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The effects of differential eye occlusion on prey and mate recognition in the brush-legged wolf spider *Schizocosa ocreata* (Hentz, 1844)

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Abstract. We examined the role of lycosid eye rows and the pairs of eyes within each row in prey detection and mate recognition by *Schizocosa ocreata* (Hentz, 1844) using two approaches: (1) occluding anterior or posterior eye rows; and (2) occluding all but a single eye pair. In response to live prey, females took longer to orient and approach crickets when the posterior eye row was occluded; responses were intermediate when the anterior eye row was occluded. With six of eight eyes occluded, spiders that could see with posterior lateral eyes (PLE-only) detected the cricket as quickly as the fully sighted spiders (controls), while spiders limited to seeing from other eye pairs took longer to orient towards prey. Orientation distance varied significantly as well, with Control, ALE-only, PME-only and PLE-only spiders responding at greater distances than AME-only spiders. To analyze the functions of the eye rows involved in mate preference in *S. ocreata*, we conducted an additional study with presentation of video playback of a courting male to females with different eye rows occluded. Females with their posterior eyes occluded took longer to orient and respond compared to controls and anterior eye row occlusion. The sum of female receptivity displays was also significantly reduced when posterior eyes were occluded, suggesting the posterior eyes may be involved in mate recognition. Taken together, these data suggest that the posterior (secondary) eyes may have an important role in detection and identification of both prey and courting males.

Keywords: Lycosidae, vision, prey detection, video playback, mate recognition
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A specialized visual system for successful processing of information is vital for the survival and reproduction of many animals (Land & Nilsson 2012). Among arthropods, the Class Arachnida utilizes only non-compound ocelli type eyes (Barth 2002; Foelix 2011). Most spiders (Order Araneae) have six or eight eyes (although some cave-dwelling species have none). Spider eyes consist of a pair of primary or principal eyes (anterior median eyes) and three pairs of secondary eyes (anterior lateral eyes, posterior median eyes, and posterior lateral eyes) (Foelix 2011; Morehouse 2020; Nentwig et al. 2022). Visual acuity varies significantly across the genera of the Araneae. While most spiders do not have high-resolution vision and some species do not have eyes at all, members of the families Salticidae (jumping spiders), Ctenidae and Lycosidae (wolf spiders) appear to be exceptions (Land 1985a; Morehouse 2020). Salticids are thought to have the most advanced and intricate visual system among spiders, demonstrating the highest visual acuity among all measured land invertebrates (Barth 1985, 2002; Harland et al. 1999; Nelson & Jackson 2012; Land & Nilsson 2012; Menda et al. 2014). Equipped with a four-tiered retina, two peak spectral sensitivities (UV and green) and three different opsins (indicative of color vision), the anterior median eyes of the salticid are optimized for a wide range of visually mediated behaviors (Land 1969a, b; Koyanagi et al. 2008; Zurek et al. 2015; Jakob et al. 2018; Cerveira et al 2021; Morehouse 2020). Consequently, salticid vision has been studied extensively in relation to sexual selection (Masta & Madison 2002), learning (Skow & Jakob 2006), and strategizing routes of prey capture (Jackson & Pollard 1996; Harland & Jackson 2000; Jakob et al. 2018).

While there have been several studies investigating the physiological capabilities of salticid and ctenid vision, there is less research analyzing lycosid vision. Lycosids, like salticids, use visual cues to successfully detect and capture prey as well as process visual components of courtship displays (Bristowe & Locket 1926; Uetz & Stratton 1983; Rovner 1996). The lycosid visual system consists of two sets of eyes consisting of posterior (PER) and anterior eye rows (AER), which differ in size and structure, and ultimately produce a complete visual field (Homann 1931; Land 1985a). The eye arrangement in lycosids is quite different than in salticids, in both arrangement and structure (see Land 1985a). The secondary eyes are equipped with a tapetum for optimizing vision in low light conditions, a surface that reflects the photons that are not initially absorbed after they passed through the photoreceptors (Schwab et al. 2002). Lycosid eyes have a high resolution and sensitivity and have a 270°–360° field of view (Homann 1931; Land 1985b; Lizotte & Rovner 1988; Kovoor et al. 1993; Rovner 1993; Ortega-Escobar & Munoz-Cuevas 1999). Although wolf spiders have better image resolution than many other spider families, their visual system is limited in the perception of color and some behavioral studies suggest they must remain stationary to detect motion (Land 1985b; Rovner 1989, 1996). In addition, anterior median eyes (AME) of wolf spiders can detect polarized light (Magni et al. 1964; Kovoor et al. 1993; Dacke et al. 2001). Despite considerable research on visually mediated behavior, lycosid eyes and the functional roles they play have only been studied in a limited number of species (DeVoe 1962; Rovner 1996; Ortega-Escobar 2006).

The wolf spider *Schizocosa ocreata* (Hentz, 1844) is an excellent model for behavioral studies, especially mate recognition and sexual selection (Dacke et al. 2001; Uetz & Roberts 2002; Hebets & Vink 2007; Roberts et al. 2007; Herberstein et al. 2014; Uetz & Clark 2014; Uetz et al. 2016). As for other wolf spider species, visual cues guide a foraging strategy in *Schizocosa* that varies between stationary sit-and-wait phases and between-patch movements (Persons & Uetz 1996, 1997). Not only do they rely on their eyesight for capturing prey, they also have conspicuous visual courtship, with decorated forelegs used in waving, raising, tapping and other displays. Discrimination of conspecifics from prey items is critical in mate recognition for *S. ocreata* as selection pressures are strong and females have been found to often mate with the male they see move first (Scheffer et al. 1996). Female *S. ocreata* have been shown to prefer males with large tufts (McClintock & Uetz 1996; Scheffer et al. 1996) and use this trait to choose high-quality mates, as tuft size is a condition-dependent trait (Uetz et al. 2002). Several studies have shown they also respond to video playback as if the moving object was real (McClintock & Uetz 1996; Uetz & Roberts 2002; Roberts et al. 2007; Moskalik & Uetz 2011; Uetz & Clark 2014; Uetz et al. 2016). Thus, this species is an interesting and useful system to analyze the functional roles of eyes of lycosids in response to not only prey capture, but also how conspecifics are recognized. In this study, we investigated possible roles of anterior and posterior eyes in prey detection/recognition and mate recognition using eye occlusion techniques.

In previous studies with another wolf spider, *Rabidosa rabida* (Walckenaer, 1837), Rovner (1993) found that the posterior eyes were involved in long distance detection and mate recognition by females, while the anterior median eyes had no distinct role (Rovner 1993). While in salticids, principal eyes (anterior median) are considered structurally different from all other secondary eyes (ALE, PLE, PME) there is some evidence to support differences among anterior eyes (ALE, AME) and the posterior eyes (PME, PLE) in wolf spiders (Melamed & Trujillo-Cenóz 1966; Trujillo-Cenóz & Melamed 1967). One difference is that lycosid AM eyes have two pairs of dedicated muscles to move the retinae behind the carapace, while the secondary eyes have stationary retinae (Melamed & Trujillo-Cenóz 1971). Posterior eyes in a species of *Pardosa* wolf spiders were shown to contain about 4000 rhabdomeres, or small light-sensitive inclusions in the receptor cells, while the anterior median and anterior lateral eyes contained 300 and 120 rhabdomeres, respectively (Foelix 2011). In addition, the anterior eyes (ALE, AME) and posterior eyes (PME, PLE) differ from each other in focal length, resolution, and sensitivity (Clemente 2010).

With these findings in mind, we tested hypotheses regarding the visual responses of *S. ocreata* to prey and male courtship when posterior eye rows (PME and PLE) or anterior eye rows (ALE and AME) were occluded. Based on the previous findings in the genus *Rabidosa,* we hypothesized the posterior eyes in *Schizocosa* would provide a similar, dominant role in female mate recognition and receptivity. In addition, at the suggestion of a previous reviewer, we conducted an additional study using a different procedure, i.e., occluding all but one set of eyes (AME, ALE, PME, or PLE) to determine the degree of involvement of eye pairs within eye rows. This complementary study only involved testing the use of eyes in prey detection and capture.

METHODS

Animal collection and maintenance.—Individual *S. ocreata* were collected from the Cincinnati Nature Center (Clermont Co., Ohio) as immature juveniles during September and October of 2011 and 2018. They were housed in individual opaque plastic deli-dish containers on a 13:11 light:dark cycle, with controlled temperature (approx. 23°C) and controlled humidity (RH approx. 60%). They were fed 2–3 small crickets weekly and given water *ad libitum*. Two weeks after maturity, we tested females with both live prey and video playback of male courtship. The video playback technique has been used extensively with this species (see Uetz & Roberts 2002; Uetz & Clark 2014; Uetz et al. 2016).

Eye occlusion technique.—Based on methodologies used in previous studies (Forster 1979; Rovner 1993), spider eyes were masked with paint. Spiders were either anesthetized with CO_2 prior to painting and kept unconscious or held under light restraint with mesh fabric during painting (controls were anesthetized and brushed with water, but not painted). Eyes were occluded under a dissecting microscope using a thin bristled paintbrush and black (Study 1) or silver (Study 2) Pelikan® PLAKA casein paint (Land 1969b; Rovner 1993). In the first study, eyes were occluded in complete rows prior to behavioral experiments. All spiders in treatment groups had the anterior eye row (AER), posterior eye row (PER), or both anterior and posterior eye rows occluded, while controls were left intact (Fig. 1A). In a second study, we occluded six of the eight eyes, leaving one pair from which the spider could see (AME, ALE, PME, or PLE) with a fifth treatment of controls with unobstructed vision (Fig. 1B). In a third study (female responses to video playback of male courtship) female eye rows (AER, PER) were occluded as above (Fig. 1A). Spiders whose face was painted with water and who retained full vision capabilities served as controls, and spiders with all eyes occluded served as an additional control.

Ethical note.—In all our studies, the paint had no apparent long-term deleterious effects. Although the paint treatment did remain intact for all behavioral trials, spiders were able to groom the paint off their eyes within twenty-four hours after the trial; spiders appeared in good health for the rest of their lifespan.

Prey detection and recognition.—*Study 1, eye row occlusion:* Females were placed randomly into one of three treatment groups. One group of spiders ($n = 26$) had their PER occluded with paint; a second group ($n = 24$) had their AER occluded with paint; and a third group ($n = 22$) was a control group painted with water but no paint (Fig. 1A). Trials were conducted in a 20.5 cm diam. circular Plexiglas® arena typical of other studies (see Uetz & Roberts 2002; Uetz & Clark 2014; Uetz et al. 2016) but modified with a clear divider between the spider and the cricket to ensure visual detection but eliminate any contact chemical or tactile cues. In this arena, spiders were exposed to a live cricket on a vibration-free substrate consisting of two slabs of granite, one underneath the area where the spider was located and one underneath the cricket's location, both layered on a second granite slab isolated with Sorbothane® vibration dampening material (Fig. 2). In this study, female spiders were starved for two weeks prior to testing. Trials were conducted < 48 hours after eye occlusion and lasted for 6 minutes. Spider behaviors displayed in response to the live cricket were recorded and scored manually from video recordings of trials for detection time (latency to orient in seconds), and recognition (approach time/frequency and frequency of lunges). Detection (latency to orient and approach cricket in seconds) was measured when the spider positioned the first two front legs perpendicular to the directional plane of the clear divider with the cricket in the background. Approach time was considered the length of time it took for the spider to walk up to the clear divider with the cricket in the background after orientation. A lunge was characterized as the spider jumping at the cricket in attempt to capture the prey item behind the clear plastic barrier.

Study 2, all but a single eye pair occluded: In the second study, females were assigned randomly into one of four treatment groups

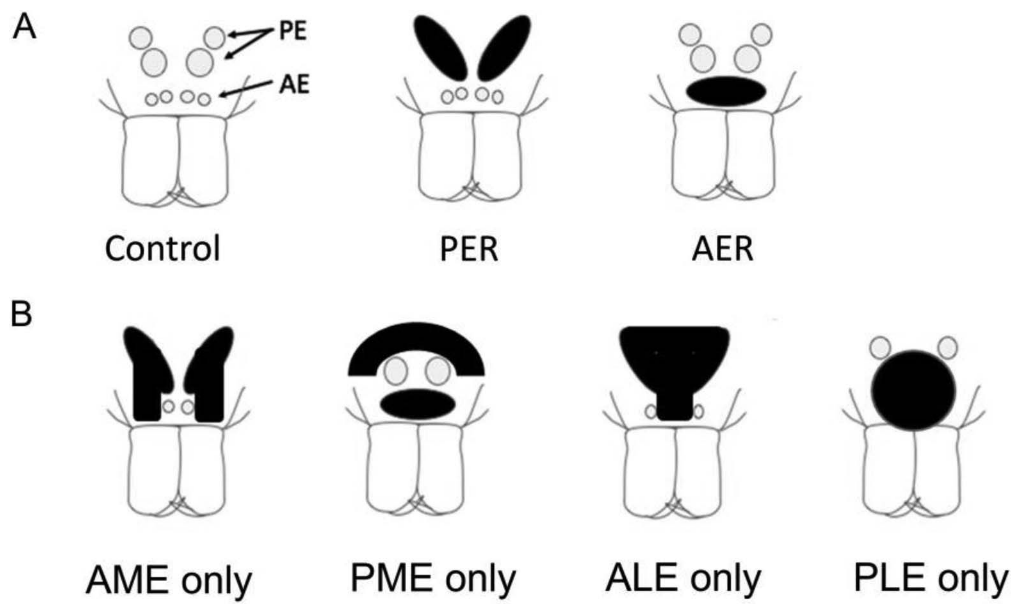

Figure 1.—Eye row occlusion diagram with each occlusion treatment. A: Study 1. Whole eye rows were occluded, while the other row was left intact: posterior eye row (PER); anterior eye row (AER). B: Study 2. Six of the eight eyes were occluded, leaving one intact pair from which the spider could see: posterior lateral eyes (PLE), posterior median eyes (PME), anterior lateral eyes (ALE), and anterior median eyes) (AME). Controls in both studies had no eyes occluded, but eyes were painted with water.

wherein we occluded six of the eight eyes (Fig. 1B), leaving one pair from which the spider could see: AME ($n = 21$), ALE ($n = 14$), PME ($n = 12$), PLE ($n = 17$). An additional randomly selected group with no eyes occluded but painted with water served as a control ($n = 23$). Mature female spiders were deprived of food for one week before prey detection/capture trials. The spiders were placed in an arena similar to the previous study (Fig. 2B). A cricket was introduced to the arena and the latency to orient and approach the cricket as well as the spider's success at capturing the cricket were recorded. In this study, because prey capture was recorded, vibration cues from crickets were not isolated with separate granite slabs, although the arena had opaque walls to

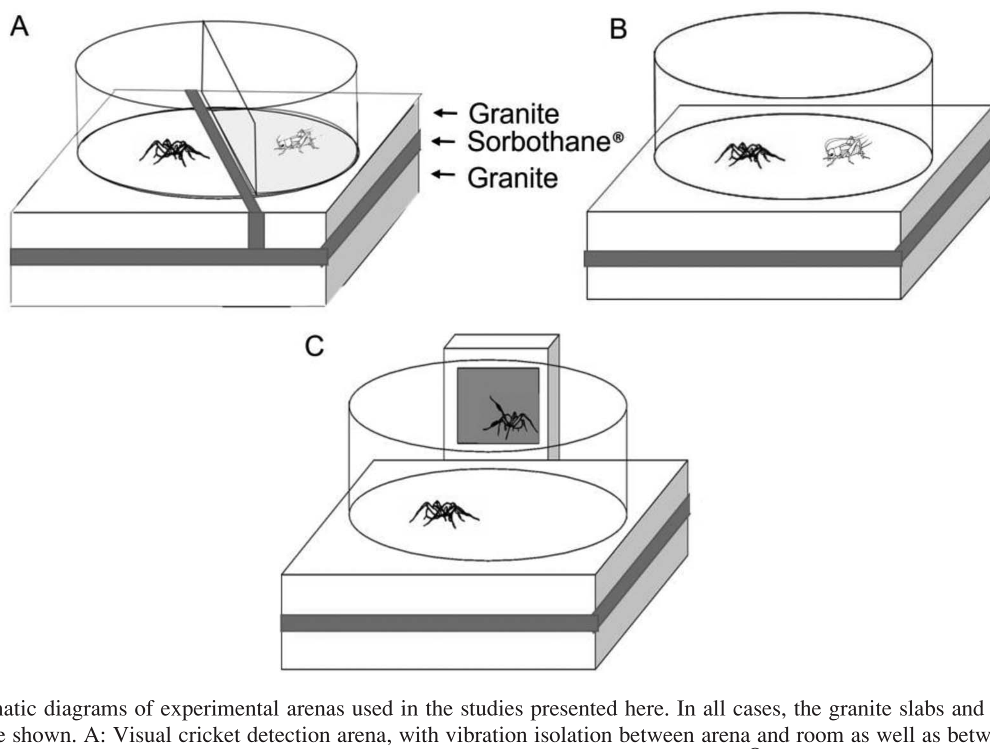

Figure 2.—Schematic diagrams of experimental arenas used in the studies presented here. In all cases, the granite slabs and Sorbothane® vibration damping material are shown. A: Visual cricket detection arena, with vibration isolation between arena and room as well as between cricket and spider. B. Cricket prey capture arena, with room vibration isolated. C. Video playback arena with Apple iPod® and room vibration isolated.

eliminate visual distraction and was placed on two layered granite slabs to eliminate extraneous vibration (Fig. 2B).

Detection and recognition of male courtship.—*Study 3 - eye row occlusion and mate recognition:* Previous studies have demonstrated an influence of reproductive age on female choosiness and therefore reproductive age was controlled (Uetz & Norton 2007). Female spiders were all selected to be of the same reproductive state and age, approximately two weeks past their final maturation molt. Three groups of females were selected at random and assigned to the same three treatment groups as the prey detection trials (PER occlusion: $n = 20$; AER occlusion: $n = 20$; control: $n = 20$). An additional control group of females was used initially to determine effectiveness of the eye occlusion technique in which all eyes were covered ($n = 9$). In this study, female spiders were fed 24 hours prior to video male courtship trials to standardize hunger and aggression as is standard procedure in previous video studies (McClintock & Uetz 1996; Uetz & Norton 2007).

Trials were conducted in a 20.5 cm diam. circular video arena as in previous studies and similar to the one above but modified for video playback (see Fig. 2C and Roberts & Uetz 2002; Uetz & Clark 2014). Approximately 48 hours after eye occlusion (as above), females were presented with playback of a 6-min video of a courting male on an iPod®, Model No. A1238 (McClintock & Uetz 1996; Uetz & Roberts 2002; Roberts et al. 2007; Moskalik & Uetz 2011; Uetz & Clark 2014; Uetz et al. 2016). Trials were recorded on digital video, which was subsequently used to score behavior. Several female behaviors displayed in response to the video were recorded. Detection time, or latency to orient in seconds was measured when the spider positioned the first two front legs perpendicular to the directional plane of the iPod® screen. Female mate recognition was measured using latency of approach and the sum of receptivity displays. Approach time was considered the length of time it took for the female to walk up to the iPod® screen. The total number of receptive display behaviors included tandem leg extensions, slow pivots, and settles, all of which are commonly observed in the study species leading to copulation (Uetz & Denterlein 1979; Scheffer et al. 1996; Norton & Uetz 2005).

Statistical analysis.—All statistical analyses were conducted using GraphPad Prism version 7.00 for Mac OS X [GraphPad Software, La Jolla California USA, www.graphpad.com] or JMP 15.0 [JMP Statistical Discovery LLC, SAS, Cary North Carolina USA, www.jmp.com]. Survival analyses were used to compare latency to orient/approach each stimulus (video of courting male or live cricket) among treatment groups (control, AER-occlusion, and PER-occlusion; as well as AME-only, ALE-only, PME-only, PLE-only and control) for female *S. ocreata* (see Jahn-Eimermacher et al. 2011). All data used in survival analyses were right-censored and singly-censored as each event recorded (orient/approach) could only occur once in a fixed period of time (6-minute trials). The significance of each survival analysis was plotted as a Failure analysis (cumulative % responding) summarized using a Log-rank (Mantel-Cox) test including Chi-square values, degrees of freedom (df), and p-values, followed by analysis with either One-Way ANOVA or non-parametric Wilcoxon tests.

For all frequency data (number of approaches, lunges, and receptivity displays), normality was assessed using the D'Agostino & Pearson normality test. Because the data were not normally distributed, comparisons among the treatment groups (control, AER-occlusion, PER-occlusion, occlusion of all eyes except single eye pairs) for each variable were performed using a Wilcoxon non-parametric test with Bonferroni correction for multiple comparisons. Variance is summarized in figures using standard error (SE) bars. SE error bars were used over standard deviation to emphasize the accuracy of the mean versus showing variation in values among the treatment groups.

RESULTS

Prey detection experiments with eye occlusion.—*Study 1, eye row occlusion:* Differences in overall prey detection of females (frequency of orientation) were not significantly different among treatments (Orient frequency: Pearson $\chi^2 = 2.550$, df = 2, $P = 0.279$). However, latency to approach prey varied significantly among treatments (Fig. 3A; Log-rank (Mantel-Cox) test: $\chi^2 = 9.164$, df = 2, $P = 0.0101$). When the PER were occluded, the female latency to approach to the live cricket was significantly longer than the control with the AER-occluded group being intermediate. The mean latency (secs) was also significant by treatment (Wilcoxon test: χ^2 [df = 2] =7.805; $P = 0.0202$), and post-hoc tests show the direction of differences was the same as the log-rank analysis of cumulative survival/failure (PER > Control: Z = 2.46799, $P = 0.0136$ PER > AER: Z = 2.15293, $P = 0.0313$; AER ≈ Control: Z = −0.4726; $P = 0.6365$) (Fig. 3B). The mean number of approaches/lunges of females toward the cricket (Fig. 3C) was also significantly different across treatment groups, with PER-occluded group having the least and AER-occluded being intermediate (Wilcoxon test: χ^2 [df = 2] = 8.5692; $P = 0.0138$). Wilcoxon post-hoc tests showed that the AER-occluded group and PER-occluded group had significantly fewer approaches than the control group (Z = 2.66099; $P = 0.0078$), although the difference between AER-occluded and PER-occluded groups was not significant (Fig. 3C).

Study 2, all but a single eye pair occluded: The cumulative distribution of prey detection latency (time it took for all spiders to orient to the cricket) varied significantly with eye treatment (Log-rank (Mantel-Cox) test: $\chi^2 = 14.59$. df = 4, $P = 0.0056$). Latency (Fig. 4A) was shortest for the controls, with 90% responding in < 50 secs. The PLE-only treatment oriented almost as quickly; within approx. 65 secs. The anterior eye treatments (AME-only, ALE-only) fell closely behind within a middle range; and the PME-only treatment took the longest to orient on average than any of the other eye groups (~ 3 min). An analysis of mean prey detection latency for treatments was significant (Wilcoxon test: χ^2 (df = 4) = 16.0137; $P = 0.0035$) and reflected this pattern (Fig. 4B). Post-hoc tests (Wilcoxon) show the spiders in the control and PLE-only treatments responded in the shortest time (< 75s); AME-only and ALE-only were intermediate (~75–100 secs). Spiders in the PME-only treatment showed the longest times on average (> 175 secs) with some taking over three min to respond (PME > Control: Z = 4.1912, $P = 0.0046$), and AME also took longer than controls (AME > Control: Z = 3.4526, $P = 0.0006$). The PLE and Control took the shortest to respond and were not significantly different from each other (PLE x Control: Z = 0.6654, $P = 0.5058$), while ALE were intermediate (ALE x Control: Z = 1.3936, = 0.1634). Subsequent survival/failure analyses showed no significant difference in the mean latency to orient between the two pairs of anterior eyes (Log-rank (Mantel-Cox) test: $\chi^2 = 0.3105$, df = 2, $P = 0.0101$), but within the posterior eyes, the PLE-only oriented significantly faster than the PME-only (Log-rank (Mantel-Cox) test: $\chi^2 = 4.909$, df = 1, $P = 0.5774$).

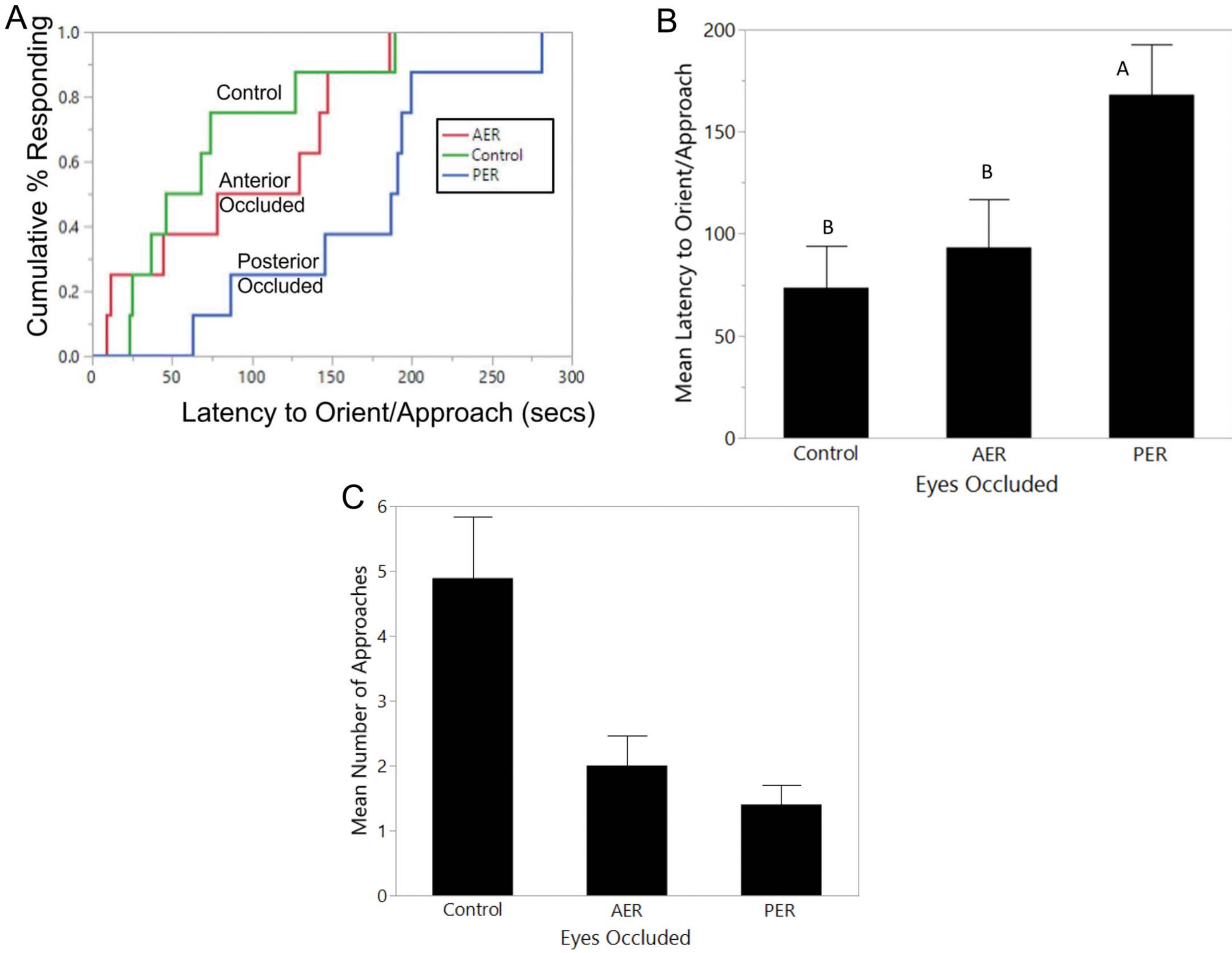

Figure 3.—Latency (seconds) to orient and approach prey for female *S. ocreata* in response to a live cricket by eye row occlusion treatment group (Study 1). A. Cumulative survival/failure probability curves; B. Mean latency in seconds (±S.E.) to orient/approach prey; C. Mean number of approaches with lunges (±SE). Differences between treatment groups from *post-hoc* Wilcoxon tests are noted in figures 3 B. and 3 C. using letters (e.g., A, B) (see text for details).

Results of orientation response over distance show a significant (Wilcoxon test: $\chi^2 = 15.3876$; $P = 0.0040$) but somewhat different pattern from that shown by latency (Fig. 5). Post-hoc tests (Wilcoxon) showed that Control and PME-only spiders response distances were not significantly different from each other (Control = PME: Z = 1.0484, $P = 0.2944$) but both responded at greater distance than other eye treatments (Control and/or PME > ALE: Z = 1.990, $P = 0.446$; Control and/or PME > AME: Z = 3.293, $P = 0.0010$; Control and/or PME > PLE: Z = 2.667, $P = 0.0076$). Spiders in the AME-only, ALE-only and PLE-only treatments were not different from each other (Fig. 5).

The mean number of approaches/lunges at crickets (Fig. 6A) varied significantly with eye treatment (Wilcoxon test: $\chi^2 = 13.056$; $P = 0.011$). The highest number of approaches/lunges was for control spiders, and lowest were the AME-only and PLE-only, which did not differ from each other (Fig. 6A). The ALE-only and PME-only treatments were intermediate, overlapping both extremes. Prey capture success, i.e., the percentage of spiders capturing crickets after orientation and approach (Fig. 6B), differed significantly across eye treatments, with control spiders having 90% or greater success (Log-likelihood test: $\chi^2 = 10.887$; $P = 0.0279$). However, there are only slight differences across eye occlusion treatments, with AME-only and ALE-only spiders capturing prey approximately 65% of the time, and the PME-only and PLE-only treatments capturing slightly less, ca. 55%).

Detection and recognition of male courtship.—*Study 3, eye row occlusion and mate recognition:* The frequency of females orienting to the video of a courting-male (% spiders responding) was not independent of eye occlusion treatment (Pearson's $\chi^2 = 40.922$, $P < 0.0001$). Spiders with all their eyes occluded did not respond, and those with PER-occluded were less likely to respond than spiders in the control and AE-occluded treatments. Because some individuals never oriented toward the video within the allotted trial time, we also analyzed data using a survivorship/proportional hazards model in order to statistically censor the data (as described above in Methods). Differences in female detection,

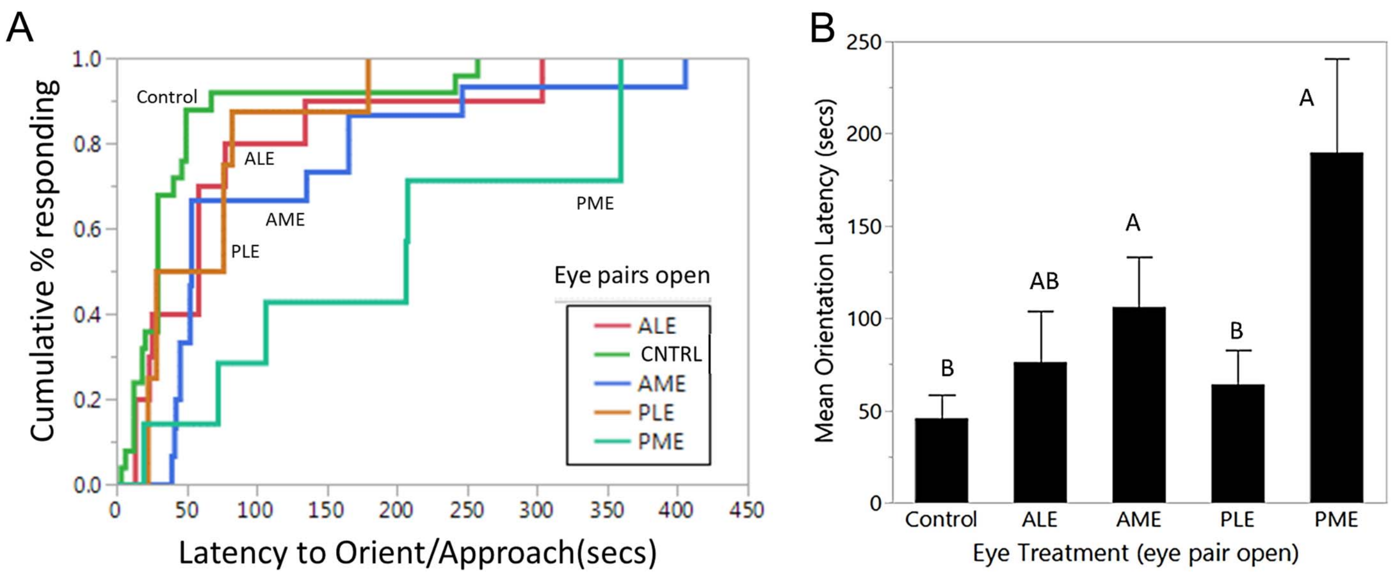

Figure 4.—Latency (seconds) to orient and approach prey for female *S. ocreata* in response to a free-moving live cricket by eye occlusion/single eye pair open treatment group (Study 2). A. Cumulative survival/failure probability curves; B. Mean latency to orient and approach prey in seconds (±SE) for female *S. ocreata* in response to a free-moving live cricket by eye treatment group. Differences between treatment groups from *post-hoc* Wilcoxon tests are noted using letters (e.g., A, B) (see text for details).

i.e., latency to orient/approach the courting male video (Fig. 7A) were significant among treatments (Log-rank (Mantel-Cox) test: $\chi^2 = 6.4926$, df = 3, $P = 0.0389$). While data from spiders with all eyes painted confirmed the efficacy of painting eliminating visual detection: to avoid bias we removed these values in further analyses. Differences in mean latency to orient (Fig. 7B) were significant (Wilcoxon: $\chi^2 = 6.3354$, df = 2, $P = 0.0421$)) which Wilcoxon *post-hoc* tests show were largely due to a significantly longer latency for spiders with PER-occluded compared to unmanipulated controls and AER-occluded treatments (PER > Control: Z = 2.138, $P = 0.0325$; PER > AER: Z = 2.193, $P = 0.0282$); values for spiders with AER occluded were close to controls (Control ≈ AME: Z = 0.2237, $P = 0.823$).

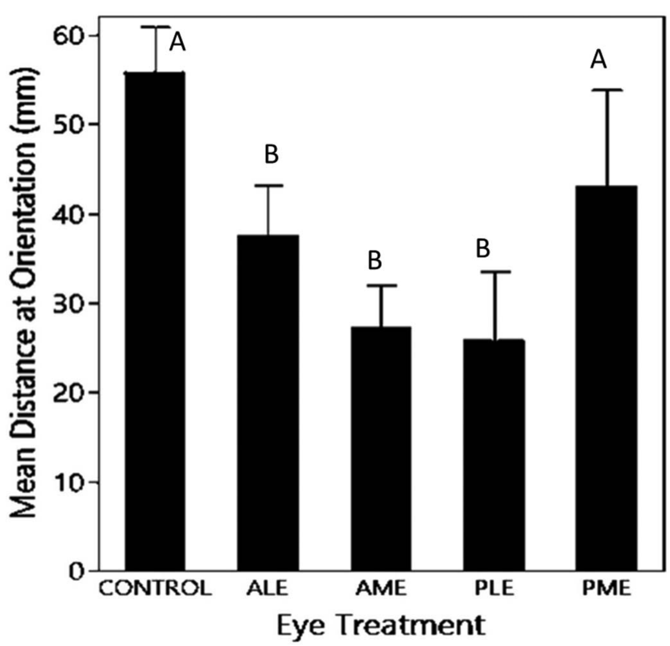

Figure 5.—Mean orientation distance (mm) of female *S. ocreata* (±SE) toward a free-moving live cricket by eye treatment group. Differences between treatment groups from *post-hoc* Wilcoxon tests are noted using letters (e.g., A, B) (see text for details).

Receptivity of female spiders: Female receptivity was measured based on the sum of receptivity displays and behaviors exhibited by the females in response to a courting male stimulus, as used in previous studies (Uetz et al. 2016). When presented with video playback of a courting male, female composite receptivity displays (Fig. 8A) varied significantly with eye treatment (Wilcoxon test: $\chi^2 = 3.863$, df = 2, $P = 0.0364$). Wilcoxon *post-hoc* tests show that receptivity scores were significantly reduced when PER were occluded (PER < Control/AER; Z = −2.588, $P = 0.0296$) and greater for controls and AER-occluded, which overlap in significance level (Control ≈ AER; Z = 1.2063, $P = 0.2277$). Interestingly, female aggression, measured as the sum of lunges and attacks directed at the video screen (Fig. 8B) also varied significantly with treatment, but in the opposite direction (Wilcoxon test: $\chi^2 = 6.553$; $P = 0.0378$). Aggression scores were greatest with the PER occluded (Wilcoxon post-hoc test: PER > Control/AER; Z = 2.236, $P = 0.0253$) and lowest with AER occluded (Control ≈ AER; Z = 0.5571, $P = 0.5774$).

DISCUSSION

While there are some fundamental similarities in structure among eye types in spiders, the diversity in arrangement, number, and size of these types across families and genera begs the question of whether eye types play different roles among spider taxa (Homann 1931, 1971; Foelix 2011; Morehouse 2020; Nentwig et al. 2022). Salticids have been studied most extensively regarding eye structure and roles of different eye types (Homann 1931, 1971; Jackson & Pollard 1996; Harland & Jackson 2000; Masta & Madison 2002; Skow & Jakob 2006; Spano et al 2012); however, there are clear

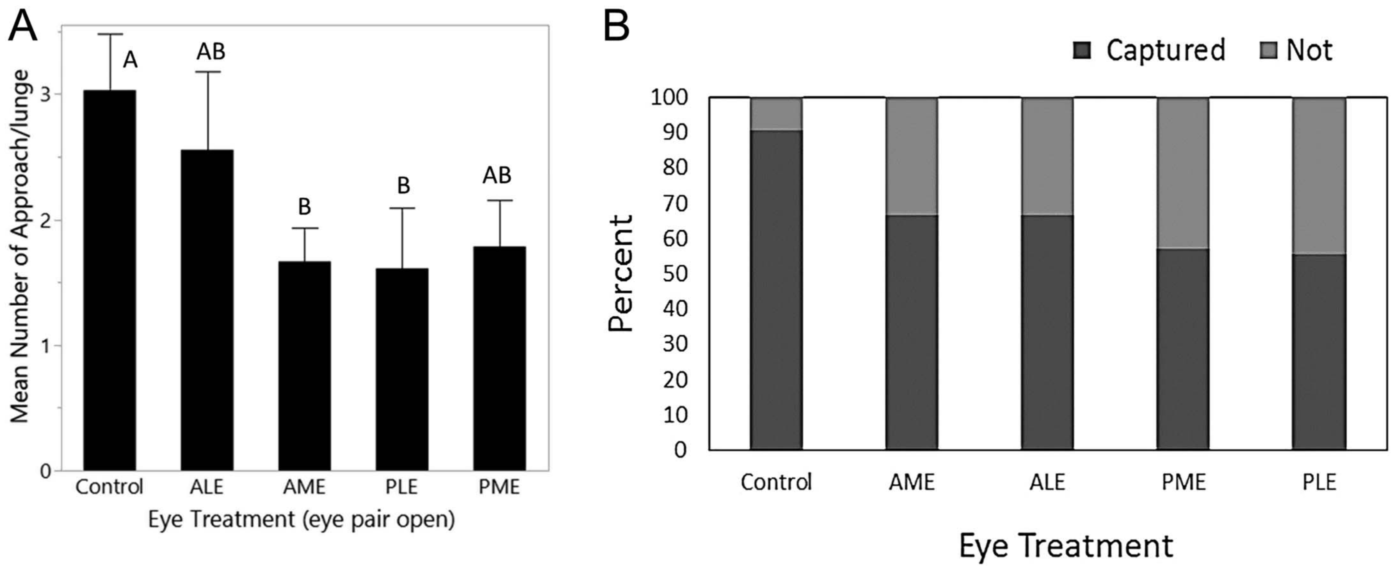

Figure 6.—Approaches and capture of free-moving live cricket prey by female *S. ocreata* by eye treatment group. A. Mean number of approaches with lunges (±SE). Differences between treatment groups from *post-hoc* Wilcoxon tests are noted using letters (e.g., A, B) (see text for details). B. Prey capture success, i.e., the percentage of spiders capturing crickets after orientation and approach.

morphological differences between salticid eye arrangement and the size distribution and eye arrangement of lycosids. In lycosids, the ALE are more similar in structure and focal length to the principal eyes than the other two pairs of secondary eyes (PME and PLE; Clemente et al. 2010; Foelix 2011; Morehouse 2020). While some behavioral evidence is available for *Rabidosa rabida* (Rovner 1996), there are no additional studies investigating if these structural similarities between posterior positioned eyes and anterior positioned eyes are reflected in behavior, particularly in a species that uses visual cues for both courtship and hunting prey. The results of this study suggest that the posterior secondary eyes (PLE and PME) may have different roles in detection and recognition of prey and courting males than the remaining pair of secondary eyes (ALE) and the principal eyes (AME).

In this study, the posterior secondary eyes of *S. ocreata* appear to influence the detection and recognition of prey. This result further supports Rovner's findings in the wolf spider *Rabidosa rabida*, confirming the posterior secondary eyes are critical in recognition as

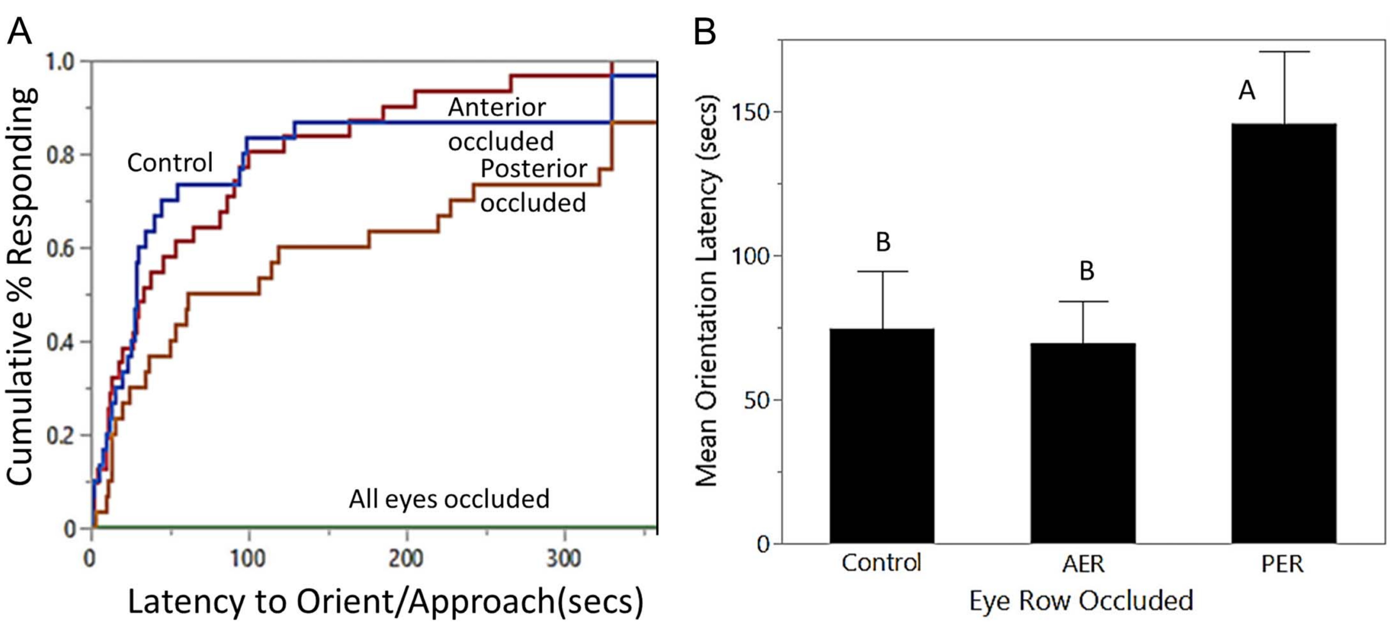

Figure 7.—Latency to orient (seconds) for female *S. ocreata* toward a courting video male by eye row occlusion treatment group. A. Cumulative survival/failure probability curves; B. Mean latency to orient in seconds (±SE). Differences between treatment groups based on Wilcoxon *post-hoc* tests are noted using letters (e.g., A, B) (see text for details).

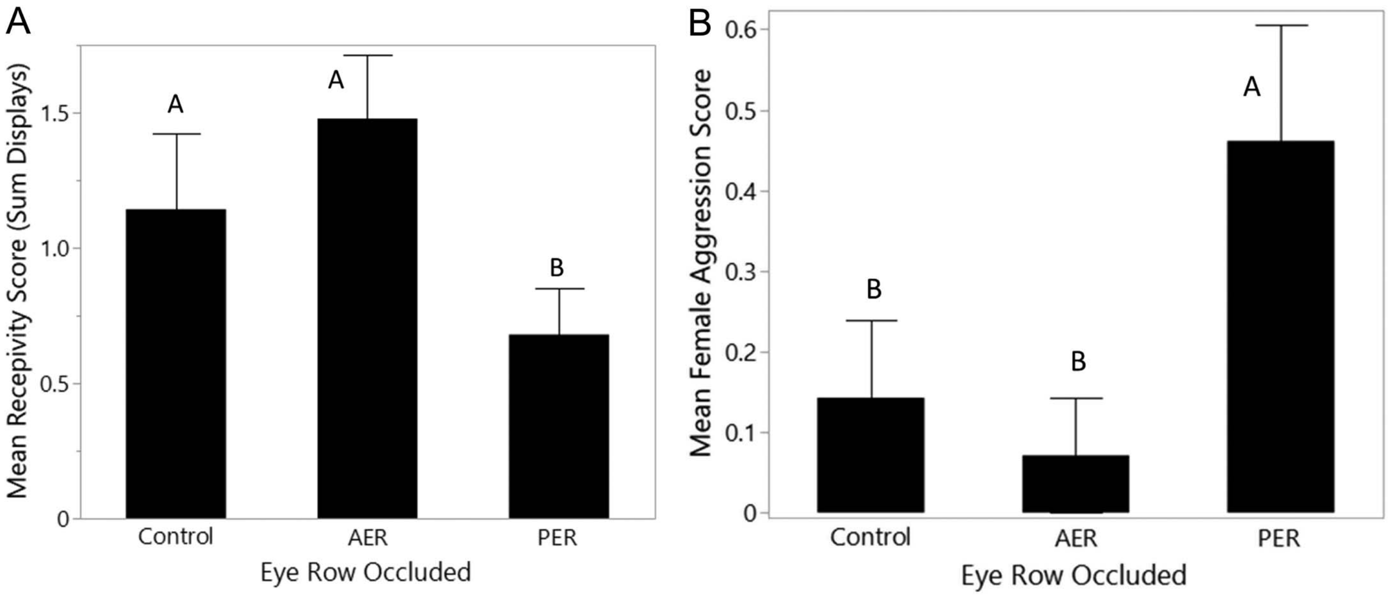

Figure 8.—A. Mean receptivity score (sum of receptivity displays) for female *S. ocreata* spiders (±SE) toward a courting male video stimulus by eye row occlusion treatment (those with all eyes painted did not respond). B. Mean aggression score (sum of leg raise, lunge behaviors) (±SE) by female *S. ocreata* spiders toward a courting male video stimulus by eye row occlusion treatment; Differences between treatment groups based on Wilcoxon *post-hoc* tests are noted using letters (e.g., A, B) (see text for details).

well as detection of visual cues (Rovner 1993). Our findings also agree with Clemente's findings in the Australian wolf spider *Lycosa leuckarti* (Thorell, 1870) (now in the genus *Tasmanicosa* Framenau & Baehr, 2016) in which posterior eyes functionally suit long-range predator and prey detection while anterior are best for distance judgment and prey capture (Clemente et al. 2010). In fact, the functionality of anterior lateral eyes in judgment distance and prey capture could partially explain why we see intermediate results when AER were occluded in our study. The AME and ALE differ substantially in size compared to the posterior eyes, with the AME being anatomically different in spider species (Homann 1971; Land 1985a). Due to their size differences alone, there is likely a different resolution, but there is also a difference in field of view in lycosids between the anterior and posterior eyes (Homann 1971; Land 1985a). These differences could be causing the intermediate effects seen (Figs. 3–5).

In isolation, the PLE were most effective in prey capture. This may be due to their position on the cephalothorax, with a range of vision of 270° or more (Land 1985a), allowing a role as a motion detector. The principal eyes in many spiders are the AME, which in some species provide the best spatial resolution. In Lycosidae, however, with smaller AME, this ability may be surpassed by the PME as shown in *Lycosa tarantula* (Linnaeus, 1758) (Kovoor et al. 2005) or the PME in this study, as well as in *Cupiennius salei* (Keyserling, 1877) (Trechaleidae) (Schmid 1998). The PME, despite slightly larger size and more visual cells, have a smaller range of vision and a longer latency to detect prey. The ALE, like the PLE and PME, detect motion, but with a more limited range of vision and fewer visual cells (Homann 1971). All these sets of eyes are considered secondary eyes anatomically and are processed by different neural pathways that converge in the same part of the brain, the mushroom body (Kovoor et al. 2005). Similar results have been seen in other Tracheleidae (formerly Ctenidae) (Strausfeld & Barth 1993). If the PME provide good spatial resolution, it might be at the expense of motion detection due to some aspect of optics or the number of cells available for vision in small animals. Also, since the neurons are more interconnected than those of other sets of eye pairs, the lack of input from the PLE may hamper the effectiveness of the PME (Kovoor et al. 2005). Thus, the poor response of the PME to orient to prey movement in our study provides behavioral evidence supporting previous physiological research.

The posterior secondary eyes are used in detecting motion in salticids and could be playing a similar role in lycosids (Foelix 2011; Morehouse 2020). Where the similarities between salticids and lycosids begin to disappear is in the roles of the third pair of secondary eyes (ALE) and the principal eyes. In salticids the AER, specifically the ALE, are effective at detecting prey and moving objects alone (Land 1971; Zurek et al. 2010; Zurek & Nelson 2012). In our study, it appears that posterior eyes alone are capable of detection and recognition of prey in lycosids without a significant level of assistance from the anterior eyes. Thus, posterior eyes appear to be influential in detecting motion and form.

The posterior secondary eyes also appear to play a significant role in detecting and recognizing conspecific courting males in *S. ocreata*. However, overlap in significance of post-hoc test ranges for mean receptivity score between AER-occluded females and controls should be interpreted with caution, as latency to orient for this treatment group was intermediate between the control and PER group. Even so, the trend in mean aggression scores is opposite that for mean receptivity score, supporting the hypothesis that the posterior eyes play a role in recognition of males. Given that there was a significant difference in female receptivity displays and aggression of spiders between PER-occluded and AER-occluded treatments, the posterior secondary eyes may not share the same function in detecting or recognizing male courtship behavior as the principal eyes.

Although our results corroborate previous studies with a different species (Rovner 1993) and extend them to *S. ocreata*, more research is needed to analyze functional roles of individual pairs in this well-studied species. Female *S. ocreata* have been shown to prefer males with large tufts (McClintock & Uetz 1996; Scheffer et al. 1996) and use this condition-dependent trait to discriminate and choose higher-quality mates (Uetz et al. 2002; Uetz & Roberts 2002; Uetz & Clark 2014; Uetz et al 2016). It would be interesting to expand this line of research to determine which eye rows are used in assessment of tuft size. Thus, further investigation is needed to examine the roles of eyes in mate assessment (e.g., evaluation of mate quality based on tuft size) or whether compensation occurs when a single eye set is occluded. It is important to note that the role of posterior secondary eyes seems to be more influential in both prey and male courtship recognition in this study. However, due to additional differences in lunging at prey and/or displaying receptive and aggressive behaviors toward the courting male videos when the posterior secondary eyes alone were occluded, it appears these eye pairs also participate to some degree in supplying sufficient information on the identification of the moving object.

While we found differences in response to distances of visual prey between principal and secondary eye pairs (AME vs. ALE, PME and PLE), our results cannot support a more specific role that the posterior secondary eyes play in detection and recognition of long range visual cues (Rovner 1996). Additional research is needed to increase the overall number of wolf spider species investigated for their acute visual system and to elucidate if this role differentiation is evolutionarily conserved across the family Lycosidae. In *S. ocreata* specifically, we might speculate this differentiation could have aided in allowing visual cues to be a strong variable in mate choice by females. As *S. ocreata* relies heavily on vision for prey capture (Persons & Uetz 1997, 1998), it would seem likely that different rows of eyes might have already been pre-adapted for specialized tasks. However, a majority of the species in the genus *Schizocosa* utilize vibratory cues as the predominant mode of communication and thus the answer to how the roles of different eyes arose in conspecific recognition may be much more complex (Hebets et al. 2013; Herberstein et al. 2014; McGinley et al. 2023; Starret et al. 2022).

Further analysis of different eye functions may be needed to provide insight regarding questions about evolution of visual courtship displays in *S. ocreata* and its absence in other *Schizocosa* species (Starret et al. 2022). Recent studies (McGinley et al. 2023) have underscored the role of the light level in the environment and visual detection of male leg pigmentation and decoration in different species of *Schizocosa*. Because *S. ocreata* is a frequent model animal for behavioral studies on complex multimodal signaling, it should be useful to investigate how visual processing has evolved in both a physiological and ecological context (Uetz & Roberts 2002; Roberts et al. 2007; Hebets et al. 2013; McGinley et al. 2023).

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LITERATURE CITED

Barth FG. 1985. Neurobiology of Arachnids. Springer, Berlin.

Barth FG. 2002. A Spider's World: Senses and Behavior. Springer, Berlin.

Bristowe WS, Locket GH. 1926. The courtship of British lycosid spiders, and its probable significance. *Journal of Zoology* 96:317–347.

Cerveira AM, Nelson XJ, Jackson RR. 2021. Spatial acuity-sensitivity trade-off in the principal eyes of a jumping spider: possible adaptations to a 'blended' lifestyle. *Journal of Comparative Physiology A* 207: 437–448.

Clemente CJ, McMaster KA, Fox E, Meldrum L, Stewart T, York B. 2010. The visual system of the Australian wolf spider *Lycosa leuckartii* (Araneae: Lycosidae): visual acuity and the functional role of the eyes. *Journal of Arachnology* 38:398–406.

Dacke M, Doan TA, O'Carroll DC. 2001. Polarized light detection in spiders. *Journal of Experimental Biology* 204:2481–2490.

DeVoe RD. 1962. Linear superposition of retinal action potentials to predict electrical flicker responses from the eye of the wolf spider, *Lycosa baltimoriana* (Keyserling). *Journal of General Physiology* 46:75–96.

Foelix RF. 2011. Biology of Spiders. Harvard University Press, Cambridge, Massachusetts.

Forster LM. 1979. Visual mechanisms of hunting behaviour in *Trite planiceps*, a jumping spider (Araneae: Salticidae). *New Zealand Journal of Zoology* 6:79–93.

Harland DP, Jackson RR. 2000. 'Eight-legged cats' and how they see - a review of recent research on jumping spiders (Araneae: Salticidae). *Strategy* 1878:231–240.

Harland DP, Jackson RR, Macnab AM. 1999. Distances at which jumping spiders (Araneae: Salticidae) distinguish between prey and conspecific rivals. *Journal of Zoology* 247:357–364.

Hebets EA, Vink CJ. 2007. Experience leads to preference: experienced females prefer brush-legged males in a population of syntopic wolf spiders. *Behavioral Ecology* 18:1010–1020.

Hebets EA, Vink CJ, Sullivan-Beckers L, Rosenthal MF. 2013. The dominance of seismic signaling and selection for signal complexity in *Schizocosa* multimodal courtship displays. *Behavioral Ecology & Sociobiology* 67:1483–1498.

Herberstein ME, Wignall AE, Hebets EA, Schneider JM. 2014. Dangerous mating systems: signal complexity, signal content and neural capacity in spiders. *Neuroscience & Biobehavioral Reviews* 46:509–518.

Homann H. 1931. Beiträge zur Physiologie der Spinnenaugen. III. Das Sehvermögen der Lycosiden. *Zeitschrift für Vergleichende Physiologie* 14:40–67.

Homann H. 1971. Die augen der Araneae, ontogenie und bedeutung fur die systematik (Chelicerata, Arachnida). *Zoomorphology* 69:201–272.

Jackson RR, Pollard SD. 1996. Predatory behavior of jumping spiders. *Annual Reviews in Entomology* 41:287–308.

Jahn-Eimermacher A, Lasarzik I, Raber J. 2011. Statistical analysis of latency outcomes in behavioral experiments. *Behavioural Brain Research* 221:271–275.

Jakob EM, Long SM, Harland DP, Jackson RR, Carey A, Searles ME, et al. 2018. Lateral eyes direct principal eyes as jumping spiders track objects. *Current Biology* 28:R1092–1093.

Kovoor J, Cuevas AM, Escobar JO. 1993. Microanatomy of the anterior median eyes and its possible relation to polarized-light reception in *Lycosa tarentula* (Araneae, Lycosidae). *Bollettino Di Zoologia* 60:367–375.

Kovoor J, Muñoz-Cuevas A, Ortega-Escobar J. 2005. The visual system of Lycosa tarentula (Araneae, Lycosidae): Microscopic anatomy of the protocerebral optic centres. *Italian Journal of Zoology* 72:205–216.

Koyanagi M, Nagata T, Katoh K, Yamashita S, Tokunaga F. 2008. Molecular evolution of arthropod color vision deduced from multiple opsin genes of jumping spiders. *Journal of Molecular Evolution* 66: 130–137. doi: 10.1007/s00239-008-9065-9

Land MF. 1969a. Structure of the retinae of the principal eyes of jumping spiders (Salticidae: Dendryphantinae) in relation to visual optics. *Journal of Experimental Biology* 51:443–470.

Land MF. 1969b. Movements of the retinae of jumping spiders (Salticidae: Dendryphantinae) in response to visual stimuli. *Journal of Experimental Biology* 51:471–493.

Land MF. 1971. Orientation by jumping spiders in the absence of visual feedback. *Journal of Experimental Biology* 54:119–139.

Land MF. 1985a The morphology and optics of spider eyes. Pp. 53–78. *In* Neurobiology of Arachnids. (Barth, FG. Ed.) Springer-Verlag. Berlin.

Land MF. 1985b. Fields of view of the eyes of primitive jumping spiders. *Journal of Experimental Biology* 119:381–384.

Land MF, Nilsson DE. 2012. Animal Eyes. Oxford University Press, Oxford.

Lizotte, RS, Rovner JS. 1988. Nocturnal capture of fireflies by lycosid spiders: visual versus vibratory stimuli. *Animal Behaviour* 36:1809–1815.

Magni F, Papi HE, Savely E, Tongiorgi P. 1964. Research on the structure and physiology of the eyes of a lycosid spider. II. The role of different pairs of eyes an astronomical orientation. *Archives Italiennes de Biologie* 102:123–136.

Masta SE, Maddison WP. 2002. Sexual selection driving diversification in jumping spiders. *Proceedings of the National Academy of Sciences U. S. A.* 99:4442–4447.

McClintock WJ, Uetz GW. 1996. Female choice and pre-existing bias: visual cues during courtship in two *Schizocosa* wolf spiders (Araneae: Lycosidae). *Animal Behaviour* 52:167–181.

McGinley RH, Starrett J, Bond JE, Hebets EA. 2023. Light environment interacts with visual displays in a species-specific manner in multimodal-signaling wolf spiders. *The American Naturalist* 201: 472-490. https://doi.org/10.1086/722830

Melamed J, Trujillo-Cenóz O. 1966. The fine structure of the visual system of *Lycosa* (Araneae: Lycosidae). *Zeitschrift für Zellforschung* 74: 12–31. https://doi.org/10.1007/BF00342937

Melamed J., Trujillo-Cenóz O. 1971. Innervation of the retinal muscles in wolf spiders (Araneae-Lycosidae). *Journal of Ultrastructure Research.* 35:359–369. ISSN 0022-5320. https://doi.org/10.1016/S0022-5320(71) 80163-6

Menda G, Shamble PS, Nitzany EI, Golden JR, Hoy RR. 2014. Visual perception in the brain of a jumping spider. *Current Biology* 24:2580–2585.

Morehouse N. 2020. Spider vision. *Current Biology* 30:R975-R980.

Moskalik B, Uetz GW. 2011. Female hunger state affects mate choice of a sexually selected trait in a wolf spider. *Animal Behaviour* 81:715–722.

Nelson XJ, Jackson RR. 2012 The discerning predator: decision rules underlying prey classification by a mosquito-eating jumping spider. *Journal of Experimental Biology* 215:2255–2261.

Nentwig W, Ansorg J, Bolzern A, Frick H, Ganske AS, Hänggi A, et al. 2022. How Do Spiders See? In All You Need to Know About Spiders (Pp. 15–22). Springer. https://doi.org/10.1007/978-3-030-90881-2_2

Norton S, Uetz GW. 2005. Mating frequency in *Schizocosa ocreata* (Hentz) wolf spiders: evidence for a mating system with female monandry and male polygyny. *Journal of Arachnology* 33:16–24.

Ortega-Escobar J. 2006. Role of the anterior lateral eyes of the wolf spider *Lycosa tarentula* (Araneae, Lycosidae) during path integration. *Journal of Arachnology* 34:51–61.

Ortega-Escobar J, Muñoz-Cuevas A. 1999. Anterior median eyes of *Lycosa tarentula* (Araneae, Lycosidae) detect polarized light: behavioral experiments and electroretinographic analysis. *Journal of Arachnology* 27:663–671.

Persons M, Uetz GW. 1996. The influence of sensory information on patch residence time in wolf spiders (Araneae: Lycosidae). *Animal Behaviour* 51:1285–1293.

Persons M, Uetz GW. 1997. The effect of prey movement on attack behavior and patch residence decision rules of wolf spider (Araneae: Lycosidae). *Journal of Insect Behavior* 10:737–752.

Persons M, Uetz GW. 1998. Presampling sensory information and prey density assessment by wolf spiders (Araneae, Lycosidae). *Behavioral Ecology* 9:360–366.

Roberts JA, Taylor PW, Uetz GW. 2007. Consequences of complex signaling: predator detection of multimodal cues. *Behavioral Ecology* 18: 236–240.

Rovner JS. 1989. Wolf spiders lack mirror-image responsiveness seen in jumping spiders. *Animal Behaviour* 38:526–533.

Rovner JS. 1993. Visually mediated responses in the lycosid spider *Rabidosa rabida*: the roles of different pairs of eyes. *Memoirs of The Queensland Museum* 33:635–638.

Rovner JS. 1996. Conspecific interactions in the lycosid spider *Rabidosa rabida*: the roles of different senses. *Journal of Arachnology* 24:16–23.

Scheffer SJ, Uetz GW, Stratton GE. 1996. Sexual selection, male morphology, and the efficacy of courtship signalling in two wolf spiders (Araneae: Lycosidae). *Behavioral Ecology and Sociobiology* 38:17–23.

Schmid A. 1998. Different functions of different eye types in the spider *Cupiennius salei*. *Journal of Experimental Biology*. 201:221–225.

Schwab IR, Yuen CK, Buyukmihci NC, Blankenship TN, Fitzgerald PG. 2002. Evolution of the tapetum. *Transactions of the American Ophthalmological Society* 100:187–200.

Skow CD, Jakob EM. 2006. Jumping spiders attend to context during learned avoidance of aposematic prey. *Behavioral Ecology* 17:34–40.

Spano L, Long SM, Jakob EM. 2012. Secondary eyes mediate the response to looming objects in jumping spiders (*Phidippus audax*, Salticidae). *Biology Letters* 8:949–951.

Starrett J, McGinley RH, Hebets EA, Bond JE. 2022. Phylogeny and secondary sexual trait evolution in *Schizocosa* wolf spiders (Araneae, Lycosidae) shows evidence for multiple gains and losses of ornamentation and species delimitation uncertainty. *Molecular Phylogenetics and Evolution* 169:107397.

Strausfeld NJ, Barth FG. 1993. Two visual systems in one brain: neuropils serving the secondary eyes of the spider *Cupiennius salei*. *Journal of Comparative Neurology* 328:43–62.

Trujillo-Cenóz O, Melamed J. 1967. The fine structure of the visual system of lycosa (Araneae: Lycosidae). *Zeitschrift für Zellforschung* 76: 377–388. https://doi.org/10.1007/BF00339295

Uetz GW, Clark DL. 2014. A tale of two spiders: investigating communication in two unique model species using video digitization and playback. Pp. 63–99. *In* Animal Behavior: How and Why Animals Do the Things They Do Vol. 3 – Integration and Application with Case Studies. (Yasukawa K, ed). Praeger-PSI.

Uetz GW, Denterlein G. 1979. Courtship behavior, habitat, and reproductive isolation in *Schizocosa rovneri* (Uetz and Dondale 1979) (Araneae: Lycosidae). *Journal of Arachnology* 7:121–128.

Uetz GW, Norton S. 2007. Preference for male traits in female wolf spiders varies with the choice of available males, female age and reproductive state. *Behavioral Ecology and Sociobiology* 61:631–641.

Uetz GW, Roberts JA. 2002. Multisensory cues and multimodal communication in spiders: insights from video/audio playback studies. *Brain Behavior & Evolution* 59:222–230.

Uetz GW, Stratton GE. 1983. Communication in spiders. *Endeavour* 7: 13–18.

Uetz GW, Clark DL, Roberts JA. 2016. Multimodal communication in wolf spiders (Lycosidae) – an emerging model for study. (Naguib M, Mitani JC, Simmons LW, Barrett L, Healy S, Zuk M (eds.)). *Advances in the Study of Behavior* 48:117–159.

Uetz GW, Papke R, Kilinc B. 2002. Influence of feeding regime on male secondary sexual characters in *Schizocosa ocreata* (Hentz) wolf spiders (Araneae: Lycosidae): evidence for condition-dependence in a visual signaling trait. *Journal of Arachnology* 30:461–469.

Zurek DB, Nelson XJ. 2012. Hyperacute motion detection by the lateral eyes of jumping spider. *Vision Research* 66:26–30. doi:10.1016/j.visres.2012.06.011

Zurek DB, Cronin TW, Taylor LA, Byrne K, Sullivan ML, Morehouse NI. 2015. Spectral filtering enables trichromatic vision in colorful jumping spiders. *Current Biology* 25:R403–404.

Zurek DB, Taylor AJ, Evans CS, Nelson XJ. 2010. The role of the anterior lateral eyes in the vision-based behaviour of jumping spiders. *Journal of Experimental Biology* 213:2372–2378.

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