Is There Division of Labor in Cooperative Pseudoscorpions? An Analysis of the Behavioral Repertoire of a Tropical Species

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Introduction

Division of labor is defined as a repeatable pattern of activity variation among workers in a group-living population (Beshers & Fewell 2001). Division of tasks based on different morphological castes or age and/or sex is well known among insects (Oster & Wilson 1978). Its main advantage is more efficient foraging and resource exploitation (Wilson 1971; Beshers & Fewell 2001). Although division of labor is well studied in Hymenoptera and termites (Gordon 1996; Franks et al. 1997; Robson & Beshers 1997; Traniello & Rosengaus 1997), there is little information on this phenomenon in other arthropods.

The most complex forms of sociality in arachnids are observed in cooperative social spiders and pseudoscorpions. These species exhibit cooperative prey capture and food sharing, cooperative nest and capture web building, overlapping generations, and cooperative parental care (D’Andrea 1987; Avilés 1997; Uetz & Hieber 1997; Lubin & Bilde 2007). No published studies have provided compelling evidence of a clear division of labor in these species.

Abstract

Division of labor is a strategy that maximizes the foraging and reproductive success of eusocial insects. Although some arachnids exhibit colony structure and social organization similar to that of hymenopterans, temporal polyethism has only been demonstrated in a few species. The social organization of cooperative pseudoscorpions Paratemnoides nidificator is similar to that of social spiders, but it involves a clear division of labor. Work allocation was experimentally investigated in colonies composed of only one developmental stage (young or adults) or by one sex (males or females), through laboratory manipulation. During 44 h of observation, more than 14,000 behavioral repetitions were quantified, distributed in 95 different types of behavioral acts, and grouped in 10 behavioral categories. The results showed that reproductive colonies of P. nidificator are maintained by gender- and age-based activities. Males and non-reproductive females performed the external cleaning of the colony and prey capture. Reproductive females take care of the juveniles and build reproductive silk chambers. Nymphs build most of the molt chambers and perform internal cleaning. In the absence of nymphs, male colonies survived 1–2 mo, while female colonies survived 3–4 mo. In nympha colonies, work is readjusted so that all maintenance tasks are executed. This is the first study clearly demonstrating division of tasks in arachnids. It suggests that specialization is an adaptative and evolutionarily old trait in this species. Unlike cooperative spiders, P. nidificator possesses physiological (e.g. reproduction, ecdysis, lifespan) and behavioral (e.g. behavioral synchrony or self-organization) characteristics that allow task specialization.
that division of labor occurs in social spiders (Lubin & Robinson 1982; Avilés 1993, 1997; Lubin 1995; Ainsworth et al. 2002; Avilés et al. 2006), although social spiders do exhibit some behavioral and ecological traits similar to those found in social insects (D’Andrea 1987; Avilés 1997). Despite the lack of empirical data on morphological castes, some cooperative spiders display behavioral differences in task allocation according to sex and age. Young or adult females can participate with greater or lesser intensity in such colony activities as prey capture, building nests and/or cobweb maintenance (behavioral attributes defined as task specialization) (Avilés 1993, 1997; Lubin 1995; Evans 2000; Lubin & Bilde 2007). According to Lubin (1995), several factors, such as inbreeding, which reduces the spread of rare alleles determining extreme reproductive altruism, may have acted to constrain the evolution of that task specialization in arachnids.

A level of social organization very similar to that of social spiders has been observed in two pseudoscorpion species of the genus Paratemnoides. This rare form of sociality among pseudoscorpions involves cooperative prey capture and food sharing, cooperative nest building, cooperative parental care and matriphagy, and collective dispersal through phoresy or colony fission (Brach 1978; Zeh & Zeh 1990; Hahn & Matthiesen 1993a,b; Tizo-Pedroso & Del-Claro 2005, 2007). In this study, we investigated whether division of labor could be a factor favoring colonial existence in Paratemnoides. Specifically, we evaluated the hypothesis that the social structure of the cooperative pseudoscorpion Paratemnoides nidificator (Balzan, 1888) (Pseudoscorpiones, Atemnidae) is maintained by task specialization.

Methods

Study Species, Collection, and Maintenance

Paratemnoides nidificator lives under the bark of Neotropical trees, particularly Caesalpinia peltaphoroides (Caesalpinaceae). Between Oct. 2003 and Oct. 2007, 35 colonies were collected around Uberlândia, State of Minas Gerais, Brazil. Colonies varied in size from five to 170 individuals and in total included more than 700 pseudoscorpions. The colonies were transported to laboratory and each one was housed in a separate Petri dish (experimental colonies). These containers were covered with red cellophane to reduce the stress caused by light. Each box also received the bark fragment from the colony collection site. The pseudoscorpions were fed in captivity every 3 d with termites (Armitthermes sp.) and beetle larvae (Palenius dermestoides; Tenebrionidae).

Behavioral Repertoire and Activity Schedule

A behavioral repertoire is a detailed description of all the behavioral characteristics of one species (Lehner 1996). To describe the repertoire of P. nidificator, one large colony (94 individuals: 30 adults, 18 tritonymphs, 12 deutonymphs, and 34 protonymphs) was housed in a Petri dish (25 cm in diameter, 3 cm high). Observation and quantification were performed under a stereomicroscope, following the ‘all occurrence sampling’ method, ad libitum (Altmann 1974), in 40-min sessions separated by 15-min intervals. The pseudoscorpions were first submitted to 10 h of qualitative observations to describe their behavioral acts and categories. Then, 42 quantitative observation sessions, totaling 28 h, were performed to describe the entire repertoire. After that time, the nymphs had totally covered the silk chambers with silk, hiding the animals, which impeded further quantitative data collection.

The number of models to assess whether a behavioral repertoire documented in laboratory conditions includes most behaviors that could be observed in nature is limited. One of them is the ‘sample coverage’ analysis, which verifies the likelihood that next behavioral act in a repertoire is a new behavior (Fagen & Goldman 1977; Lehner 1996). The ‘sample coverage’ is calculated through the expression: \( \theta = 1 - \left( \frac{N_1}{i} \right) \), in which \( N_1 \) is the number of behaviors observed only once, and \( i \) is the number of total behavioral acts observed. When \( \theta \) is close to 1, the probability that the next behavioral act will be a new behavior is relatively low. The repertoire can be considered virtually complete when \( \theta \) ranges between 0.90 and 0.99.

A very recent model, adapted from the species richness accumulation curve, was proposed by Dias et al. (2009) as an alternative to evaluate the behavioral repertoire by contrast with the expected values yielded by Clench equation (see Soberón & Llorente 1993). Here, we generated the behavioral curve according to Dias et al. (2009) and compared it with sample coverage analysis (Lehner 1996).

The activity schedule of P. nidificator (foraging period preference) was also determined by counting the number of animals moving (engaging in any kind of movement lasting at least 2 min) or at rest during 10 min every 2 h, throughout 48 h first in the wet season (April–Sept.), then in the dry season (Oct.–Mar.), to verify differences in behaviors frequency in relation to seasons.
Division of Labor and Activity Patterns

To examine task distribution among colony individuals, the pseudoscorpions were divided into four experimental groups, with eight replications each, thus comprising 32 colonies manipulated to contain a specific set of individuals: Control – non-manipulated colonies composed by individuals of all ages and sexes; Male only – colonies composed only of adult males; Nymph only – colonies composed only of nymphs; and Female only – colonies composed only of adult females. Each experimental group was maintained in 10-cm-diameter Petri dishes containing one bark fragment and 7–10 individuals. Despite the fact that experimental colonies had a small number of individuals relative to the colony assayed for behavioral repertoire analysis, it is common to find in the field colonies of 5–10 individuals, with all ages and sex represented. These small colonies in field conditions behave typically like a colony in initial phase of establishment. Under laboratory conditions, small colonies enable more accurate observation of behaviors.

The colonies were kept in the laboratory for 3 wks, time enough for the pseudoscorpions to build their silk chambers. After this time, each colony was observed during 10 h, following the ‘all occurrence samples’ method (Altmann 1974). These 10 h of observation were divided into sessions of 30 min. Observations were carried out in a maximum period of 3 mo in each colony, totaling 80 h for each treatment. The behaviors observed were classified into new groups of tasks that were behaviors related to colony maintenance, i.e. behaviors directly related to the basic tasks of colony maintenance such as cleaning, prey capture, building of silken chambers, and parental care. The frequencies of each of these tasks were quantified for each experimental colony, and a flowchart was developed from the mean values of task performance (behavioral sequences) for each treatment. The mean values of task performance were compared using a G test to assess possible treatment effects on maintenance tasks.

Results

Activity Schedule and Behavioral Repertoire

The 14 431 behavioral repetitions for all the developmental stages (protonymphs, deutonymphs, tritonymphs, and adults) yielded a behavioral repertoire of the reproductive colony of *Paratemnoides nidificator* comprising 95 distinct behavioral acts, classified into 10 behavioral categories (Table 1). The most diversified category was communication with 36 different behaviors based on large variation of pedipalps vibration (see fully detailed repertoire in Table S1).

![Table 1: Behavioral repertoire of *Paratemnoides nidificator* described from 34 h of observation of one reproductive colony with 94 individuals (30 adults, 18 tritonymphs, 12 deutonymphs, and 34 protonymphs) maintained in laboratory. Details of each behavioral type compounding the behavioral categories can be found in Table S1](image-url-here)
distribution pattern yielded an $r^2 = 0.998$, $a = 21.847$, and $b = 0.206$. The total behavioral acts recorded fitted 89% of the estimated behaviors; the behavioral estimation was 106 behavioral acts and would require 43 observation sessions to reach 90% of the estimated behaviors. The frequencies of each behavioral category for the juvenile and adults were organized in Table S1.

**Division of Labor in Reproductive Colonies**

Males and non-reproductive females (young or old females, inseminated or not, but for some reason did not reproduce) execute similar tasks, mainly prey capture and external silk chamber maintenance (debris removal) (Fig. 2a). Males and non-reproductive females also provide direct and indirect parental care by allowing immatures to feed on their prey or by directly offering food to nymphs. Reproductive females engage in parental care and provide protection (against natural enemies), nursing, and food to their brood. Reproductive females also contribute to colony physical growth by building new reproductive chambers, which might later serve as communal chambers, but this production is smaller than that of nymphs (around 35% of the colony chambers). Because the young individuals build most of the colony chambers, they are mainly responsible for the physical expansion of the colony. They also cooperate to internally maintain the nest by removing exuvia and prey remains (Fig. 2a). Tritonymphs rarely contribute to prey capture. Details of tasks performance and its mean values in each experimental age and gender groups can be found in Tables S2 and S3.

**Experimental Colonies and Task Organization**

Experimental colonies behaved differently from the control colonies. There was a highly significant treatment effect on task performance ($G = 201.57$; df = 12; $p < 0.0001$). Non-control colonies lacked some maintenance behaviors. In the Male-only colonies, the animals were unable to build or clean chambers. Thus, pre-existing chambers (built by nymphs) were littered with feces and prey carrion, facilitating the proliferation of fungi (Fig. 2b). Male-only colonies only survived 2 mo (67 ± 33 d, n = 8). In Female-only colonies, few silk chambers were constructed (2 ± 1, n = 8). Only previously inseminated females were able to build chambers, but only to shelter their embryos and nymphs. Colony stability was only reached after protonymphs emerged (Fig. 2c). Adult females did not perform nest maintenance behaviors, and Female-only colonies survived an average of 3 mo (92 ± 38 d, n = 8).

By contrast, Nymph-only colonies performed all the maintenance behaviors observed in normal reproductive colonies, but with some differences. They exhibited activity adjustments and assumed functions that they do not normally perform; they captured prey cooperatively, built silk chambers, and engaged in both forms of debris removal (inside and outside of chambers) (Fig. 2d).

**Discussion**

The hypothesis that *P. nidificator* colonies display division of labor and that the colonies are organized through an age-based system of labor division was confirmed by this study. This pseudoscorpion has
one of the most elaborate behavioral repertoires known among social invertebrates, and our study provides the first clear evidence of division of labor in arachnids. The observation of division of labor in *P. nidificator* provides new information not only on the Pseudoscorpiones order, but also on sociality in arachnids. The analysis of the behavioral repertoire revealed that *P. nidificator* has a very complex behavioral structure. It was expected that the diversity of behavioral acts would be related to the species social complexity (Lehner 1996). Among South American arachnids, behavioral repertoire studies in species of Opiliones and Scorpiones also revealed the occurrence of some degree of sociality. Scorpions *Tityus serrulatus* Lutz & Mello, 1922 (Buthidae) constitute larger aggregations, but have a lower social relation and a poorly diversified behavioral repertoire (Mineo et al. 2003). The gregarious harvestmen *Discocyrtus oliveroi* (Gonyleptidae) and *Mischonyx cuspidatus* (=*Ilhaia cuspidata*) (Gonyleptidae) constitute groupings with richer repertoires and more extensive social relationships (Elpino-Campos et al. 2001; Pereira et al. 2004). The social amblypygi *Damon diadema* have a more complex social organization than scorpions, harvestmen, and several other spiders (Walsh & Rayor 2008). In relation to these species, *P. nidificator* had a more diversified repertoire, especially with regard to the number of behavioral acts related to social interactions.
The sample coverage analysis confirms that the behavioral repertoire of *P. nidificator*, in all its development stages, can be considered complete. The analysis based on the behavioral accumulation curve corroborated these results. According to the model by Dias et al. (2009), the asymptote was not reached and the estimated value was slightly higher. The final analysis indicated that 89% of the estimated behavioral acts were observed, which allows us to consider the repertoire as nearly complete.

**Pedipalp Signaling as Communication Behavior**

In his classic study of pseudoscorpion biology, Weygoldt (1969: 25) postulated ‘It seems probable, therefore, that they have some behavioral device by which they recognize each other or, more scientifically expressed, by which the predatory aggression is suppressed in members of the same species.’ In fact, the pedipalps have some communication function. Previous research has established that body, leg, and mainly pedipalp vibrations function in courtship and in mediating aggressive interactions among adults (Weygoldt 1969; Zeh & Zeh 1997; Andrade & Gna- spini 2003). Communication behavior based on pedipalp vibrations was the most diversified behavioral category exhibited by *P. nidificator* (Table I and Table S1), possibly with purposes other than courtship and reproduction. For example, the signaling among nymphs could be related to non-aggressive contests for access to food.

**Division of Labor in Spiders**

Division of labor is well studied in eusocial insects (Wilson 1971; Hölldobler & Wilson 1990; Beshers & Fewell 2001), but has not previously been unambiguously demonstrated in social arachnids. In Araneae, task specialization appears not to occur, and all colony members are able to perform the tasks needed for colony maintenance (Whitehouse & Lubin 2005). D’Andrea (1987) suggested that the social complexity of some spider species would provide the conditions required for the evolution of task specialization. Studying *Achaearanea wau* (Theridiidae), Lubin (1995) did not observe evidence of division of labor, and Ainsworth et al. (2002) did not find differences in prey capture behavior of *Stegodyphus mimosarum* (Stegodyphus) in relation to the size of the spiders. Evans (2000) obtained suggestive evidence for division of labor showing that subadult males of *Diaea ergandros* (Eresidae) contributed to the building of capture webs.

Although division of labor is not very clear in spiders, evidence of task partitioning exists. For example, in the cooperative spider, *Anelosimus eximius* (Theridiidae), competition for food can result in reproductive and non-reproductive females in the same nest. Several authors have reported that tasks that involve more risks and performance outside the nest are preferentially executed by adult females (e.g. foraging), while juveniles usually repair webs, a less vulnerable activity (Christenson 1984; Avilés 1993; Lubin 1995).

According to Lubin (1995), inbreeding would be a factor restricting the evolution of division of labor. Endogamic reproduction, as occurs in cooperative spiders, would favor low genetic variation and contribute to homozygosity, limiting the expression of genes for extreme altruism that would only be expected to occur in heterozygotes (Michod 1980, 1993; Lubin 1995). Beside these reproductive and genetic constraints, task specialization could diminish individual reproductive success in cooperative spiders (Lubin 1995).

In spider colonies, all females are totipotent. However, there are traces of reproductive asymmetry that could result in reproductive skew (Rypstra 1993; Gonzaga & Vasconcellos-Neto 2002; Whitehouse & Lubin 2005; Avilés et al. 2006). Thus, the absence of division of labor can result from the selection for a ‘strategy of colony growth’ and not for specialization in work. Division of labor would reduce the number of reproductive females and consequently the foundation of new colonies (Avilés 1993; Lubin 1995).

**Division of Labor in *Paratemnoides nidificator***

Communication and individual recruitment are key elements for the division of tasks among social hymenopterans (Wilson 1971; Beshers & Fewell 2001). Our findings demonstrated a great diversity of communicative behaviors, but do not clarify how communications mediate the division of labor. However, tasks do not appear to be regulated by recruitment mechanisms. Rather, the synchrony among individuals, without clear communication during tasks, suggests that work can be regulated by self-organization. In other words, similar traits, added to the physiological and behavioral characteristics of the development stage or sex, stimulate similar behavioral responses (Hemelrijk 2002a,b). Although communication behaviors have been the most frequent and diversified ones, they possibly are not related to the division of tasks.

There are some similarities and differences between the task distribution of *P. nidificator* and that...
of social spiders. In the case of Aebutina binotata, there is also an asymmetrical performance of colony tasks (Avilés 1993). Nymphs also perform maintenance tasks more frequently than adults. However, Avilés (1993) did not evidence division of labor among individuals of the same age. By contrast, P. nidificator have an elaborate organization with groups of individuals performing group of tasks based on their morphological or physiological limitations. The reallocation of tasks by nymphs, when no adults are present, reinforces both the adaptive importance of this organization and nymph behavioral plasticity, allowing colony survival.

Our findings show that the behavioral flexibility of P. nidificator nymphs is an important attribute in colony maintenance and stability. However, we still need to explain what mechanisms cause the behavioral restrictions or stimulate the changes in task performance by nymphs. The role of juveniles in colony work and their capability to adjust task in absence of a caste also occurs in termites. In experimental colonies of lower termite Reticulitermes fukienensis (Rhinotermitidae), in absence of large workers, medium-sized workers adjusted their behaviors to perform tasks mainly performed by large workers (Crosland & Traniello 1997). In the absence of workers, nymphs of Coptotermes formosanus (Rhinotermitidae) are capable of feeding and surviving by themselves, despite the existence of soldiers specialized for feeding (Crosland & Su 2006). In social Hymenoptera, age polyethism is common and early instars have morphological and physiological characteristics that predispose them to carry out colony tasks not performed by adults (Wilson 1971; Robinson 1992). Similar to termites (Noirot & Pasteels 1987), pseudoscorion nymphs are small version of adults with little morphological limitations to task cooperation. However, the small size of nymphs, shortened palp segments, and body fragility make juveniles less efficient in all tasks compared with adults. Variation in task performance is therefore expected, and we suggest that behavioral flexibility has important consequences for the maintenance of colony cohesion in Paratemnoides.

Why do Male Paratemnoides nidificator Share Work and Take Care of Brood?

Only exceptionally do males of invertebrate social species participate in colony work. Their sole contribution to the colony is gene transmission (Wilson 1971; Beshers & Fewell 2001; but see also Santos & Del-Claro 2009). Some factors that could limit male work include lower lifespan than females, premating dispersion when they reach adult age, and significant deviation of sex ratio in favor of females.

Isopterans are among the few examples of animals whose male work. In this group, both sexes engage in similar tasks in the colony. The sex ratio in isopteran species is typically close to one (Roisin 2001). Males and females have similar longevity, morphology, and behaviors (Thorne 1997). Males of the wasp Ropalidia marginata can contribute to feeding nestlings in the period of colony growth, before dispersion, but not as efficiently as females (Sen & Gadagkar 2006). Among spiders, young males of Diaea sp. can help weave the web that shelters the colony (Evans 2000).

Paternal care is also uncommon among invertebrates and is generally correlated with paternity assurance (Zeh & Smith 1985). Males providing care could increase their chances of being selected by females and their paternity rates (Queller 1997; Whittingham & Dunn 1998; Kvarnemo 2005). Among arachnids, parental care is more frequent and best understood in harvestmen (Machado et al. 2004; Requena et al. 2009). However, several factors could favor the evolution of parental care and the division of labor in P. nidificator. Among them are a close to one sex ratio (2/3); equal male and female life expectancy (approximately 3 yrs of adult life); similar body size in males and females; and ability of both sexes to produce poison, enabling males to be nearly as successful as females in hunting, defending, and maintaining the colony, and dispersion of both sexes is very limited and males can remain in the mother colony during their entire life (Tizo-Pedroso & Del-Claro 2007; Del-Claro & Tizo-Pedroso 2009). All these features constitute a complex system in which each characteristic interacts with the others to offer favorable conditions for the development of a division of labor that includes males making a significant contribution to colony maintenance (Fig. 3).

The same factors that have favored the evolution of male work could also favor the development of male brood care. Paternity uncertainty does not seem to be a fundamental requirement in the evolution of paternal care in this system, because the colonies have restricted flux of individuals among them. Thus, high relatedness among males and between males and nymphs in a colony is likely. Therefore, males can obtain indirect fitness benefits by taking care of related nymphs. The participation of males in colony work and cooperative parental care appears to be a strategy to organize and maximize colony productivity. As a consequence,
reproductive females can concentrate their effort on reproductive activities, which increases colony growth and originates new propagules to disperse and found new colonies.

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Literature Cited


Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Behavioral repertoire of Paratemnoides nidificator (Pseudoscorpiones, Atemnidae), based on 34 h of observations of a colony with 94 individuals (30 adults, 18 tritonymphs, 12 deutonymphs and 34 protonymphs) maintained in captivity.

Table S2. Mean value of maintenance tasks performance in experimental colonies of Paratemnoides nidificator.

Table S3. Mean value of maintenance tasks performance in experimental colonies of Paratemnoides nidificator in activities of age and gender division of labor. The abbreviations indicate respectively: (M) males; (RF) reproductive females; (nRF) non-reproductive females; (N) nymphs.

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