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A characterization of social interactions across age and sex in the amblypygid *Paraphrynus laevifrons*

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Abstract. Although our basic knowledge regarding the natural history of amblypygids (commonly called whip spiders; Order: Amblypygi) is expanding, there is much about these mysterious animals that remains unknown and unexplored. In particular, we know relatively little about social interactions and potential communication displays within and across sexes, despite observed variation in species studied to date. To acquire basic knowledge about amblypygid communication, we quantified the behavior of juvenile and adult *Paraphrynus laevifrons* (Pocock, 1894) across social interaction types. Specifically, we staged the following adult interaction types – male-female, female-female, and male-male - as well as juvenile-juvenile interactions. Adults performed an array of behavior during social interactions. Some of the observed behaviors are similar to those reported in other species, while others we describe here *de novo*. Across adult social interaction types, we found no significant differences in social interaction duration nor escalation. Adult social interactions were more likely to escalate to pedipalp grappling the larger the size of the interacting pair. Juveniles rarely engaged in social interactions, and those that did occur were shorter than adult social interactions; juvenile social interactions never escalated beyond antenniform leg touching, a behavior associated with examining an individual's surroundings. We discuss the behavioral repertoire of social interactions in *P. laevifrons* in relation to what is known from other amblypygid species.

Keywords: Social behavior, behavioral ecology, arachnids, communication, whip spider

The literature examining arachnid social interactions is extensive (reviewed in Pinto-da-Rocha et al. 2007; Beccaloni 2009; Foelix 2011), especially in reference to spider intersexual interactions (e.g., Elias et al. 2003; Girard et al. 2011; Herberstein et al. 2014) and intrasexual interactions (e.g., Taylor et al. 2001; Elias et al. 2008; McGinley et al. 2015a, b). These studies often address fundamental questions regarding sexual selection, signal evolution, and speciation (see Miller et al. 1998; Masta & Maddison 2002; Elias et al. 2006; Schwartz et al. 2014; Anderson & Hebets 2016; Watts et al. 2019), and thereby have broad implications in evolutionary biology and ecology. While this rich literature on spider social interactions continues to expand, there is unfortunately much less known about conspecific interactions in those arachnid groups with less-known species diversity (the "smaller arachnid orders," Harvey 2003) such as amblypygids.

Amblypygids (Order: Amblypygi) are relatives of spiders that are distinguished, in large part, by their extraordinarily long first pair of legs (i.e., antenniform legs), which they can use during social interactions (reviewed in Santer & Hebets 2011a). While our understanding of amblypygid biology continues to expand, there is much about these mysterious animals that remains unknown and unexplored. Of the 151 extant amblypygid species, only 5 species from 3 genera have recently been studied in terms of conspecific communication (Chapin & Hebets 2016).

Our knowledge of intersexual communication in amblypygids comes from a relatively small numbers of species whose courtship and mating behavior have been described through the foundational work of Peter Weygoldt (reviewed in Weygoldt 2000), along with opportunistic observations from the field (Chapin & Hebets 2016). These studies demonstrate that amblypygids from different families and genera perform diverse arrays of behavior during social interactions, some of which may last for upwards of eight hours. These interactions include elaborate, stereotyped movements of the antenniform legs and pedipalps, wherein males use these putative tactile and near-field sound signals to direct females towards a spermatophore that they had deposited on the substrate (e.g., *Damon medius* (Herbst, 1797), Weygoldt 1999).

Most studies of amblypygid social interactions have focused on intrasexual, or agonistic, interactions (see *Phrynus marginemacultatus* C.L. Koch, 1840; Fowler-Finn & Hebets 2006; Rayor & Taylor 2006; Santer & Hebets 2008, 2011b; *Phrynus longipes* (Pocock, 1894), Chapin 2015; Chapin & Hill-Lindsay 2015; *Heterophrynus longicornus* (Butler, 1873), Porto & Peixoto 2013). Specifically, intrasexual interactions have been explored relatively recently in three species—two within the genus *Phrynus* (Family: Phrynidae; *Phrynus marginemaculatus,* Fowler-Finn & Hebets 2006; Santer & Hebets 2008; Walsh & Rayor 2008; *Phrynus longipes,* Chapin 2015) and one in the genus *Damon* (Family: Phrynichidae; *Damon diadema* (Simon, 1876), Walsh & Rayor 2008). These interactions often include both animals examining each other with their antenniform legs, holding their pedipalps in stereotyped positions, and vibrating their antenniform legs over each other's walking legs. Antenniform leg vibrations produce near-field sound (Santer & Hebets 2008) and the duration of this display is correlated with agonistic contest outcome (Fowler-Finn & Hebets 2006). Individual size and territory ownership have also been studied in relation to intrasexual interaction outcome (Chapin & Hill-Lindsay 2015; Chapin & Reed-Guy 2017). These latter studies also suggest that the potential risks associated with intrasexual interactions – in the form of cannibalism – vary across species. For example, cannibalism is regularly observed during agonistic contests in *P. longipes* (Chapin & Reed-Guy 2017), but rarely observed in *D. diadema* (Rayor & Taylor 2006) and never observed in *P. marginemaculatus* (Fowler-Finn & Hebets 2006; Rayor & Taylor 2006).

Studies of juvenile conspecific interactions and potential communication are rare in amblypygids (but see Rayor & Taylor 2006; Walsh & Rayor 2008), as is the case for most communication research. In the amblypygid *Damon diadema,* however, careful observations of juveniles suggest that they may be capable of kin recognition, as measured by a greater number of tolerant touches with their antenniform legs (Walsh & Rayor 2008). Similar observations across conspecific interactions in non-kin may provide insight into basic amblypygid natural history, such as whether juveniles maintain territories. These observations could additionally contribute to our understanding of how social behavior develops throughout an individual's ontogeny.

Here we provide a quantitative comparison of adult–adult intersexual and intrasexual, and juvenile-juvenile conspecific interactions in the amblypygid *Paraphrynus laevifrons*. We also provide the first descriptions of social behavior in this species (and in the genus *Paraphrynus* as a whole). Specifically, we (1) describe and compare the behavior present in adult-adult intersexual (male-female, "MF"), adult-adult intrasexual (female-female, "FF"; male-male, "MM"), and juvenile-juvenile ("JJ") social interactions, (2) compare social interaction presence and duration across interaction types, and (3) compare social interaction escalation and outcome across interaction types. Our goals were to identify whether these characteristics of social interactions differed between adult males, adult females, and juveniles, and whether any behaviors are specific to particular social interaction types.

METHODS

Animal collection and maintenance.—*Paraphrynus laevifrons* were collected from Las Cruces Biological Station, Coto Brus County, Costa Rica in January 2016 (first trial block) and June 2016 (second trial block) and maintained at the University of Nebraska-Lincoln, USA. *Paraphrynus laevifrons* can be found abundantly along creeks and trail sides within the secondary growth, tropical wet forest at Las Cruces Biological Station (Corey & Hebets 2017).

We maintained individuals in the first trial block in a large animal room housing other arachnids from the time of their collection (January 2016) until May 2016. Each individual was housed in a plastic cage (Pioneer Plastics) with dimensions of either 5.5 cm x 5.5 cm x 7.5 cm, 9 cm x 9 cm x 11 cm, or 18.9 cm x 13.5 cm x 9.5 cm depending on the individual's size. Cages rested on metal grating in 63.5 cm x 39.4 cm x 15.2 cm plastic tubs (Tupperware) filled with water. We maintained humidity in each cage by placing a cotton dental wick half way through a 0.5 cm diameter hole at the bottom of each cage. The bottom of wick rested in water in the tub, thus providing moisture to the cage.

Upon collecting the second batch of individuals, we moved all amblypygids to a temperature-controlled room. In this new room, we placed each individual in a 22.9 cm x 16.2 cm x 16.5 cm plastic terrarium (Exo Terra® Faunarium PT2255) with window mesh covering all sides; a 2.5 cm layer of coconut fiber substrate (Zoo Med); a 10.2 cm x 16.2 cm x 0.64 cm piece of cork tile (Quartet); and a small petri dish filled with water. We maintained humidity in the room by refilling three humidifiers (two Crane Ultrasonic Cool Mist Humidifiers, one Bemis Model 821 000 humidifier) every 1–2 days. Temperature was kept at 26°C. In instances where the temperature dropped due to the heat being shut off in our laboratory building, we added space heaters to keep the temperature as close to 26°C as possible; no experimental trials were conducted during this time. All individuals were maintained on 12-hour day/night cycle (light from 0600–1800 hours, dark from 1800–0600 hours) consistent with the day/night cycle in their natural habitat. Coincident with initial housing set-up, we measured the cephalothorax width (CW) at the widest point of each *P. laevifrons* (in mm) using digital calipers.

Behavioral trials.—In order to observe social interactions between pairs of *Paraphrynus laevifrons*, we simultaneously introduced two individuals to an experimental arena under laboratory conditions. Due to unavoidable limitations on collecting and transporting specimens, we used each *P. laevifrons* in multiple trials to increase sample size (see **Statistical analyses** subsection below); however, we never repeated pairings between individuals for behavioral trials in this experiment, as social interactions between amblypygids can be affected by prior experience between individuals (Fowler-Finn & Hebets 2006). We observed and scored the behavior of both individuals in all pairings.

Since amblypygids are nocturnal (Weygoldt 2000), we ran all behavioral trials in darkness during their normal lights-off cycle—i.e., between 1800 and 0100 hours. We filmed all social interactions with infrared cameras (Sony HDR-SR10 Handycam). Before a trial, we took individuals from their housing room to the experimental room. Since we commonly find *Paraphrynus laevifrons* on vertical surfaces (Corey & Hebets 2017), we built a vertical experimental arena within which to run behavioral trials. The arena had a vertical 81.3 × 50.8 cm corkboard-climbing surface; bordered by a 14.0 cm tall acrylic frame that *P. laevifrons* was unable to climb. The vertical climbing surface consisted of two panels of 40.6 cm x 50.8 cm Elmer's Cork Foam Board that individuals could easily grip and climb (Corey, pers. obs.). We attached an 81.3 cm x 36 cm acrylic panel to the base of the arena at an approximately 70° angle to prevent individuals from climbing onto the ground and out of the arena (Supplemental Video S1, online at http://dx.doi.org/10.1636/JoA-S-19-049.s1). We simultaneously removed the two amblypygids from their cages and placed them on opposite ends of the experimental arena (by placing each individual's cork parallel to the arena's climbing surface). If individuals did not readily move to the desired location, we gently touched the sides of their walking legs to guide them. A behavioral trial began as soon as both individuals were climbing inside of the arena.

For each trial, we defined the start of a social interaction as the time in which individuals were within mutual antenniform leg length and one or both individuals demonstrate directed antenniform leg movement towards the other individual. We defined the end of a social interaction to be the time in which both individuals were outside the range of two mutual antenniform leg lengths. In cases where individuals could not move beyond two mutual antenniform leg lengths due to the acrylic wall, we considered a social interaction over if one or both individuals attempted to climb on the acrylic wall, or walked into the wall and made contact with their bodies or antenniform legs. In some social interactions that escalated to pedipalp grappling, one or both individuals were unable to retreat or were clearly injured ($n = 3$). In such instances, we separated individuals immediately and returned them to their housing.

Due to the availability of animals, we structured our experiment in two trial blocks. In the first block (21 March 2016 – 8 April 2016), we ran JJ behavioral trials and pilot adult behavioral trials with individuals from a first collection. In the second block (17 July 2016 – 17 October 2016), we ran adult behavioral trials (MF, FF, MM) with individuals from a second collection. We ran 21 juvenile *P. laevifrons* in the first block, and 16 adult female and 22 adult male *P. laevifrons* in the second block. For the first block, we ended trials after one social interaction occurred, regardless of trial length. We ran trials in the second block until the amblypygids had completed one social interaction (regardless of duration), or until a maximum of 30 minutes had elapsed without any social interaction.

Juvenile amblypygids in the first block were randomly paired together, as their sex could not be confidently identified and we had no *a priori* expectation about juvenile size and aspects of social interactions. Amblypygids in the second block were size-matched and paired blind to social interaction type, as we found in pilot trials that size-matched contests encompassed a greater range of behaviors (as in related amblypygid species, see Fowler-Finn & Hebets 2006) – especially a greater variety and frequency of antenniform leg vibrations and pedipalp movements (see Table 1) – and our main goal was to describe the behavioral repertoire of *P. laevifrons* social interactions.

Determining age and sex.—We used measurements of cephalothorax width to separate individuals into juvenile or adult age categories based on prior studies reporting the size at which related amblypygid species reach reproductive maturity– i.e., <10 mm = juvenile and >10 mm = adult (Weygoldt 2000; Corey & Hebets 2017). Amblypygids molt and grow continuously throughout their entire lives and they lack clear external characters of sexual maturity (Weygoldt 2000); we also could not confidently identify sexual maturation via internal genital morphology (see description below). Given this constraint, we followed previous suggestions by Weygoldt (2000) regarding the likely conservative (for this relatively large species; maximum cephalothorax width in this study = 16.2 mm) size-based age class determination. We recognize that our method of inferring age class based on size could lead to the incorrect categorization of individuals, and interpret our results with this in mind.

As mentioned previously, the sex of *Paraphrynus laevifrons* is difficult to determine based on external morphology alone. While some amblypygid species are sexually dimorphic, particularly in reference to pedipalp size (McArthur et al. 2018), *P. laevifrons* is not conspicuously so. In related amblypygid species, the shape of the genital opercula on the ventral side of the opisthosoma can be used to identify the sex of an individual—trapezoidal in females, oval in males (Hebets 2002). However, we have found this method to be inconsistent for *P. laevifrons*, as subsequent sex identification using internal morphology (using invasive procedures following behavioral trials) found a number of individuals to be misidentified for pilot trials. Thus, we paired adult *P. laevifrons* blind to sex in this experiment, and identified sex via internal genital morphology only after behavioral trials were completed. To determine an individual's sex, we lifted the genital opercula of live amblypygids under CO_2 anesthesia using soft forceps, after which we could identify internal genital morphology. If amblypygids died in the laboratory prior to the completion of behavioral trials for this experiment, we dissected them to identify internal genital morphology. Our inability to accurately identify all individuals by sex in the first block and our purposeful blind pairing and scoring of individuals in the second block ensured that behavior scoring was done naively with respect to social interaction type.

Quantifying and comparing behavior.—We described behavior after watching and annotating dorsal- and lateral-view video footage from 80 different behavioral trials (TBC). The lateral-view camera footage allowed for more precise discrimination of antenniform leg movements and contact points. We referenced published ethograms of amblypygid social behavior to identify previously described behavior. We had to observe a given behavior in a minimum of 5 distinct social interactions to include it in our formal description (Table 1).

We defined social interaction 'duration' as the difference in time between our determined start and the end of the interaction (see **Behavioral trials** above). We scored 'escalation' as an ordinal variable (1–3) based on the presence of specific behaviors – i.e., 1 = *examine* – individuals made contact via antenniform leg touches; 2 = *display* – individuals opened their pedipalps and/or performed antenniform leg vibrations; 3 = *grapple* – individuals made physical contact and grappled each other with their pedipalps. Behavioral trials where individuals were within mutual antenniform leg length but did not make contact with their antenniform legs were given a score of 0 and excluded from escalation analyses. If one or both individuals performed the requisite behavior(s), the social interaction pairing was assigned the given escalation score. For descriptions of these behaviors, see Table 1. While social interactions could move back and forth from different stages of escalation, they generally followed the above-mentioned pattern—e.g., grappling was typically preceded by some period of antenniform leg touches, opened pedipalps, and antenniform leg vibrations. We scored each social interaction at the highest level of escalation observed during it.

Statistical analyses.—We created behavioral repertoires for each type of interaction we examined (MF, FF, MM, JJ). This part of the study was mainly descriptive, so we did not include statistical analyses, but we did examine the presence/absence of particular behaviors across interaction types.

We used a one-way ANOVA to test if the duration of social interactions (log-transformed) could be predicted by adult social interaction type. We omitted behavioral trials from this analysis where adult amblypygids did not interact, and where we separated individuals that had been grappled into submission ($n = 3$, see **Behavioral trials**), because we did not have a clear measure of the end time of the social interaction. After finding no differences in duration across adult interaction types (see RESULTS), we used a general linear model to secondarily explore if interaction duration could be predicted by the average size of the interacting pair (measured in cephalothorax width, in mm) across all adult social interactions. We used ordinal logistic regression to compare social interaction escalation across MF and MM social interactions. We excluded FF social interactions from this analysis and did not examine an effect of size on escalation to a given category due to limited sample size. In order to have sufficient sample

Table 1.—Ethogram of behavior observed during social interactions in *Paraphrynus laevifrons*. Behaviors are organized by their presence in escalating stages of interactions, from lowest to highest (see *Quantifying and Comparing Behavior* subsection in Methods for details). Each of the following behaviors were observed a minimum of 5 times, in distinct pairings, across all behavioral trials that we conducted for which we recorded both dorsal and lateral views of social interacts with sufficient video quality ($n = 80$ trials). We referenced recently published ethograms for behaviors that have been previously defined in specific detail, and note behaviors described for the first time in this study to our knowledge. We define a focal individual as the amblypygid performing a given behavior of interest during a social interaction (the putative signaler), and the target individual as the amblypygid that behavior is directed towards (the putative receiver). Therefore, both amblypygids can be both focal and target individuals – at the same or different times – throughout a social interaction.

Category	Behavior Name	Description	References
Antenniform Leg Touch	Antenniform Leg Tap	Focal individual makes contact with target individual using the distal end of their antenniform leg(s).	"Contact" (Fowler-Finn & Hebets 2006); "Touch other amblypygid" (Walsh & Rayor 2008); Supplemental Videos S1 and S2
	Antenniform Leg Probe	Focal individual makes contact with target individual using any more proximal region of the antenniform leg(s), e.g., flexes the center of an antenniform leg around target's leg.	"Contact" (Fowler-Finn & Hebets 2006); "Touch other amblypygid" (Walsh & Rayor 2008); Supplemental Videos S1 and S2
Pedipalp Position	Pedipalps Closed	Pedipalps are held in resting position in line with chelicerae, such that patella and trochanter are approximately parallel.	Agonism low" (Walsh & Rayor 2008); Supplemental Video S1
	Partial Pedipalp Open	Pedipalps are held open such that the patella – trochanter joint forms an acute angle.	"Partial pedipalp open" (Fowler-Finn & Hebets 2006, Santer & Hebets 2008); "Agonism low" (Walsh & Rayor 2008); Supplemental Video S1
	Full Pedipalp Open	Pedipalps are held open such that the patella and trochanter joint form an angle $\geq 90°$	Fowler-Finn & Hebets 2006, Santer & Hebets 2008; "Agonism high" (Walsh & Rayor 2008); Supplemental Video S1
Antenniform Leg Vibration	Pulse	Focal individual vibrates antenniform leg(s) while they are not completely outstretched.	*This study*; Supplemental Video S3
	Flick	Focal individual laterally vibrates completely outstretched antenniform leg(s) towards target individual.	"Antenniform leg flick" (Fowler-Finn & Hebets 2006); Supplemental Video S4
	Whip Crack	Focal individual rapidly moves completely outstretched antenniform leg(s) in perpendicular plane towards target individual.	*This study*; Supplemental Video S4
	Whip Flap	Focal individual rapidly moves entire antenniform leg(s) back and forth in anteroposterior plane with its body.	Weygoldt (2002) description; Supplemental Video S3
Misc.	Groom	Focal individual grooms its antenniform leg(s) using its pedipalps and chelicerae.	(Walsh & Rayor 2008)
	Body elevated off substrate	Focal individual straightens walking legs more than during typical resting posture, such that the body is no longer in contact with the substrate.	(Walsh & Rayor 2008); Supplemental Videos S1, S3, and S4
	Pedipalp Reach	Focal individual slowly reaches towards target individual using one or both pedipalps. Focal individuals may or may not make direct contact with target individual.	"Bat/swat palps" (Walsh & Rayor 2008); Supplemental Videos S1 and S3
	Pedipalp Strike	Focal individual rapidly moves towards target individual and uses pedipalps to attempt to hold it, as in prey capture.	"Grab" (Walsh & Rayor 2008); Supplemental Video S5
	Pedipalp Grappling	Both interacting individuals make contact and grapple with their pedipalps.	"Pedipalp contact" (Fowler-Finn & Hebets 2006); Supplemental Videos S1 and S5

size to explore an effect of size on social interaction escalation, we combined observations from MF, FF, and MM trials, and measured the probability that social interactions escalated to pedipalp grappling as a binomial response. Specifically, we used logistic regression to test if the average size of the interacting amblypygids affected the probability that adult social interactions escalated to grappling.

Given that we re-ran individual *P. laevifrons* in multiple trials, we examined our data in three subsets in order to increase our confidence in our observed patterns while limiting confounds from repeated measures of individuals. Specifically, we examined (a) "combined trials" – the first two behavioral trials that individuals participated in, for individuals that were run in at least two or more trials, (b) "all trials" – which include all behavioral trials conducted during this experiment, and (c) "first trials" – only the first behavioral trial that individuals participated in. Unfortunately, pairing amblypygids randomly with respect to sex in the second trial block led to a strongly biased dataset; regardless of how we subset our data, we have far fewer FF behavioral trials than MF or MM behavioral trials. We based our results and conclusions in this study from the (a) "combined trials" subset ($n = 17$ MF, 3 FF, 13 MM, 19 JJ), which provides sufficient sample sizes to make statistical comparisons between groups while limiting pseudoreplication; see Supplementary Table S1 (online at http://dx.doi.org/10.1636/JoA-S-19-049.s6). We compared our findings from each analysis using all three data subsets and found no qualitative differences in our results (data not shown). Statistical analyses were performed in R (version 3.4.2) and SPSS (IBM, Trial Version, Build 1.0.0.1275).

RESULTS

Description of behaviors.—Adult *Paraphrynus laevifrons* present an array of behaviors during social interactions, some of which have been reported in previous studies on amblypygids and several we describe here *de novo* (Table 1; Supplementary Videos 1-5, online at http://dx.doi.org/10.1636/JoA-S-19-049.s1 through http://dx.doi.org/10.1636/JoA-S-19-049.s5). We describe specific types of antenniform leg vibrations – "pulse" and "whip crack" (Supplementary Video S4) – as novel behavior. In comparison to adults, the behavioral repertoire in juvenile *P. laevifrons* is much more limited. Specifically, we did not observe partially/fully opened pedipalps, antenniform leg vibrations, pedipalp grasps, or pedipalp grappling in JJ interactions. Adult *P. laevifrons* often maintain contact with each other using their antenniform legs throughout social interactions. Adult *P. laevifrons* perform antenniform leg vibrations that we categorize based on general intensity, orientation, and degree of antenniform leg extension. Additionally, the behaviors observed in adults were found in each interaction type—i.e., no behavior was specific to a particular social context.

Social interaction presence and duration.—There were no differences in social interaction duration across adult interaction types (ANOVA; $F_{2,\ 25} = 0.816$, $P = 0.454$). When considering adult interaction types together (i.e., combining observations from MF, FF, and MM trials), the average size of an interacting pair did not affect social interaction duration (general linear model; $t = 0.975$, $P = 0.338$). All but two adult behavioral trials (31 of 33) led to social interactions, while juvenile *Paraphrynus laevifrons* engaged in social interactions in only three of 19 behavioral trials (15.8%). Juvenile-juvenile social interactions were shorter on average than adult social interactions (Fig. 1, Table 2).

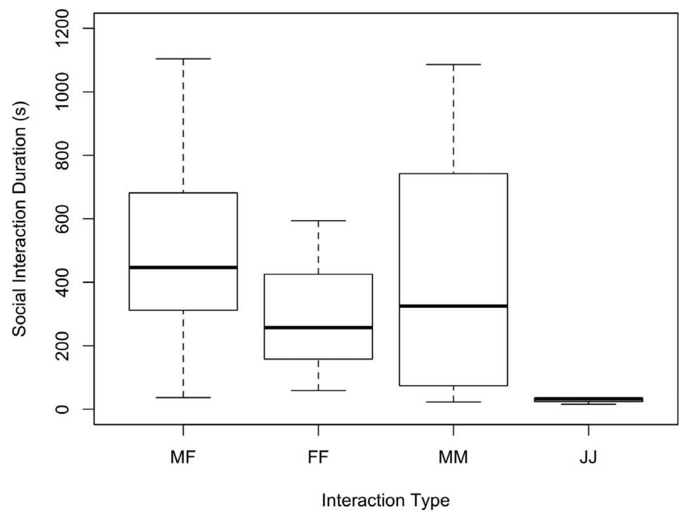

Figure 1.—*Paraphrynus laevifrons* social interaction duration across four different social interaction types (MF = adult male and adult female, FF = two adult females, MM = two adult males, JJ = two juveniles). Juvenile interactions were shorter than adult social interactions, but there were no differences in duration between adult social interaction types. Durations shown here are from behavioral trials from the combined trials subset (see *Methods, Statistical Analyses*) that led to social interactions (n = 17 MF, 3 FF, 11 MM, 3 JJ).

Social interaction escalation.—The large majority of social interactions between adult *P. laevifrons* included putative display behavior (i.e., opening pedipalps and/or antenniform leg vibrations), and nearly half of all social interactions escalated to pedipalp grappling (Table 2). When comparing only MF and MM behavioral trials that led to social interactions, there were no differences in the probability of escalation to putative displays (i.e., opening pedipalps and/or performing antenniform leg vibrations) and pedipalp grappling, regardless of interaction type (ordinal logistic regression; log odds = 0.47, SE = 0.765, Wald statistic = 0.369, $P = 0.543$). When analyzing MF, FF, and MM social interactions together, the probability of escalation to grappling as a binomial response increased with increasing average size of the size-matched interacting pair (logistic regression; log odds = 0.48, SE = 0.222, $P = 0.019$; Fig. 2). Social interactions between juvenile *P. laevifrons* never escalated beyond antenniform leg touching.

DISCUSSION

Social interactions between adult *Paraphrynus laevifrons* consist of an array of distinct behaviors used across inter- and intrasexual social contexts. We broadly define social interactions as any form of interaction between conspecifics (Wolf et al. 1999), regardless of whether communication through the use of evolved signals and responses occurs (Scott-Phillips 2007). None of the observed behavior was exclusive to a particular interaction type amongst adults. There were no

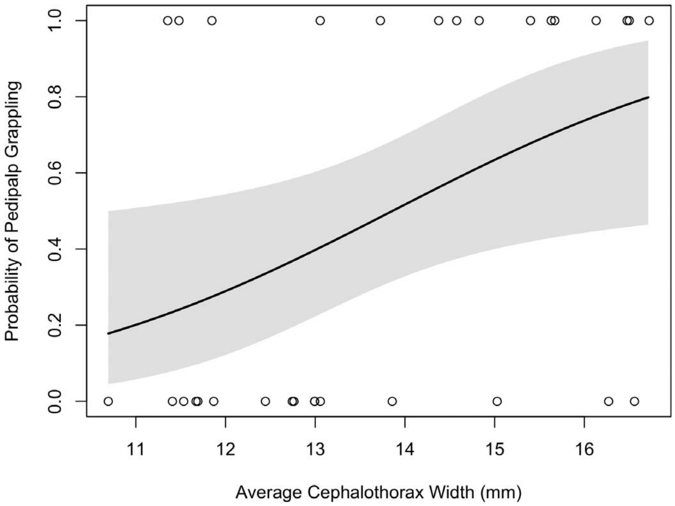

Figure 2.—Social interactions between size-matched adult *Paraphrynus laevifrons* were more likely to escalate to pedipalp grappling with increasing average size of the interacting pair (measured in cephalothorax width, mm), regardless of social interaction type ($n =$ 17 MF, 3 FF, 11 MM, "combined trials" subset).

significant differences in social interaction duration or escalation between MF, FF, and MM social interactions. Social interactions between smaller pairs of *P. laevifrons* were less likely to escalate towards pedipalp grappling than interactions between larger individuals. However, since we could not determine with absolute certainty if the amblypygids in our study were in fact juveniles or adults (see METHODS, **Determining age and sex** subsection), it is possible that this pattern could be driven by juveniles – which are less likely to engage in and potentially escalate social interactions – being included in "adult" social interactions.

We did find clear differences in the presence, duration and escalation of social interactions between juveniles, compared to social interactions between adults. Juveniles were less likely than adults to engage in social interactions with each other in our experimental arena. Those juvenile social interactions that did occur were shorter than adult social interactions and rarely escalated beyond antenniform leg touching, a behavior associated with examining an individual's surroundings (Walsh & Rayor 2008). Pilot field observations of *P. laevifrons* social interactions suggest that all of the behavior present in this laboratory experiment are also exhibited in social interactions in nature. We did not observe any behaviors during field social interactions that we did not also observe in this study (Corey, pers. obs.).

Open pedipalps and antenniform leg vibrations are putative communication signals in *P. laevifrons* social interactions, given their similarity to signals in other studied amblypygid species (e.g., Santer & Hebets 2008, 2011b). Determining the specific function(s) of such behaviors is beyond the scope of this study, but we can nonetheless hypothesize about potential functions. For example, opening pedipalps may be a display of weapon size (e.g., Sneddon et al. 1997), intent to attack (e.g., Adamo & Hanlon 1996), and/or a preparatory movement for attack or defense (e.g., Popp 1987). In *P. laevifrons,* as well as other previously studied amblypygid species, individuals use their pedipalps in grappling, which is the highest stage of interaction escalation short of cannibalism (Fowler-Finn & Hebets 2006; Chapin & Hill-Lindsay 2015). We observed individuals using their antenniform legs to touch an opponent's pedipalps, which could function in detecting the target's pedipalp position and thereby collect information about their size and/or aggressive state (Foelix & Hebets 2001). Future work focused on the responses of target individuals to a focal individual's behaviors will allow us to disentangle their potential function (e.g., Egge et al. 2011).

Antenniform leg vibrations function in intraspecific signaling in at least one amblypygid species, and likely serves the same or similar functions in others (Fowler-Finn & Hebets 2006; Santer & Hebets 2008). Prior work on *Phrynus marginemaculatus* demonstrates that antenniform leg vibrations in intrasexual agonistic contests produce air particle movement signals that are detected by sensory hairs (i.e., trichobothria) on the patella of the walking legs. Signalers position the majority of antenniform leg vibrations over their opponent's patellar trichobothria, and contest outcome is predicted by antenniform leg vibration duration (Santer & Hebets 2008). Furthermore, the duration of antenniform leg vibrations no longer predicted agonistic contest outcomes when trichobothria were experimentally removed (Santer & Hebets 2011b). Similar to this previous work, we found that antenniform leg vibrations are common in adult *P. laevifrons* social interactions. Unlike in *Phrynus marginemaculatus* (Santer & Hebets 2008), however, *Paraphrynus laevifrons* do not consistently perform antenniform leg vibrations directly over their target's patellar trichobothria (or any other body part).

Table 2.—Summary of *Paraphrynus laevifrons* social interaction metrics across interaction types. The data below are from the "combined trials" subset, the first two behavioral trials in which a given individual was run (see *Methods, Statistical Analyses* for details). *Interaction Type* = age/sex pairing of *P. laevifrons* introduced during social interaction trials; $n =$ number of social interaction trials run; *# Interacting* = number of pairs engaged in social interactions during trials, defined as being within mutual antenniform leg length and one or both individuals performing antenniform leg touches; *# Displaying* = number of interacting pairs in which one or both individuals engaged in putative display behavior, including opening pedipalps and/or antenniform leg vibrations; *# Grappling* = number of interacting pairs in which individuals made physical contact and grappled each other with their pedipalps; $\bar{x}$ *Duration* ± *SD (Median Duration)* = mean duration of social interactions (in seconds) ± standard deviation, with median duration in parentheses.

Interaction Type	*n*	*# Interacting*	*# Displaying*	*# Grappling*	$\bar{x}$ *Duration* ± *SD (Median Duration)*
Male – Female	17	17	17	9	520.3 ± 330.75 (446.5)
Female – Female	3	3	2	1	303.3 ± 270.49 (257)
Male – Male	13	11	10	5	454.4 ± 417.04 (293)
Juvenile – Juvenile	19	3	0	0	28.3 ± 10.79 (33)

We described at least four types of antenniform leg vibrations in *P. laevifrons* based on the position of the focal individual's antenniform legs in relation to itself and its target (Table 1). One of these types of antenniform leg vibration ("Antenniform Leg Vibration Flick") has been quantified in behavioral experiments in two other amblypygid species (*P. marginemaculatus,* Fowler-Finn & Hebets 2006; *Phrynus longipes*, Chapin & Hill-Lindsay 2015), and has been reported as "antenniform leg fencing" in many others (reviewed in Weygoldt 2000). Using supplemental high-speed videography, we also found that *P. laevifrons* make direct contact with their opponents – i.e., antenniform leg touches (Table 1) – while performing antenniform leg vibrations. Antenniform leg vibrations in *P. laevifrons* might therefore encompass tactile signals, air particle movement signals, or both.

The lack of differences in duration, escalation, and behavioral repertoire between adult intra- and intersexual interactions was unexpected. Previous descriptions of amblypygid intersexual interactions report courtship durations up to eight hours in some species (reviewed in Weygoldt 2000), while intrasexual interactions, in contrast, last on the scale of seconds to minutes (Fowler-Finn & Hebets 2006; Chapin 2015). Prior to this study, one of us (T.B.C.) observed a putative mating pair of *P. laevifrons* in nature interacting for over 3 hours. This pair additionally could have interacted before and after this window for an unknown period of time. At a minimum then, we expected intersexual interactions to last longer than intrasexual interactions. Our failure to substantiate this expectation could have many explanations. First, we may have failed in providing adequate conditions for mating behavior. Captive amblypygid breeders successfully stage matings by introducing males to terraria with ample refuges that females have been kept in for several days. Given the success with this protocol, females may require familiarity with their surroundings, and potentially some form of territory ownership, to be receptive to mating (Casto pers. comm., McMonigle 2013). It is also possible that we performed our experiments outside of the breeding window of *P. laevifrons*. Unknown to us at the start of this experiment, our previous work infers the primary mating season of *P. laevifrons* to be between October and January, with some individuals continuing to mate throughout the year (Hebets 2002; Corey & Hebets 2017). Finally, as we acknowledge previously, it is also possible that juveniles were included in our adult interaction category, and therefore did not demonstrate mating behavior. Future studies using different experimental conditions will be required to more thoroughly understand mating behavior in *P. laevifrons*.

Juvenile *P. laevifrons* never performed the full array of behaviors that we observed in adult social interactions. Only three of 19 juvenile pairings lasted beyond achieving a mutual antenniform leg length distance. Once individuals achieved this distance and presumably detected the other animal, they often hastily retreated in opposite directions. From our data, it is difficult to assess if these results were simply due to a lack of motivation to interact with an unfamiliar individual in our laboratory setting, but we find this unlikely. Observations made during fieldwork as well as results from other systems leads us to hypothesize that juvenile *P. laevifrons* are less likely to interact with unfamiliar conspecifics than adults.

Specifically, we often observed juvenile *P. laevifrons* retreating into a refuge upon encountering another amblypygid in the field (Corey, Hebets, pers. obs), and individual *P. laevifrons* are more abundant in areas with greater potential refuges (Corey & Hebets 2017). Rapid retreats into refuges is an antipredator behavior and such behavior may be more likely in juvenile amblypygids encountering conspecifics due to their risk of cannibalism. Adult and large juvenile *P. laevifrons* have been observed to cannibalize smaller juveniles (Corey pers. obs.). Furthermore, we found in our current study that the likelihood of social interactions between adults escalating to grappling increased with increasing size of the interacting pair. If larger adult *P. laevifrons* are more aggressive in general, then juveniles could avoid cannibalism risk by rapidly retreating from larger conspecifics. Similar results have been found in other arthropods. In the aquatic insect *Notonecta hoffmani,* for example, juveniles are more vulnerable to predation than adults, and young juveniles are even more vulnerable than older juveniles. Accordingly, young juvenile *N. hoffmani* show more pronounced antipredator behavior than older juveniles in response to cues from cannibalistic adults (Sih 1982). We similarly propose that greater risks of predation and cannibalism could select for juvenile *P. laevifrons* to retreat upon encountering unfamiliar conspecifics more often than adults. Purposeful field observations and experiments would be required to test this, and to determine the frequency and composition of juvenile social interactions in nature.

Our current work expands the taxonomic breadth of amblypygid behavioral ecology by providing foundational information on social behavior and putative communication in *Paraphrynus laevifrons*. It is clear that social interactions between *P. laevifrons*, especially adults, are potentially complex and can provide ample avenues for future study on the unique sensory and communication systems of amblypygids. Our identification of novel behavior and differences in social interactions from other amblypygid species continue to nuance our understanding of this unique and mysterious arachnid order.

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SUPPLEMENTARY MATERIAL

Audio portion of video recordings only contained background noises, and have been removed to decrease file size.

Video S1.—An overview of *Paraphrynus laevifrons* social interaction escalation. Dorsal view of one female and one male *P. laevifrons* interacting in a vertical arena. Online at http://dx.doi.org/10.1636/JoA-S-19-049.s1

Video S2.—Antenniform leg touches (Table 1) during a *Paraphrynus laevifrons* social interaction. Dorsal and lateral views of two adult male *P. laevifrons* in a vertical arena. Online at http://dx.doi.org/10.1636/JoA-S-19-049.s2

Video S3.—Antenniform leg vibrations (Table 1) during a *Paraphrynus laevifrons* social interaction. Dorsal view of an adult female and male *P. laevifrons* in a vertical arena. Online at http://dx.doi.org/10.1636/JoA-S-19-049.s3

Video S4.—Antenniform leg vibrations “whip crack” and “flick” (Table 1) during *Paraphrynus laevifrons* social interaction. Lateral view of two adult female *P. laevifrons* interacting in a vertical arena. Online at http://dx.doi.org/10.1636/JoA-S-19-049.s4

Video S5.—Pedipalp strike and grappling (Table 1) during a *Paraphrynus laevifrons* social interaction. Lateral view of two adult males *P. laevifrons* interacting in a vertical arena. Online at http://dx.doi.org/10.1636/JoA-S-19-049.s5

Supplementary Table S1. Online at http://dx.doi.org/10.1636/JoA-S-19-049.s6