

The influence of regurgitation by sisters on male growth in the subsocial spider *Anelosimus viera* (Araneae, Theridiidae)

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Summary

Food exchange by the mother to her brood is a frequent phenomenon in social arthropods. In the subsocial spiders of the genus *Anelosimus* Simon, 1891, maternal regurgitation to offspring plays an important role in the survival of the colony. Regurgitations also occur between subadult males and females in *Anelosimus viera* Agnarsson, 2012. In order to examine the effects of supplementary food on the subadult male's development towards maturity, we carried out two types of trial: groups of subadult males and female siblings and non-siblings were weighed, and cephalothorax and leg I in adult males were measured. Our results suggest that the supplementary food obtained by males from sisters allows the acquisition of larger size. Besides weight gain, males fed by females reached larger sizes of leg I femur and tibia. Leg I could be important in intrasexual competition, given that males with larger legs are more likely to win agonistic encounters. This also has positive implications for access to females.

Introduction

The concept of social spiders refers to a group of individuals occupying a continuous and discrete web structure called a nest or colony (Buskirk 1981). None of the social spiders have reproductive castes, which imply that all members of a social group work are able to reproduce (Buskirk 1981). Some exceptions were found by Vollrath (1986) in *Anelosimus eximius* (Keyserling, 1884) and Marques, Vasconcelos-Netto & Britto de Mello (1998) in *Anelosimus jabaquara* Levi, 1956. Sociality in spiders implies an extended period of maternal care (Buskirk 1981). The focus is not only on the reproductive success, but also on cooperative capture (Whitehouse & Lubin 2005). Social behaviour includes improvement in population fitness by group selection (Aviles 1997) and a more nearly equal food distribution.

Food exchange between mother and her brood is a frequent phenomenon in eusocial arthropod species (Wilson 1980) and, in some cases, regurgitation has been mentioned (Marques, Vasconcelos-Netto & Britto de Mello 1998; Viera *et al.* 2007). Regurgitation is an important step on the pathway to sociality, allowing communal life through prolonged maternal care behaviour (Buskirk 1981).

In social and subsocial spiders from the genus *Anelosimus* the mother regurgitates to the offspring, playing an

important role in the survival of the colony (Aviles 1997), including in our study species (Viera *et al.* 2007).

Anelosimus viera lives in nests made up of one or more adult females with their offspring, reaching up to 100 individual (Viera *et al.* 2007). It is morphologically similar to *A. studiosus* (Levi, 1956) but was determined as a different species by molecular studies, combined with behavioural data (Agnarsson 2012).

Each nest is composed initially of a female with her brood, later joining with other nearby nests to form large colonies with several territorial adult females (Viera *et al.* 2007). This species, despite being tolerant to juveniles and males of the same species and other species, shows intolerance among adult females, causing a dispersion phase (Viera *et al.* 2007). Regurgitation in this species by the mother to its brood also occurs among subadult females toward subadult males (Viera, Ghione & Costa 2005). This activity involves energy and nutrient expenditure from the subadult female favouring the male. A basic question in this process is why the females are performing an act that involves a decline in their fitness.

Food supplementation by subadult females would help males in their growth, allowing them to moult faster and/or to acquire larger body structures. We have shown the importance of male size (as indicated by the length of the first leg) in battles with other males (Albo, Viera & Costa 2007), but acceleration of development by supplementary food did not occur (Gómez & Viera unpublished data). In order to examine the effects of supplementary food on the development of subadult males, we determined whether the food given by females produced larger males, both in terms of body mass and the size of body parts. We also wanted to know if regurgitation occurs more often between related individuals, involving kin recognition.

Methods

Twenty-seven nests of *A. viera* were collected from middle and lower tree branches of two places in Montevideo, Instituto de Investigaciones Biológicas Clemente Estable (34°53'15"S 56°08'33"W) and Parque Rodó (34°54'48"S 56°10'02"W). We used nests from two different places in order to avoid the possibility of their being siblings. Each nest had one adult female (the presumed mother) and numerous juvenile males and females. Individuals were kept in laboratory conditions with controlled temperature (24 ± 2°C) and relative humidity (70%). Juveniles from each nest were reared in a Petri dish (15 cm diameter, 1.5 cm height) until they reached the subadult stage, keeping the identity of each nest. For the experiments, subadults were confined in smaller Petri dishes (5.5 cm diameter, 1.5 cm height). Three groups were created: A (siblings) five subadult males and five subadult females from the same nest (9 replicas), B (non-siblings) with five related subadult males and five related subadult females from different nests (6 replicas) and C with five males alone (4 replicas), to test male growth without females.

Groups of males and groups of females from each group were separated a week before the experiment; when all males were deprived of food, females were fed *ad libitum*

with *Drosophila* spp. The groups of males and the groups of females were weighed separately using a precision scale (Ainsworth AC-800: 0.001 g accuracy) before being placed together. After 48 hours deprivation of food and water, males and females from each group were weighed again. Initial and final weights were compared using the non-parametric Mann-Whitney and Kruskal-Wallis tests (no normality and homogeneity of variances were found), in the PAST package (Hammer, Harper & Ryan 2004). After the final weight, females and males were separated and were fed *ad libitum* in the same laboratory conditions as above.

When males reached adulthood, 24 from group A, 5 from group B, and 10 from group C were preserved individually in 70% alcohol to be measured. Length and width of carapace, and length of the femur and tibia of leg I were measured. According to Uetz, Papke & Killinc (2002) feeding differences in males are evidenced in the carapace and the size of leg I. These structures were photographed with a Nikon Coolpix P5100 digital camera, and measured using the program ImageJ (Ferreira & Rasband 2010). Normality was tested using the Shapiro-Wilk test, and homogeneity of variances was verified by the Levene test. These measures were compared using a one-way ANOVA, followed by Tukey's tests using PAST software (Hammer, Harper & Ryan 2004).

Results

Occasional regurgitations from subadult females towards subadult males in group A ($n = 10$) were observed during the experiments. This did not occur in group B, in which nine males which requested fluid food from females were rejected by the females without aggressive interactions.

The initial weights of the different groups of males showed no significant differences ($H = 2.357$, $p = 0.307$). On the other hand, final weights (A, B and C) of males showed significant differences among experimental groups ($H = 4.89$, $p = 0.039$); males of group A gained more weight. Major differences were found between group A and B ($U = 9$, $p = 0.017$) but individuals of group B lost weight in both sexes. Females did not differ significantly in their initial and final weights among the experimental groups ($U = 24$, $p = 0.703$; $U = 24$, $p = 0.496$ respectively) (Table 1).

Male carapaces did not differ significantly among the groups regarding length ($F = 1.011$, $p = 0.374$, $n = 39$) and width ($F = 0.4038$, $p = 0.670$, $n = 39$). Differences were found in the lengths of the femur ($F = 3.724$, $p = 0.033$, $n = 39$) and tibia ($F = 7.566$, $p = 0.001$, $n = 39$) among experimental groups. The males of group A showed the longest leg I. Differences in the length of the femur ($Q = 3.451$, $p = 0.050$) and tibia ($Q = 5.358$, $p = 0.001$) were found between group A and B (Table 2).

Discussion

Males usually make a big investment in searching for females, competition for access to females, courtship, and copulation (Parker 1984). Lubin (1986) found that, in the

Weight (g)	Group A	Group B	Group C
	Mean \pm SD (n = 9)	Mean \pm SD (n = 6)	Mean \pm SD (n = 4)
♂ initial	0.011 \pm 0.003	0.018 \pm 0.009	0.015 \pm 0.005
♂ final	0.018 \pm 0.009	0.010 \pm 0.002	0.012 \pm 0.005
♀ initial	0.013 \pm 0.005	0.013 \pm 0.008	-
♀ final	0.011 \pm 0.003	0.010 \pm 0.001	-

Table 1: Initial and final weights of males and females of *Anelosimus vieirae* from the three experimental groups: **A** female and male siblings; **B** unrelated female and male; **C** control.

social spider *Achaeaearanea wau*, male conflicts involved vibrations, persecutions, ritualized fights, and biting, causing injuries and even death of individuals in laboratory conditions of confinement. Albo, Viera & Costa (2007) observed in *A. vieirae* three types of male agonistic encounters involving persecutions, ritualized fights and bites, and in a few cases the individuals were killed. In these instances of fighting, males with the greatest leg I size won. These authors also found that males fight even when courtship does not take place, suggesting that fights also occur in female territories and to gain access to subadult or adult females. Our results suggest that the food supplement obtained by males from their sisters allowed them to acquire larger body size, and larger femur and tibia of leg I. Because leg I is essential in intrasexual competition in males, the dietary supplement will have important implications in contests to access females. In this regard, studies of the social spider *Achaeaearanea wau* (Levi, Lubin & Robinson 1982) suggested that, during fights, males struggle to push through their legs I, and to push each other and try to bite, although there no injuries were documented (Lubin 1986). These push and release movements with leg I also occur in *A. vieirae* and, often, a large pressure while pushing is enough for the opponent to withdraw from the conflict (Albo, Viera & Costa 2007). Therefore, the food supplement provided by their sisters is a fundamental contribution to the preparation of their brothers for fighting. In spite of the cost involved in the food donation, the sisters obtain indirect benefit by increasing the fitness of their brothers.

Differences in weights and sizes of leg I observed among males in groups A and B, and the observed rejections of males by females of group B, could suggest the existence of kin recognition. Kin recognition in subsocial spiders has been poorly studied, except by Schneider & Bilde (2008) who found benefits of cooperation in *Stegodyphus lineatus* siblings compared to non-siblings.

So far, evidence of recognition between groups (nests) was documented in our study species, but no individual recognition (Cayafa, Viera & Costa 2011). In that study, males of *A. vieirae* were unable to discriminate nests of

Size (mm)	Group A	Group B	Group C
	Mean \pm SD (n = 24)	Mean \pm SD (n = 5)	Mean \pm SD (n = 10)
Carapace length	1.406 \pm 0.056	1.372 \pm 0.025	1.385 \pm 0.065
Carapace width	1.042 \pm 0.054	1.023 \pm 0.057	1.029 \pm 0.035
Femur length	1.855 \pm 0.155	1.690 \pm 0.054	1.756 \pm 0.129
Tibia length	1.713 \pm 0.161	1.431 \pm 0.079	1.592 \pm 0.167

Table 2: Size of selected body traits of *Anelosimus vieirae* from the three experimental groups. **A** female and male siblings; **B** female and male no related; **C** control.

their sisters or their sisters' chemical stimulus, in spite of the presence of chemical stimuli from the spiders and/or the spider web. In *A. viera*, weight loss resulted in a lower survival of males in group B; in spite of the small sample size, this could suggest some kind of recognition. Maybe males and females lost weight and the low survivor rate in males of group B, not only by the absence of regurgitation, but by the stress from food solicitation behaviour by the males and subsequent non-aggressive rejection behaviour. More studies are needed to explain the low survival rate of males in group B, and the absence of cannibalism in starved individuals, as observed in *Stegodyphus lineatus* (Bilde & Lubin 2001).

A. viera, like other species of the same genus, have asynchronous maturity: males reach adulthood one or two moults before females (Viera *et al.* 2007). Adult males must leave the maternal nest to find adult females, this being a strategy to avoid inbreeding in subsocial spiders (Bukowski & Avilés 2002). Regurgitation between siblings would have a role in accelerating the growth of males, and thus the dispersion latency, in this and other species of *Anelosimus*, and reduce inbreeding as well (Bukowski & Avilés 2002). According to Avilés (1997), clarifying aspects of inbreeding are very important in these subsocial animals, and help to understand the evolution of sociality.

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