those that had not had any palp amputation (Welch's t = -1.15; p=0,25). Using ImageJ, we calculated cephalothorax width (as a measure of male size) and the total white area of each palp separately. When testing the handicap hypothesis, it is important to control for variation in sexual characters driven by variation in male body size (Cotton et al. 2004). We then calculated percentage of palp area covered by the white patches. Whenever we had access to both palps, we used arithmetic means of those variables for analysis.

Statistical analysis

Normality of variables and homocedasticy of models were checked graphically. Correlations between pairs of variables were calculated with Pearson's correlation and Generalized Linear Models (GLM) with appropriate distributions and variance functions. Speed was transformed to log scale to achieve normality whenever necessary. All analyses were performed in R using the ICC, rptR, and nlme packages (R Core Team, 2014).

Results

Male size and weight were strongly correlated (R = 0.852, p < 0.0001, N=53), indicating that larger animals are heavier. Body size and white patch area were also correlated (R=0.423, p=0.001, N=56), indicating that part of the variance in patch area is due to variation in body size. However, percentage of white patch cover and body size were not correlated (R=-0.04, p=0.75, N=56). Since percentage of cover and total patch size were correlated (R=0.557, p<0.0001, N=59), we used the percentage of cover as a measure of white patch area corrected for body size. White patch area and percentage of white patch cover were entered in separate models to avoid collinearity problems.

Speed was entered as a response variable in a model with Gaussian distribution and fixed variance structure to correct for heterogeneity of variances, with white patch cover as explanatory variable. The correlation between these variables, however, was not significant (β=-0.061, p=0.86, N=53, Table 1). A similar model, but with constant variance structure and Gamma distribution were built with percentage of cover as an explanatory variable. Again, the model was non-significant (β=0.981, p=0.89, N=53, Table 1).

Table 1-Generalized Linear Models regressing predator escaping speed in the jumping spider *Hasarius adansoni* and size of palpi white patch cover and percentage of palp area covered by white patch.

Sexual trait (Predictor)	β	Variance function	Distribution function	p
White patch cover	-0.061	Fixed ($\sigma^2 * X$)	Gaussian	0.86
Percentage of white patch cover	0.981	Constant (σ^2)	Gamma	0.89

Discussion

We found no evidence that *Hasarius adansoni* follows the handicap hypothesis, interpreting it as originally proposed. Males with larger white patch area were also larger in body size, but did not escape predatory attacks more quickly than males with smaller white patch area. A measure of white patch area controlled for body size (i.e.: percentage of white patch cover) also was not correlated with predation escaping capacity. Distance traveled while running from a threat was non-repeatable across males, showing that this is not a consistent individual feature, which would make selection on this trait by female choosiness unlikely.

The general acceptance of the handicap hypothesis resulted from a few modeling seminal studies (Grose 2011), but empirical evidence substantiating the hypothesis is scarce (Kotiaho 2001). In fact, there is as much evidence supporting the hypothesis (e.g.: Møller & Nielsen 1997; Papeschi & Dessi-Fulgheri 2003; Friedl & Klump 2005; Woods Jr et al. 2007; Hoefler et al. 2008; Schmidt & Belinsky 2013) as there is rejecting it (e.g.: Garcia & Contreras 1994; Kotiaho et al. 1998; Godin & McDonough 2003; Johnson & Basolo 2003; Hadfield et al. 2006; Lindström et al 2006; Møller et al. 2006; Hernandez-Jiøenez & Rios-Cardenaz 2012, reviewed by Kotiaho 2001; Cotton et al 2004 and Grose 2011). Evidence available from those studies show that secondary sexual characters usually attract predators; however, differential costs are much less common in nature. Although it is unknown if *H. adansoni* male palps attract predators, it is clear from our data that males with bigger white patches do not have differential costs.

One important gap in the theory is the fact that escaping capacity is not always genetically determined. For instance, in the spider *Pardosa milvina*, males that court more are able to better escape predators. Although this is in line with the handicap hypothesis, these characters can be easily manipulated with feeding regimen during development (Hoefler et al. 2008). In such a species with no parental care, it seems implausible that a female could gain advantages from male escaping capacity if this has not a strong genetic component, and thus sexual selection must be driven by other mechanisms (e.g.: fisherian run-away process).

In multiple signals displays, it is possible that some signals follow the handicap hypothesis while others do not. Theory predicts that multiple signals may send different information about mate quality (Johnstone 1995; Candolin 2003). Thus, it is plausible that some signals may conform to the handicap hypothesis and convey information about predator avoidance capacity, while others convey some other kind of information. For instance, in the spider *Hygrolycosa rubrofasciata*, males that drum at a higher rate do not escape predators any differently when compared to low drumming rate males (Lindström, et al. 2006). However, other display characteristics that might be important in mate choice (e.g.: drumming sound amplitude) could be more related to escaping capacity. An even more extreme example of different information sent by different signals occurs in the bird *Euplectes ardens*, in which tail length is sexually selected by females and carotenoid patch coloration is sexually selected by other males, during male-male aggressive interactions (Pryke et al. 2001). This introduces the challenge of testing the handicap hypothesis in such model systems, especially if multiple sexually selected traits are not strongly correlated.

In the particular case of *H. adansoni*, we measured the size of the white patch shown to females during display as jumping spiders are visually guided. We cannot rule out the possibility that reflectance of the patch or the vibration behavior, also performed in courtship (this thesis), are related to predation escaping capacity. However, we are confident that the white patch area located on the palps, presented to females during courtship of this highly visual species, did not evolve in the context of the handicap hypothesis.

This does not mean, however, that the white patches are not under sexual selection. It is possible that fisherian processes are at work, if females have an innate preference for males with large white patches (see Burley & Symanski, 1998 for a similar example in birds). Direct benefits are also possible in the form of sperm load (e.g.: Weir & Grant, 2010), if males with large white patches carry and transfer more sperms to females. Another possibility is that such a secondary sexual character represents a cost that differs from predation risk, such as parasite load or decrease in inmmunocompetence (Hamilton & Zuk, 1982; Folstad & Karter, 1992). If some of these other processes are at work, female could still benefit from mating with males with larger patches (although current experiments show that this is not the case; this thesis).

Wolf spiders (Lycosidae) are the most well studied spiders with respect to the handicap hypothesis (Cotton et al. 2004), while jumping spiders are underrepresented. Given the highly multimodal displays typical of many jumping spiders, we believe there is an unexplored venue for studies of the handicap hypothesis, in order to test if and how attraction of predators and, especially, differential costs apply to specific components of the display (e.g.: visual versus acoustic displays). This study is a first step in that direction, explicitly testing the differential costs condition of the handicap hypothesis in one of the secondary sexual characters used in the multimodal display of a species never studied in this context.

We concluded that this important mating signal used in *H. adansoni* is not related to predation escaping capacity and there is no evidence that such character has evolved following the handicap hypothesis, even when controlling for variation in body size.

References

Alatalo, R. V., Höglund, J., Lundberg, A. 1991. Lekking in the black grouse-a test of male viability. *Nature*, 325, 155-156.

Andersson, M. 1994. Sexual Selection. New Jersey, Princeton University Press.

Burley, N. T., Symanski, R. 1998. “A Taste for the Beautiful”: Latent aesthetic mate preferences for white crests in two species of australian grassfinches. *The American Naturalist*, 152, 792-802.

Candolin, U. 2003. The use of multiple cues in mate choice. *Biological Reviews*, 78, 575-595.

Cotton, S., Fowler, K., Pomiankowski, A. 2004 Do sexual ornaments demonstrate heightened condition-dependent expression as predicted by the handicap hypothesis? *Proceedings of the Royal Society of London B*, 271, 771-783.

Fisher, R. A. 1930. The genetical theory of natural selection. Clarendon, Oxford University Press.

Folstad, I., Karter, A. J. 1992. Parasites, bright males, and the immunocompetence handicap. *The American Naturalist*. 139, 603-622.

Friedl, T. W., Klump, G. M. 2005. Sexual selection in the lek-breeding European treefrog: body size, chorus attendance, random mating and good genes. *Animal Behaviour,* 70, 1141-1154.

Garcia, C. M., Jimenez, G., Contreras, B. 1994. Correlational evidence of a sexually-selected handicap. *Behavioral Ecology and Sociobiology*, 35, 253-259.

Godin, J. J., McDonough, H. E. 2003. Predator preference for brightly colored males in the guppy: a viability cost for a sexually selected trait. *Behavioral Ecology*, 14, 194-200.

Grose, J. 2011. Modelling and the fall and rise of the handicap principle. *Biology and Philosophy*, 26, 677-696.

Hadfield, J. D., Burgess, M. D., Lord, A., Phillimore, A. B., Clegg, S. M., Owens, I. P. F. 2006. Direct versus indirect sexual selection: genetic basis of colour, size and recruitment in a wild bird. *Proceedings of the Royal Society of London B,* 273, 1347-1353.

Hamilton, W. D., Zuk, M. 1982. Heritable true fitness and bright birds: a role for parasites? *Science*, 218, 384-387.

Hernandez-Jimenez, A., Rios-Cardenas, O. 2012. Natural versus sexual selection: predation risk in relation to body size and sexual ornaments in the green swordtail. *Animal Behaviour*, 84, 1051-1059.

Hoefler, C. D., Persons, M. H., Rypstra, A. L. 2008. Evolutionarily costly courtship displays in a wolf spider: a test of viability indicator theory. *Behavioral Ecology*, 19, 974-979.

Johnson, J. B., Basolo, A. L. 2003. Predator exposure alters female mate choice in the green swordtail. *Behavioral Ecology*, 14, 619-625.

Johnstone, R. A. 1995. Honest Advertisement of multiple qualities using multiple signals. *Journal of Theoretical Biology*, 177, 87-94.

Kotiaho, J. S. 2000. Testing the assumptions of conditional handicap theory: costs and condition dependence of a sexually selected trait, 48, 188-194.

Kotiaho, J. 2001. Costs of sexual traits : a mismatch between theoretical considerations and empirical evidence. *Biological Reviews*, 76, 365-376.

Kotiaho, J., Alatalo, R. V., Mappes, J., Parri, S., Rivero, A. 1998. Male mating success and risk of predation in a wolf spider: a balance between sexual and natural selection? *Journal of Animal Ecology*, 67, 287-291.

Levi, H. W., Levi, L. R. 1990. A Golden Guide: Spiders and Their kin. New York: Golden Press.

Lindström, L., Ahtiainen, J. J., Mappes, J., Kotiaho, J. S., Lyytinen, A., Alatalo, R. V. 2006. Negatively condition dependent predation cost of a positively condition dependent sexual signalling. *Journal of Evolutionary Biology*, 19, 649-656.

Møller, A. P., Nielsen, J. T. 1997. Differential predation cost of a secondary sexual character: sparrowhawk predation on barn swallows. *Animal Behaviour*, 54, 1545-1551.

Møller, A. P., Nielsen, J. T., Garamszegi, L. Z. 2006. Song post exposure, song features, and predation risk. *Behavioral Ecology*, 17, 155-163.

Nakagawa, S., Schielzeth, H. 2010. Repeatability for Gaussian and non-Gaussian data: a practical guide for biologists. Biological Reviews, 85, 935-956

Papeschi, A., Dessi-Fulgheri, F. 2003. Multiple ornaments are positively related to male survival in the common pheasant. *Animal Behaviour*, 65, 143-147.

Petrie, M. 1994. Improved growth and survival of offspring of peacocks with more elaborate traits. *Science*, 371, 598-599.

Pryke, S. R., Andersson, S., Lawes, M. J. 2001. Sexual Selection of multiple handicaps in the red-collared widowbird: female choice of tail length but not carotenoid display. *Evolution*, 55, 1452-1463

R Core Team (2014). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Schmidt, K. A., Belinsky, K. L. 2013. Voices in the dark: predation risk by owls influences dusk singing in a diurnal passerine. *Behavioral Ecology and Sociobiology*, 67, 1837-1843.

Weir, L. L., Grant, J. W. A. 2010. Courtship rate signals fertility in an externally fertilizing fish.*Biology Letters*. 6, 727-731.

Woods Jr., W. A., Hendrickson, H., Mason, J., Lewis, S. M. 2007. Energy and predation costs of firefly courtship signals. *The American Naturalist*, 170, 702-708.

Sluys, V. M. 1991. Dieta de *Tropidurus itambere* Rodrigues (Sauria; Iguanidae) na Fazenda Manga, município de Valinhos, São Paulo. (Masters dissertation) – Instituto de Biologia, Universidade Estadual de Campinas, 65p.

Zahavi, A. 1975. Mate selection-A selection for the handicap. *Journal of Theoretical Biology*, 53, 205-214.

Conclusão geral

A seleção sexual é uma importante vertente da seleção natural, dando origem a fenótipos morfológicos e comportamentais diversos (Andersson 1994). Diferentes modelos de seleção sexual preveem diferentes caminhos e benefícios evolutivos pelos quais tais fenótipos serão selecionados. Nessa tese, o comportamento reprodutivo de *Hasarius adansoni* foi descrito pela primeira vez, e foram encontradas diferentes características que aparentemente evoluíram pela seleção sexual, com destaque para o *display* sexual dos indivíduos e as manchas brancas presentes nos pedipalpos dos machos.

Não foi identificado, contudo, nenhum padrão de preferência a nível populacional para a espécie, tampouco foi encontrado qualquer benefício evolutivo em termos de qualidade e sobrevivência da prole para aqueles animais que copularam com pares de diferentes qualidades. A premissa de custos diferenciais da hipótese da desvantagem, proposta por Zahavi (1975), foi explicitamente testada e também não foi corroborada, o que demonstra que, ao menos para a característica estudada (i.e.; tamanho da mancha branca), a espécie não segue a hipótese.

Embora preferências a nível populacional não tenham sido observadas, foram identificadas fortes preferências a nível individual para fêmeas de *H. adansoni*. O padrão geral encontrado foi de que fêmeas com maior propensão para engajar em comportamento sexual, preferem machos maiores, enquanto fêmeas com menor propensão para se engajar em comportamento sexual, preferem machos menores. A razão pela qual tais diferenças individuais ocorrem ainda não é clara, mas é possível que fêmeas com genéticas e experiências prévias distintas possuam escolhas distintas no que tange a parceiros sexuais.

Referências bibliográficas

Andersson, M. 1994. Sexual Selection. New Jersey, Princeton University Press.

Zahavi, A. 1975. Mate selection-A selection for a handicap. *Journal of Theoretical Biology*, 53, 205-214.