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# SHORT COMMUNICATION

# Proximate cues governing egg sac discrimination and recognition in the wolf spider *Pardosa milvina* (Araneae: Lycosidae)

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**Abstract.** Female lycosids carry their egg sacs on their spinnerets until spiderlings emerge but spiders are occasionally found carrying shells, dirt, or other objects on their spinnerets, suggesting recognition errors can occur. We investigated some proximate cues that may influence egg sac recognition and discrimination in the wolf spider *Pardosa milvina* (Hentz 1844). We tested the ability of female *P. milvina* to discriminate among egg sacs based on size, texture, and contrast. We also tested the ability of *P. milvina* to discriminate between its own or a conspecific's egg sac, and the ability to discriminate between an egg sac that had just been removed and an egg sac that was removed seven days earlier. When given a choice, females significantly chose their own egg sac over plastic beads of equal mass, preferred large plastic beads equal in mass to an egg sac over small plastic beads, round over faceted beads, and showed a non-significant tendency to attach black rather than white beads of equal mass. When given a choice between two conspecific egg sacs, spiders more often rejected those that had been removed from the mother seven days earlier than those that had been freshly removed. Spiders were unable to recognize their own egg sacs versus a conspecific's. Although spiders recognize egg sacs from non-egg sacs based on mass, texture, and presumably odor when given the choice, acceptance of non-egg sacs was common when no real egg sac was available. Also, females would not reattach their own egg sac once an artificial one had been attached. Attachment of any object on the spinnerets apparently ceases searching or attachment behavior.

**Keywords:** Chemical recognition, dropping, artificial egg sac, lycosid

Adult female wolf spiders attach their egg sacs to their spinnerets and transport them as they move through the environment. The wolf spider, *Pardosa milvina* (Hentz 1844), is especially active (Walker et al. 1999; Samu et al. 2003), and females carry egg sacs that average 72% of the female's post-reproductive weight (Colancecco et al. 2007). Because of the large relative size of the egg sac and high activity level of these spiders, female *P. milvina* may be especially prone to dropping or losing the egg sac. Misidentification and subsequent adoption of another spider's egg sac is known to occur in some wolf spider species (Fujii 1980; Wagner 1995). There are also many anecdotal observations of adult female wolf spiders carrying other objects on their spinnerets such as pebbles, snail shells, pieces of cork, bits of soil, small seeds, rabbit droppings, thread pellets, bread pieces, and wads of paper or cotton (O'Connor 1896; Fabre 1912; Locket & Marsh 1957; Bristowe 1958; Fuji 1980). Such observations suggest that misidentification of egg sacs may be common despite the large potential fitness consequences of such errors. We investigated some of the proximate cues that may be used by female *P. milvina* to recognize its egg sac and also measured the frequency of errors in choosing various spherical objects that are not egg sacs. We measured the influence of five factors that may govern egg sac recognition including size, texture, brightness contrast, discrimination of their own vs. a conspecific egg sac, and time elapsed since egg sac removal.

## METHODS

Adult female *P. milvina* with egg sacs were collected in August and September from corn and soybean fields in Selinsgrove, Snyder County, Pennsylvania. Spiders were individually housed in 150-ml (= 5-oz) plastic containers with moistened peat moss and were fed a mixed diet of house cricket nymphs (*Acheta domesticus*) and fruit flies (*Drosophila melanogaster*) twice weekly. All test spiders received approximately equal quantities of both food types while being housed. We conducted a series of independent choice experiments with a sample size of 50 spiders for each of six experiments and 40 spiders for a seventh experiment. Each spider was used only once (i.e., a total of 340 females with egg sacs was used as test subjects for the experiments. We obtained extra egg sacs from an additional 40 females. These egg sacs were used as one of the egg sac choices, but these females were not used as test subjects.

Prior to a choice test, we placed each spider in a glass vial on ice for one minute to slow the spider and used entomological forceps to then remove the egg sac. After egg sac removal, the spider was immediately placed in a 166-ml (= 45-dram) plastic vial (10 cm high × 5 cm diameter), with a choice between two objects. Seven separate experiments were performed, six of which involved simultaneous presentation of two objects. The paired choices were as follows: 1) its own egg sac vs. a randomly selected conspecific egg sac removed from another female at the same time, 2) its own egg sac vs. a white plastic bead of approximate equal mass (11 mg) and diameter to that of a natural egg sac (3 mm), 3) a black plastic bead vs. a white plastic bead of equal mass (each 11 mg, 3mm diameter), 4) a small black plastic bead (2 mg, 1.8mm diameter) vs. a large black plastic bead approximately equal in mass and diameter to a female egg sac (11 mg, 3 mm diameter), and 5) a multi-faceted black plastic bead vs. a round black plastic bead of approximately equal mass and diameter (10.9 mg and 11 mg respectively, both 3 mm diameter). In a sixth experiment, we used a sequential presentation. A female was given its own egg sac immediately after it had attached a white plastic bead (11 mg, 3mm diameter) to its spinneret to determine if it would exchange the attached bead. Except for the choice test of small and large plastic beads, the artificial egg sacs consisted of plastic beads approximately equal in mass and diameter to that of a female's egg sac (female *P. milvina* egg sac = 13.1 mg, SE ± 01.6 mg, $n$ = 15). To minimize possible effects of time, spider age, or egg sac developmental stage, we ran all six of the experiments concurrently. For a seventh experiment, we used an additional 80 spiders with egg sacs not used in any previous experiment. Forty females were used as sources for egg sacs, and the remaining 40 were used as test subjects. We randomly selected and removed 40 egg sacs from females and placed the egg sac individually for seven days within separate sealed plastic vials. Each

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Table 1.—Preference for one of two objects by adult female *P. milvina* that had recently had its egg sac removed. The first binomial test (Choice/No choice) *P*-values are based on significant differences from a 50% probability distribution for accepting one of the objects offered (attaching an object) versus ignoring both objects within the allowed time. The *P*-values for the second binomial test (among egg sacs chosen) compare only the frequency of choosing between the two objects of those females that made any choice at all. The seventh test recorded the number of females that would accept their own egg sac after attaching a plastic bead to their spinnerets.

| Experiment | Choice | Choice | No. choice made | Binomial Test Choice/No. choice | Binomial test among egg sacs chosen |
|---|---|---|---|---|---|
| 1. Own vs. Conspecific | Own: 20 | Conspecific: 15 | 15 | 0.0020 | 0.0945 |
| 2. Own vs. Bead | Bead: 1 | Own: 36 | 13 | 0.0003 | < 0.0001 |
| 3. Black vs. White | Black: 21 | White: 13 | 16 | 0.0044 | 0.0540 |
| 4. Small vs. Large | Small: 5 | Large: 12 | 33 | 0.0087 | 0.0472 |
| 5. Round vs. Faceted | Round: 13 | Faceted: 5 | 32 | 0.0160 | 0.0327 |
| 6. Fresh vs. Seven-day old | Fresh: 32 | 7-day old: 4 | 4 | 0.0008 | < 0.0001 |
| 7. Attached vs. Own | Didn't switch: 49 | Switched: 1 | * | * | < 0.0001 |

166-ml vial was previously rinsed with alcohol and allowed to dry prior to placing the egg sac in the container. After 7 days, we then removed another 40 egg sacs from an additional 40 females. Each female from the second set of 40 spiders was then offered a choice between either the freshly removed egg sac of another female or the egg sac that had been removed from a conspecific seven days earlier.

For all experiments, test objects (egg sacs or plastic beads) were placed immediately adjacent to each other along the edge of the vial where the spider traveled. The position of each pair was alternated for every trial to minimize any potential bias due to placement. Spiders were observed for 15 min, after which we counted the number of individuals that attached each object. A positive choice was scored if the egg sac or bead was attached to the spinnerets with silk and the abdomen rose to a normal upright position within the 15-min period. Failure to attach the egg sac during the 15-min trial was scored as a no choice. The results were analyzed using two sets of binomial tests. Among all choice experiments, we used a binomial test comparing individuals that made a choice of either test object offered or made no choice at all. For this analysis, we tested whether the spiders showed a significant preference for attaching objects or ignoring them. We also used a binomial test to test for a significant preference among those individuals that attached one of the two objects offered. In this test, individuals that made no choice were dropped from the analysis.

## RESULTS AND DISCUSSION

*Pardosa milvina* females showed significant differences in their tendency to ignore or attach various objects depending on the pair of test objects presented. Spiders attached objects to their spinnerets significantly more than 50% of the time when they were given choices between their own egg sacs and those of conspecifics. They also preferred to attach an object when the choice was its own egg sac or a white bead, black bead or a white bead, or the choice between a freshly removed egg sac or one that had been removed seven days earlier (Table 1). However, when spiders were given a choice between a small and large artificial egg sac, or a round versus faceted artificial egg sac, they were significantly more likely to ignore both objects (Table 1).

Females showed no significant preference for their own versus a conspecific's egg sac when given a choice (Table 1); however, a power test with a beta error level of 50% suggests a possible sample size too small to reliably accept the null hypothesis. A sample size of 188 rather than 35 would be needed to demonstrate a non-significant effect. Spiders also showed no significant preference between a black and white artificial egg sac of equal size, but there was a non-significant tendency to prefer black over white plastic beads (Table 1). However, here too, a power test suggests an insufficient sample size to accept the null hypothesis. Based on a 50% beta error level, a sample size of 69 would be required to reliably accept the null hypothesis. Females showed a small but significant preference for a large artificial egg sac equal in size to a natural egg sac compared to a smaller artificial egg sac. They also preferred a round plastic bead to a faceted one of equal mass (Table 1). Females attached their own egg sac significantly more often than a plastic white bead of approximately equal mass (Table 1); however, out of 50 females that had already attached a white plastic bead, only one female dropped it and reattached her own egg sac when given a choice. Females that were given a choice between another female's egg sac that was freshly removed or one that was removed seven days earlier, showed a highly significant preference for the freshly removed egg sac (Table 1).

Regardless of the choices available, some females failed to reattach any object even when offered their own egg sac, but the rejection rates tended to be lower when at least one of the choices was a real egg sac. The rejection rate of all offered objects varied considerably, from a low of 10% for individuals offered fresh or older conspecific egg sacs to a high of 66% for individuals offered large or small plastic beads. It is unknown if rejecting egg sacs after removal is a generally maladaptive response. It is possible that various egg sac parasites may cause the female to drop her egg sac during parasitism. Females that then reject these parasitized egg sacs may benefit by depositing and brooding a second egg sac rather than carrying one that was parasitized.

Females were unable to discriminate between their own and another female's egg sac. This is surprising, since kin recognition of offspring is known to exist in *P. milvina* (Anthony 2003). Anthony (2003) found that *P. milvina* females with egg sacs or with recently dispersed spiderlings preferentially avoided preying on their own offspring. This study, combined with our data, suggests that a cuticular compound or other substance intrinsic to the spider itself may be important in kin recognition and that the egg sac surface, egg surface, or both may interfere or inhibit such chemical recognition. Other studies have documented that the sicariid, *Loxoceles gaucho* (Gertsch 1967) and salticid *Portia labiata* (Thorell 1887) can discriminate between their own egg sac and that of a conspecific (Clark & Jackson 1994; Japyassú et al. 2003). However, in both of these studies, the web itself seemed to be an important factor in discrimination, rather than the egg sac per se and in *L. gaucho*, egg sac adoption was common as measured by time near the egg sac.

Although *P. milvina* exhibit some ability to discriminate between egg sacs and non-egg sacs, errors were frequent. *Pardosa milvina* appear to have a weak preference based on size, relative shape, and perhaps brightness contrast when attaching an object after egg sac loss. Spiders do have a strong preference for their own egg sacs over round plastic objects of equal mass and contrast, suggesting that mass and contrast alone were not the primary means of discrimination, but that texture, size, and relative shape provided composite information for recognition. Despite a strong preference for attaching real egg sacs rather than artificial ones, when females lack a real egg sac as a choice, attachment of artificial ones was quite common. Acceptance of artificial egg sacs varied considerably from a low of 34% for small versus large plastic beads to a high of 68% when the choice was black versus white plastic beads.

It remains unclear if the fitness costs of lost egg sacs are high, but certainly carrying a misidentified object would incur significant energetic and fitness costs. Lycosid egg sacs usually require that the mother open them to release the spiderlings; thus, the loss of an egg sac would result in the complete failure to produce offspring from that clutch. Further, a female is unlikely to produce another egg sac when an object is already attached to her spinnerets (Wagner 1995). During our study, many females quickly produced a second egg sac within as little as two days after the first was removed. However, we found that females that attached other objects failed to produce another egg sac. In a short breeding season, this delay may have significant consequences for reproduction.

Poor egg sac recognition implies that selection pressure on discrimination is weak. Either the cost of such recognition errors is far lower than we believe or the accidental loss of an egg sac is a very rare event and of little evolutionary consequence. Despite observing high rates of artificial egg sac attachment under laboratory conditions, few field-caught wolf spiders are found with foreign objects on their spinnerets. Out of 382 wolf spiders initially collected for this study, only two spiders (0.5%) had objects other than an egg sac attached to their spinnerets (these were not used in this study). In one case, it was a small, unidentified seed and in the other case, it was a small bit of soil attached to the spinnerets. Since errors appear to be common under laboratory conditions, this suggests that females either rarely have their egg sacs become detached or that few objects sufficiently resemble an egg sac for a mistake to be made during the critical period in which females search for them. Alternatively, artificial objects may be attached somewhat frequently, but may not remain on long enough to be observed under field conditions.

We suggest caution in interpreting the apparently low frequency of egg sac loss and attachment of artificial egg sacs found in our field census. Our field data are based on a static frequency of 0.5% (i.e., that 0.5% of all egg sac carrying females are carrying non-egg sacs at any given time). This is quite different than assuming that 0.5% of all females lose their egg sacs or that 0.5% of all females attach other objects to their spinnerets. As our study indicates, many females made no choice once their egg sacs were removed. These females would be indistinguishable from gravid females that have not produced egg sacs, post-reproductive females that had spiderlings already dispersed, or virgin females. Thus, egg sac loss may be considerably more frequent than can be reliably measured in the field. Further, if females that carry artificial egg sacs are more likely to drop them later, as is the case with *P. milvina* (Colancecco et al. 2007), this will further underestimate the true frequency of artificial egg sac carrying. We must also be very cautious in equating the frequency of field observations with the evolutionary significance of the behavior. Copulation, parasitism, and feeding (including cannibalism) are also infrequently observed among field populations of cursorial spiders. Although observed infrequently, these behaviors are under strong selection and likely occur at much higher frequencies than can be easily observed. Our field observations could be erroneously interpreted to mean that only 0.5% of wolf spiders lose their egg sacs and reattach foreign objects. More likely, it means that, like copulation, feeding, and parasitism, it is of short duration and females with egg sacs may seek refuge. Our field data may also be biased toward encumbered females that engage in risky behaviors.

Colancecco et al. (2007) found that female *P. milvina* readily drop artificial egg sacs but rarely drop real ones while subduing large prey or escaping larger predatory wolf spiders. They also found that once artificial egg sacs were dropped, females failed to search for them or reattach them during a 2-h period. This study suggests that females may preferentially drop artificial egg sacs over real egg sacs and may use a post-attachment tactile cue to evaluate artificial egg sacs. However, Colancecco et al. (2007) also suggested that while grappling with prey or predators, spiders may become displaced from their egg sacs, making lost egg sacs more difficult to locate. Our result showed that females will rarely drop artificial egg sacs when offered their own back, which suggests that reattachment itself inhibits searching and additional reattachment behavior.

During our study, the spiders usually contacted both offered objects before making a choice and only rarely immediately chose the first object they contacted. Even when choosing between their own egg sacs and conspecifics' egg sacs, the spiders would often contact both egg sacs before choosing one. Females responded qualitatively differently to artificial egg sacs. Spiders tended to contact artificial beads with outstretched legs more often than with real egg sacs. With real egg sacs, females would tend to touch the objects less with outstretched legs but instead grasp, hold, or pick up the egg sacs with their chelicerae and pedipalps more often before attachment. Many times the spider would pick up different objects, manipulate them and drop them, or just hold them for the duration of the fifteen minutes suggesting that acceptance of egg sacs may require a longer time interval. Colancecco et al. (2007) allowed female *P. milvina* 12 h to accept or reject a single round black plastic bead very similar to those used for this study. Acceptance rates were between 52–69% for this study, suggesting that a longer period of time may have modestly increased overall acceptance rates of artificial egg sacs.

Females did show a remarkable ability to distinguish between egg sacs that were recently removed from those that had been removed a week earlier. We were uncertain of the precise mechanism by which females made these evaluations. Fungi or bacteria may have attacked the week-old egg sacs and provided an odor cue. However, we saw no indication of fungus or decomposition on the outside surface of these egg sacs. This is also unlikely given that the container was sterilized prior to placing the egg sac in a sealed vial. It is also possible that the eggs within the sac require periodic turning to remain viable as is necessary with some tarantula species (Marshall 1996; Saul-Gershenz 1996). In this case, females may have been able to evaluate viability by assessing if the eggs moved within the sac and remained non-agglutinated. Females periodically added loose silk to their egg sac. This fresh silk or a pheromone embedded in it could also potentially provide a means to distinguish freshly removed egg sacs from older ones.

Female lycosids clearly use composite information rather than a single cue to recognize egg sacs. Size, texture, shape, and odor are almost certainly involved. However, the strong preference for fresh compared to older egg sacs suggests that odor cues may be a particularly strong means of recognition. Additional studies that examine chemical recognition of egg sacs or state-dependent value of searching and attachment (i.e., female body condition or age) may prove fruitful.

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