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Males of a subsocial spider choose among females of different ages and the same reproductive status

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Most sexual preference studies test female choice between different males or test male choice between females with different reproductive status, but few studies test male preference towards females of different ages within the same reproductive status. In subsocial spiders, males can share the nest with subadult and adult females of different ages. We exposed a male of *Anelosimus* cf. *studiosus* to three kinds of females simultaneously: a subadult female, a recently moulted female and a mature adult female (both virgins) comparing male preferences and female interactions. Males courted mature and recently moulted females indiscriminably, but preferentially mated with mature females, while subadult females were not courted. Mature females did not interfere with male courtship towards other females whereas some recently moulted females unsuccessfully attempted to interfere with male courtship towards mature females. In spite of these interferences, no female-female fighting was observed. Taking into account the fact that males court and pseudocopulate with subadult females in uni-female nests, this research using multi-female nests suggests that male preferences show plasticity according to the social context. The preference for copulation with mature females could be due to possible mechanical problems associated to the moult, constraining attempts by males to mate with recently moulted females. This hypothesis and our findings suggest that male may recognize female state and age of adulthood.

KEY WORDS: *Anelosimus,* subsocial spiders, male preferences, mature, recently moulted females, subadult females.

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INTRODUCTION

In many spiders, adult males cohabit with subadult females until they reach adulthood (Pollard & Jackson 1982, Christenson 1984, Jackson 1986, Lubin 1986, Costa 1993, Fahey & Elgar 1997, Holdsworth & Morse 2000). Subadult female attractiveness can vary during the penultimate instar, with females becoming more attractive just before the final moult (Schneider & Lubin 1998). Some males prefer subadult instead of adult females (Watson 1991, Eberhard 1996) mating as soon as they mature to assure their paternity (Austad 1984, Eberhard et al. 1993, Bukowski & Christenson 1997).

Some studies of solitary spiders show that mated females are less sexually receptive than virgin females (Aisenberg & Costa 2005) and old females are less sexually receptive than young virgin females (Fernández-Montraveta & Ortega 1990). According to Roberts & Uetz (2005), in *Schizocosa ocreata* the receptivity of virgins varies with the female adult age showing the highest sexual receptivity after the first week of maturation.

In social spiders individuals live in communal webs and show a primary sex ratio biased towards females reaching 10:1 in *Anelosimus eximius* (Avilés 1986, 1997). Females of the subsocial spider *Anelosimus arizona* become sexually receptive 10 days after maturation according to Bukowski & Avilés (2002). These authors found that old virgin females are more attractive to males than young virgin females and that males obtain most of their matings with old females after brief courtships. In the social theridiid *Achaearanea wau*, recently moulted females are not sexually receptive, but males attempt to mate with them opportunistically. However, due to the progressive hardening of the female exoskeleton, males of this species have mechanical difficulties in performing palpal insertions until the cuticle hardens (Lubin 1986).

Anelosimus cf. *studiosus* is a subsocial spider of Uruguay. Individuals remain in a communal web until sexual maturation and have a sex ratio of 2:1 biased towards females (Viera et al. 2006). They mature asynchronously and can adopt two reproductive tactics: remaining in the maternal nest (multi-female nest) or moving away as subadults and building individual nests (uni-female nest) (Viera et al. 2007). Preliminary observations under laboratory conditions show that subadult females are as sexually receptive as adult females and easily adopt a mating posture in uni-female nests. Moreover, subadult females in uni-female nests elicit pseudocopulations (Albo et al. 2007).

No published studies of social spiders that focused on testing male preference towards females of different ages but of the same reproductive status were found. The objective of the present research is to test the male sexual preferences of *Anelosimus* cf. *studiosus* in a multi-female scenario. The male behaviour elicited by virgin females of different ages of maturity and development instars were analysed. We discuss the possible implications of these behaviours for mating strategies.

MATERIAL AND METHODS

Sixty-eight juveniles of *Anelosimus* cf. *studiosus* were collected from different nests of a "Canelón" tree (*Rapaneae laetivirens*, Myrsinaceae), located at the Institute of Biological Sciences (IIBCE), Montevideo, Uruguay (34°53'15"S, 56°08'33"W). Individuals were bred under laboratory conditions, with a room temperature of 23 °C (± 2.6 SD). Spiders were kept in individual Petri dishes (3.5 cm internal diameter, 1 cm height) and were fed ad libitum with fruit flies *Drosophila* spp.

Multi-female nests were used for testing male sexual preferences. "Recently moulted females" were considered as those that had moulted a few hours prior to the experiments, their body was light brown indicating that the exoskeleton had not yet hardened. "Mature females" were those that had moulted 48 to 72 hr prior to the experiments, their body was dark brown and completely hard. Only virgin females were used. Because subsocial females build individual retreats a few centimetres away from each other in a multi-female nest (C. VIERA & F.G. COSTA unpublished data), one mature female, one recently moulted female and one subadult (penultimate) female were placed together in a Petri dish. After 48 hr, one adult male was introduced. Seventeen trials were performed, recording all the interactions among individuals from the introduction of the male until no sexual behaviour was observed for a period of 1 hr.

For statistical analyses, the Past Statistical package (HAMMER et al. 2004) was used. For testing normality and homogeneity of variances, the Shapiro-Wilk and Levene tests were used, respectively. For comparing frequencies, we used Mann-Whitney U-test, Chi-square test for independent samples (with Yates correction). Voucher specimens were deposited at the Entomological Collection of the Faculty of Sciences, Uruguay.

RESULTS

All the males (n = 17) courted at least one female. Although mature and recently moulted females were courted, no courtship was observed towards subadult females. Males courted a single female more frequently than two females simultaneously ($\chi^2 = 7.53$, $P = 0.006$). Nine males courted only mature females and four courted only recently moulted females. Four males courted two females simultaneously, one mated with both females consecutively (first with the recently moulted female) and the other three mated only with the mature female. One male courted only one female but consecutively mated with both adult females: first with the mature and later with the recently moulted female. No differences in courtship occurrence between mature and recently moulted females were found ($\chi^2 = 1.99$, $P = 0.158$). No differences were found between courtship duration towards recently moulted and mature females ($P = 0.294$) (Table 1).

Fifteen males mated with a single female, 13 mated with a mature female and 2 with a recently moulted female. Only 2 males mated consecutively with two females. These results show clearly that males mated with a single female more frequently than with two females ($\chi^2 = 16.9$, $P < 0.0001$) and they mated more frequently with mature females than with recently moulted females ($\chi^2 = 11.93$, $P = 0.0006$). Copulation duration was longer with mature females than with recently moulted ones ($P = 0.04$) (Table 1). No statistical differences were found in the number of palpal insertions between mature

Table 1.

Courtship and mating durations (in minutes) and number of palpar insertions. Values are given as mean ± standard deviation together with the number of data points in parentheses.

	Courtship duration (min)	Mating duration (min)	Number of insertions
Mature female	14.6 ± 20.6 (n = 13)	60.3 ± 46.4 (n = 15)	5.8 ± 4.8 (n = 15)
Recently moulted female	27.8 ± 26.3 (n = 8)	16.5 ± 29.1 (n = 4)	2.3 ± 1.8 (n = 4)

and recently moulted females ($P = 0.06$), but a tendency for more insertions with mature females was observed (Table 1). Males demonstrated difficulties in engaging the bulb parts when they mated with recently moulted females and insertions were apparently briefer in comparison to matings with mature females.

In 10 cases females actively looked for the male while it was courting or copulating with another female. These females performed abdominal and leg vibration on the silk, touched the male with their forelegs and assumed an acceptance posture. Four subadult females and two recently moulted females approached the male-mature female couple during courtship, but their mating was not interrupted. Two recently moulted females successfully interfered with courtship and another two recently moulted females interrupted matings with mature females. Mature females did not interfere with male-recently moulted female couples during copulation. When we analysed female interactions, we found differences in the interference frequencies among the three types of female ($\chi^2 = 20.72$, $P < 0.001$, df = 2). In the pair-wise comparisons, recently moulted females interfered more actively than mature females ($\chi^2 = 5.06$, $P = 0.02$) whereas no differences in the interference frequencies were found between recently moulted and subadult females ($\chi^2 = 0.14$, $P = 0.71$) nor between adult and subadult females ($\chi^2 = 2.55$, $P = 0.11$). No fights were observed among females nor between a female and the male.

DISCUSSION

The experimental design simulating a multi-female nest of *Anelosimus* cf. *studiosus*, allows males to choose between females. Although recently moulted and mature females were shown to be receptive and attractive when males courted both simultaneously, males preferred to copulate with the mature females. Males did not court subadult females as observed in *Anelosimus arizona*, where males prefer adult instead of subadult females (KLEIN et al. 2005). Consequently, males gain immediate fertilizations, but assume costs of sharing paternity with other males because mature females could have mated previously. This situation is possible in *Anelosimus* cf. *studiosus,* because males

would probably remain in the multi-females nest, where females of different ages coexist due to their asynchronous maturation (VIERA et al. 2007). However, more studies on this issue are necessary in order to elucidating male strategies and sperm priority.

Both attractive and unattractive penultimate females have been reported for spiders (WATSON 1991, EBERHARD 1996, KLEIN et al. 2005). The apparent unattractiveness of penultimate females conflicts with the findings of ALBO et al. (2007) that penultimate females in uni-female nests are very attractive and sexually receptive, eliciting intense male courtship, and male-male competition. Moreover, penultimate females can perform pseudocopulations with males attempting to insert the palps without success. In this study, the results suggest that subadult females, do not attract males in the multi-female nest due to the presence of adult females, that are preferred by males. These preferences are in accord with preliminary observations in this species (VIERA & ALBO 2002), in which males in multi-female nests courted adult females more frequently than subadult females, although both females were equal receptive and adopted a copulatory position.

Mature females attracted males more efficiently than recently moulted females. Although we did not quantify female sexual activity, the preference for mature females could have been determined by their more active behaviour towards the males. Additionally, this attractiveness could be due to the presence of contact sex pheromones associated with the female silk reported by VIERA & GHIONE (2003), that could be predominant and more intense in mature females, or by a hierarchical female order, as found in close-related species, where mature females expel other adult or subadult females from the maternal web (BRACH 1977). Finally, this preference could be determined by the physiological status of female (i.e., time of maturation).

The absence of differences in courtship frequencies towards the two adult females suggests that these females are similarly attractive to the male. This fact differs from observations in *A. arizona* by BUKOWSKI & AVILÉS (2002) who found shorter courtship duration towards old females, compared to courtship towards young females. FERNÁNDEZ-MONTRAVETA & ORTEGA (1990) also reported that in *Lycosa tarentula fasciiventris* old females are less receptive than young adult females. In *Schizocosa ocreata* the receptivity of unmated females varies with age after the adult moult, showing the highest receptivity at the end of the first week of adulthood (ROBERTS & UETZ 2005).

We found that most males mated with a single female, this could be due to the brief experimental time that we used. Although the differences of age between females in our research is small compared to other studies (FERNÁNDEZ-MONTRAVETA & ORTEGA 1990, BUKOWSKI & AVILÉS 2002), these differences were enough to elicit differences in male mating behaviour. Moreover, we found longer copulations with mature females and despite no significant differences in the number of palpar insertions, we observed a tendency to perform more insertions when mating with mature females. Males that mated with recently moulted females had difficulties in inserting their palps, similar to the difficulties of *Acharaneae wau* males (LUBIN 1986). Recently moulted females are still hardening their exoskeleton while males attempt to insert their palps, which could result in possible sticking of the palp in the female genital zone. As a

consequence, males would prefer mature females, in spite of courting both female types. This hypothesis implies that males are able to recognize female state and their age of adulthood.

Summarizing, we suggest that male preferences show plasticity according to the social scenario. Uni-female nests present male with the alternative of waiting for a virgin female or of moving away to search for mature females. In contrast, multi-female nests, that include several females in different instars, offer the male several mates with low risk of predation, but increased costs for mate guarding (described for this species by ALBO et al. 2007) and of competition with other males. Further studies are necessary in order to elucidate these issues.

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