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SHORT COMMUNICATION

Comparative reproductive output of two cellar spiders (Pholcidae) that coexist in southwest Ohio

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Abstract. Species differ in their reproductive abilities, which may affect their success. In southwest Ohio, populations of *Pholcus phalangioides* (Fuesslin, 1775) (Araneae: Pholcidae) have largely been replaced by *Pholcus manueli* Gertsch, 1937 (Araneae: Pholcidae). We suspected that differences in reproductive output underpinned the success of *P. manueli*. We documented the reproductive success of both species in the laboratory. Female *P. phalangioides* mated more readily and were more likely to produce eggsacs than *P. manueli*, but the timing of eggsac production and spiderling emergence were similar. The eggsacs of *P. phalangioides* contained smaller numbers of larger eggs but similar numbers of spiderlings emerged from the sacs of both species. We uncovered a negative relationship between egg size and number for *P. phalangioides,* but not for *P. manueli*. Our results do not explain the relative success of *P. manueli*, however, we have uncovered differences in the reproductive strategies utilized by these ecologically-similar congeners.

Keywords: Invasive species, tradeoffs, reproduction

Limited resources dictate that animal reproductive strategies involve tradeoffs where investment in one beneficial fitness parameter precludes the full development of other aspects (Saeki et al. 2014). One example is the spider *Loxosceles rufescens* (Dufour, 1820), whose slow heart rate, low metabolism, and long lifespan facilitates its dispersal around the world. However, these same features mean it has limited mobility and often does not spread from the initial point of introduction (Nentwig et al. 2017). Common tradeoffs that are documented include those between size and number of eggs or offspring (Macip-Ríos et al. 2012), number of offspring and the growth or survival of those offspring (Sikes 1998; Cronin et al. 2016), and investment in current vs. future reproductive success (Rios-Cardenas et al. 2013). The decision to allocate energy or effort in one way or another can determine the success of populations and specific differences in reproductive strategies or reproductive potentials may be what allows one species to displace another or to buffer a species against displacement or extinction (Allen et al. 2017).

Historically, *Pholcus phalangioides* (Fuesslin, 1775) was the dominant pholcid species in southwest Ohio but, over the last 10 years, *Pholcus manueli* Gertsch, 1937 has displaced *P. phalangioides* and established robust populations across the region (A.L. Rypstra, personal observation). As congeners, the two species are superficially similar in appearance and both species build their irregular tangle webs under ledges and in the corners of buildings and barns (Jackson & Brassington 1987; Cutler 2007). However, adult, *P. phalangioides* are much larger (>6 mm in body length) than adult *P. manueli* (<5 mm in body length) (Cutler 2007). In general, the global success of *P. phalangioides* has been attributed to its behavioral flexibility, ability to invade the webs of other species (Jackson & Brassington 1987; Jackson 1992), the subdivision and genetic structure of its populations (Schäfer et al. 2001), and the plasticity in growth and body size (Miyashita 1988a, b; Uhl et al. 2005; Wilder 2013). Essentially nothing is known about the biology of *P. manueli*, especially the traits that are enabling it to take over areas with well-established *P. phalangioides* populations.

The purpose of this study was to document aspects of the reproductive output of *P. phalangioides* and *P. manueli* to determine if they could account for the success of *P. manueli* in displacing *P. phalangioides*. If *P. manueli* had higher reproductive output than *P. phalangioides* found in the same area, that difference could explain why *P. manueli* was able to replace *P. phalangioides*. Specifically, we predicted that the *P. manueli* would produce more offspring in a shorter time frame than *P. phalangioides*. In addition, we expected that the *P. manueli* would not need to make a trade-off between egg size and number that would be evident for *P. phalangioides*.

Spiders of both species were collected from buildings and barns around Butler and Hamilton Counties in southwest Ohio, USA between September 2013 and September of 2017. We housed spiders individually in cylindrical translucent plastic containers; those for *P. manueli* were 9 cm tall with a diameter of 13 cm and those for *P. phalangioides* were 12 cm tall with a 15 cm diameter. We kept the spiders in a climate-controlled room set on a 12:12 L:D cycle, 25°C, and 50% humidity and fed them two crickets, *Gryllodes sigillatus*, approximately equal in length to the spider (3 mm or 6 mm) once per week. We mated spiders by adding a male to a container where a female was housed and monitored the interaction. If the spiders acted aggressively toward one another, the male was removed. If mating occurred, we waited until the male and female separated and removed the male. The females were monitored so that the appearance of an eggsac and the emergence of spiderlings could be recorded.

As part of our effort to maintain laboratory populations of these species, we regularly attempted to mate animals in the laboratory. Of these, 41 pairs of *P. manueli* and 44 pairs of *P. phalangioides* were known to have molted to adulthood in the laboratory and, thus, were virgins. In all cases, the animals were mated 2–3 weeks after completing the final molt. After mating, we monitored the females for 30 days and recorded eggsac production and spiderling emergence. The number of females of each species that produced eggsacs as well as the number of those eggsacs that successfully produced spiderlings were compared using Fisher exact tests. The number of spiderlings emerging from eggsacs were compared using the Mann Whitney U Test.

Only 16 of the 41 *P. manueli* virgins we mated produced eggsacs, which was significantly less than the 40 of the 44 *P. phalangioides* virgins who deposited eggs (Table 1). Some spiderlings emerged from all of the *P. manueli* eggsacs but spiderlings appeared from only 34 of the 40 eggsacs produced by *P. phalangioides* (85%) (Table 1). The number of spiderlings per sac was highly variable for both species, ranging from 1 to 60 for *P. phalangioides* and 1 to 55 for *P. manueli,* and there was no overall difference between the two species in offspring number (Table 1).

The low reproductive output of virgin *P. manueli* in these initial observations was not supportive of our original hypothesis. Nevertheless, we noted that adults of both species are long lived (2–3 years

Table 1.—The reproductive output of *Pholcus manueli* and *Pholcus phalangioides* in our study. Values include the actual count or the average ± the standard error.

Category	*Pholcus manueli*	*Pholcus phalangioides*	Test Statistic	DF	P-value
Mating of virgin spiders					
Total number mated	41	44			
Number producing eggsac (%)	16 (39%)	40 (91%)	Fisher		<0.0001
Number of spiderlings emerged	16.1±4.2	18.6±3.5	Z=0.57	1	0.5650
Number eggsacs to hatch (%)	16 (100%)	34 (85%)	Fisher		0.1676
Mating of field caught adults					
Total number mated	66	38			
Number producing eggsac (%)	38 (58%)	29 (76%)	Fisher		0.0597
Time to 1st eggsac (d)	17.0±2.2	13.7±1.52	Z=0.07	1	0.9418
Number of 1st eggsacs hatched (%)	22 (58%)	21 (72%)	Fisher		0.3047
Time to hatch (d)	13.1±0.6	15.0±1.1	Z=1.42	1	0.1558
Number of spiderlings emerged from 1st eggsac	21.5±2.9	19.2±3.2	Z=0.66	1	0.5065
Number producing additional eggsacs (%)	13 (34%)	18(62%)	Fisher		0.0285
Time between 1st and 2nd eggsacs	23.8±6.4	22.7±8.7	Z=0.86	1	0.4100
Number of 2nd eggsacs to hatch (%)	3 (23%)	5 (28%)	Fisher		1.000
Egg data of field caught adults					
Sample size	17	19			
Clutch size	36.4±1.8	24.4±1.9	Z=3.57	1	0.0004
Egg diameter	0.57±0.01	0.83±0.01	Z=5.17	1	<0.0001

as adults in the laboratory) and reproductive adults can be found in our field sites during all months of the year (A.L. Rypstra and A. D. Berry, personal observation). In addition, individuals of both species willingly mate repeatedly in the laboratory (A.L. Rypstra and A. D. Berry, pers. observ.). While nothing is known regarding the mating system of *P. manueli,* sperm mixing and last male sperm priority seem to be the rule for other pholcid species (Uhl 2000; Schäfer & Uhl 2002; Calbacho-Rosa et al. 2013). Notably, the mating system of *P. phalangioides* involves multiple sequential matings (Uhl 2000; Schäfer & Uhl 2002) and females have a preference for experienced males (Hoefler et al. 2010). Given this background on pholcid mating, we considered the reproductive success of mature animals who likely had mated in the field before they were brought to the laboratory. We reasoned that their nutritional status and condition would be more reflective of what led to their relative success in the field and we hoped that documenting the reproductive success of these middle-aged animals would give us another angle on the differences between these two species.

Adult spiders were collected from area buildings and barns between September 2016 and March 2017 and housed in the laboratory for at least one week. We mated 66 *P. manueli* and 38 *P. phalangioides* pairs by introducing the male into the container housing the female, identical to the ones described above. If we observed aggression, we removed the male. If the spiders did not begin to mate within 20 minutes, we removed the male and introduced a new one from our laboratory population. If the second pair did not mate, then the female was returned to the population stored in the lab and we offered her the opportunity to mate again several days later. In this way, we eventually succeeded in getting all spiders to mate. The spiders were left alone until mating had completed, after which the male was removed and returned to his home container. We then returned both spiders to the environmental chamber described above. We checked females for eggsacs every day and recorded if and when the female produced an eggsac, the date spiderlings emerged, and the number of spiderlings that emerged. Once the eggsacs had hatched, we left the spiderlings with the mother until they had molted once, typically 7–10 d after emergence, and removed them. We continued to monitor the females until death and recorded the same information on all subsequent eggsacs. We compared the likelihood that one or more eggsacs (2–4) were produced, and the frequency with which spiderlings emerged from eggsacs of each species using Fisher exact tests. We compared the time it took for the spiderlings to emerge, the time between eggsacs (the time from the emergence of spiderlings from one eggsac to the production of the next), and the number of live spiderlings produced by each eggsac with Mann-Whitney U tests.

There were only a few differences in the reproductive parameters we documented (Table 1). *Pholcus phalangioides* females were more receptive as it took only 47 mating attempts to get the 38 field caught females mated (81%) whereas it took 149 trials to secure 66 mated *P. manueli* females (44%). *Pholcus phalangioides* appeared to be more likely to produce an eggsac than *P. manueli* females but the difference was not significant at the $P = 0.05$ level (Table 1). However, the established species was significantly more likely to produce a subsequent eggsac than the new arrival (Table 1). Neither the timing of eggsac production nor the time between its appearance and the emergence of spiderlings were different between the two species (Table 1). Similarly, the likelihood that the eggsac hatched and the number of spiderlings emerging from the eggsacs did not differ significantly between *P. manueli* and *P. phalangioides* (Table 1). Between 5 and 29 spiderlings emerged from *P. phalangioides* sacs and between 6 and 43 from *P. manueli* and there were no species differences in the production of live offspring (Table 1).

In order to determine if there was a trade-off between the number of eggs produced and the size of the eggs, we collected another 17 *P. manueli* and 19 *P. phalangioides* between October 2015 and April 2017. Three of these females (1 *P. manueli* and 2 *P. phalangioides*) produced eggsacs before we had the opportunity to mate them. We mated the rest of the females using the procedures described above. Five days after the eggsac appeared, we removed it and opened the sac to separate the eggs. We counted the eggs in each eggsac and randomly selected 7 eggs from each sac. We measured the egg diameter using a filar micrometer with an accuracy of 0.01 mm attached to a dissecting microscope (Wild Heerbrugg, Switzerland). We calculated an average egg size for each clutch. We compared egg size and egg number between species in separate Mann-Whitney U tests. We explored the effects of egg size and the species involved on the number of eggs in an eggsac using Poisson regression. We conducted the regression with and without the three eggsacs that were produced from field matings.

Pholcus manueli produced larger clutches than *P. phalangioides* but *P. phalangioides* clutches contained larger eggs (Table 1). Interestingly, clutch size was related to egg size in our Poisson regression ($\aleph^2$

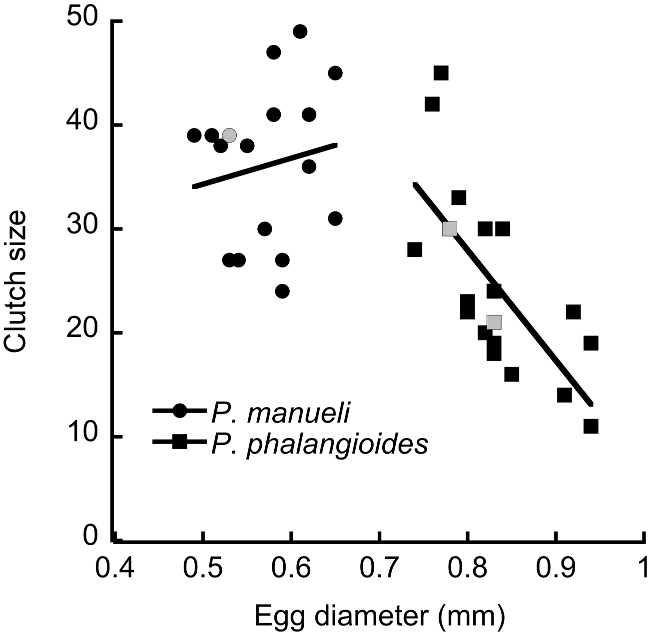

Figure 1.—The relationship between egg number and egg size for field caught adult *Pholcus phalangioides* and *Pholcus manueli*. Most data were collected from the first clutch produced after animals were mated in the laboratory. Gray symbols are data points for eggsacs produced by field-caught animals before they had the opportunity to mate in the laboratory.

$= 11.47$, $P = 0.0007$), however the species producing the eggsac was not significantly related to egg number in this analysis ($\aleph^2 = 0.10$, $P = 0.7484$). Instead, there was a highly significant interaction between the species of the female producing the eggsac and the size of the eggs it contained ($\aleph^2 = 18.6$, $P < 0.0001$). This interaction emerged because there was a negative relationship between egg size and egg number for *P. phalangioides* but there was no correlation between egg size and egg number for *P. manueli* (Fig. 1). This relationship was strong and was not affected by the data from the three individuals whose eggsacs resulted from mating that occurred before we collected them (Fig. 1).

These results hint at some differences in the reproductive strategies for these two congeners yet the differences do not necessarily help us understand how *P. manueli* has managed to replace *P. phalangioides* throughout our region. Female *P. phalangioides* readily mated in the laboratory and, overall, they were much more likely to produce eggsacs after a single mating than were *P. manueli* females. In addition, *P. phalangioides* females were more likely to produce multiple eggsacs and, even though the success of these later eggsacs was low, the offspring produced would still contribute to their population size. Thus, the laboratory data we collected from virgin and field collected adult individuals makes it difficult to argue that reproductive success can account for the success that *P. manueli* has had in displacing *P. phalangioides*.

Although *P. manueli* females produced larger clutches than *P. phalangioides* (Fig. 1), similar numbers of spiderlings emerged from the eggsacs of each species (Table 1), which likely offsets the advantage of producing more eggs. Nevertheless, the variability in the number of spiderlings emerging from eggsacs was very high for both species. This variation was particularly remarkable for our observations of the matings of virgin spiders since they had been in the laboratory on a standard feeding schedule for at least one month prior to mating. In a study of *P. phalangioides*, Hoefler et al. (2010) found that males with prior experience had higher mating success than those who had never been exposed to female silk. However, we typically hold animals in the laboratory in individual containers so as to control for mating and prevent cannibalism. It may be that these protocols impact the mating system and the propensity for males to transfer, or females to accept, sperm, which might account for the high variability in offspring production we observed.

We uncovered a negative relationship between egg size and number for *P. phalangioides* yet no such relationship emerged for *P. manueli* (Fig. 1). Theoretically, such a trade-off between making more eggs vs. making larger eggs should occur when there are constraints on resources (Bernardo 1996; Roff & Fairbairn 2007). Presumably larger eggs translate to larger offspring which potentially increases their success (Walker et al. 2003; Warne & Charnov 2008). In addition, some of the cost of the smaller clutch sizes we observed for *P. phalangioides* is offset by the fact that individuals in our sample were more likely to produce additional eggsacs. Thus, these data alone would also cause us to predict that *P. phalangioides* would be more successful than *P. manueli*. On the other hand, the larger clutch size of *P. manueli* was not related to egg size. This result suggests that this species maximizes clutch size within the constraint on some minimum viable offspring size (Marshall & Gittleman 1994). Alternatively, it is possible that the excess eggs in the sacs of *P. manueli* are trophic eggs that support the success of the developing spiderlings and offset the fact that the eggs themselves are smaller in size. Trophic eggs have been reported for a variety of arthropod species, including some spiders (Perry & Roitberg 2006). In any event, these data suggest that there should be less variation in offspring performance for *P. manueli* than for *P. phalangioides*.

Our data failed to support the hypothesis that a higher reproductive potential aided *P. manueli* in displacing and excluding *P. phalangioides*. In fact, the higher reproductive rate and the larger egg size could give *P. phalangioides* an advantage over its competitor. Having a numbers advantage can be critical for both invading species and those native species trying to resist displacement (Blackburn et al. 2015). Thus, there must be some other aspect of the biology of *P. manueli* that is leading to its success in replacing *P. phalangioides*. Since virtually nothing is known regarding the basic biology and behavior of *P. manueli*, the information we report here may help frame subsequent studies that address their successful invasion, ecology, and evolutionary history.

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