

# Dim-light vision in jumping spiders (Araneae, Salticidae): identification of prey and rivals

Ana M. Cerveira<sup>a</sup>; Robert R. Jackson<sup>a,b</sup>; Ximena J. Nelson<sup>a\*</sup>

<sup>a</sup>School of Biological Sciences,  
University of Canterbury,  
Private Bag 4800, Christchurch 8140, New Zealand

<sup>b</sup>International Centre of Insect Physiology and Ecology (ICIPE)  
Thomas Odhiambo Campus  
P.O. Box 30, Mbita Point, Kenya

AM Cerveira present address  
CESAM - Centre for Environmental and Marine Studies,  
Faculdade de Ciências, Universidade de Lisboa,  
1749-016 Lisboa, Portugal

\*corresponding author

**KEY WORDS:** Camera eyes, Spatial acuity, Visual sensitivity, Intraspecific-display behavior, Prey-choice behavior, Spartaeinae

## SUMMARY STATEMENT

Investigating the capacity for vision under low ambient light levels by two salticid spider species, we found low-light vision comparable to nocturnal spiders.

## ABSTRACT

Jumping spiders (family Salticidae) are known for their intricate vision-based behavior during encounters with prey and conspecific individuals. This is achieved using eyes specialized for discerning fine detail, but there has been minimal research on the capacities that salticids might have for visual performance under low ambient light levels. Here we investigate the capacity of two salticid species, *Cyrbia algerina* from Portugal and *C. ocellata* from Kenya, to perform two specific visual tasks under low ambient light levels. We used lures made from spiders and midges in prey-identification experiments and mirror images (virtual conspecifics) in rival-identification experiments. These experiments were implemented under a range of ambient light levels (234 cd m<sup>-2</sup>, 1.35 cd m<sup>-2</sup>, 0.54 cd m<sup>-2</sup>, 0.24 cd m<sup>-2</sup>). In most instances, *C. algerina* and *C. ocellata* were proficient at performing both of these visual tasks when ambient light was 234 cd m<sup>-2</sup> and 1.35 cd m<sup>-2</sup>, and a minority performed these tasks at 0.54 cd m<sup>-2</sup>, but none succeeded when light level was 0.24 cd m<sup>-2</sup>. *C. algerina* and *C. ocellata* made vision-based discriminations under low ambient light levels previously associated with nocturnal species.

## INTRODUCTION

Most animals have eyes, but there is considerable variation in these eyes' structure, functioning and size, and these variations may reflect the different functions required of them (Land & Nilsson 2012). To see well under low-light levels, an animal needs especially good sensitivity; yet to be proficient at discerning visual detail of objects, an animal needs especially good spatial acuity. However, a well-known trade-off that applies to eyes in general is that features that increase sensitivity tend to reduce spatial acuity and vice versa (Land & Nilsson 2012). Spatial acuity depends on the quality of the image delivered to the retina and on how fine-grained the retinal mosaic is, but sensitivity depends on the reliability with which photoreceptors can capture photons. The trade-off comes about because, for a given light intensity, the number of photons that arrive during the photoreceptor's integration time is a stochastic phenomenon and, as light gets dimmer, the level of uncertainty in photon capture inevitably increases (Barlow, 1956; Warrant, 1999). Sensitivity can be improved by putting larger photoreceptors into the retina; however, owing to the retinal mosaic becoming coarser, this improvement in sensitivity is achieved at a cost to spatial acuity.

The sensitivity-acuity trade-off becomes especially serious for animals that rely on seeing considerable spatial detail under dim light, and this can be especially problematic for small animals. When an eye is small, having large photoreceptors, which can improve sensitivity through increased area for photon capture (spatial summation), may work against achieving a fine-grain retinal mosaic that would improve spatial acuity; yet some of the best examples of high-performance spatial vision under low-light come from insects and spiders (e.g., Kelber et al., 2006; Fenk and Schmid, 2010; Warrant and Dacke, 2011; Honkanen et al., 2014). Instead of camera-type eyes like those of spiders, most arthropods rely on compound eyes made up of multiple smaller light-gathering lenses (facets). Although details vary considerably, structural adaptations by which animals compensate for the trade-off between sensitivity and spatial acuity include preserving a fine-grain retinal mosaic through having long rhabdoms that are proficient at capturing photons while also being narrow. Other compensation mechanisms are neural, such as spatial and temporal summation, which sum photons in space and time, respectively (Warrant, 1999; Warrant et al., 2004; Frederiksen et al., 2008).

When discussing visual systems, jumping spiders (Salticidae) are of particular interest because it is among salticids that we find both some of the most intricate vision-based predatory strategies (Nelson and Jackson, 2011) and intraspecific display behavior (Crane, 1949; Jackson and Pollard, 1997; Girard and Endler, 2014). Salticids have a visual system consisting of a pair of large camera-type forward-facing (antero-medial) eyes, called the 'principal eyes', and three

pairs of smaller camera-type eyes, collectively called the ‘secondary eyes’, positioned to the side or behind the principal eyes (Homann, 1928). Although the secondary eyes have multiple functions (Land 1972, Zurek et al., 2010; Harland et al., 2012; Zurek and Nelson, 2012; Jakob et al., 2018), they are best known for their role in motion-detection proficiency (i.e., tasks requiring good temporal acuity) and for mediating the orientation behavior by which the salticid brings the corneal lenses of the principal-eye into alignment with salient objects in the environment (Land, 1971, 1972; Jakob et al., 2018). Based on observations of hunting behavior (e.g., Forster, 1982), and eye structure (Land, 1969), it is widely-assumed that salticids are diurnal (e.g., Foelix, 2011). Commensurate with a diurnal lifestyle by which they are often characterized, salticids rely primarily on the exceptional spatial acuity of their principal eyes to discern fine detail (Land, 1969; Blest et al., 1990). In fact, the best spatial acuity known for a salticid’s principal eyes surpasses that known for any other animals of comparable body size (Williams and McIntyre, 1980; Land and Nilsson, 2012; Harland et al., 2012). However, the assumption that salticids are diurnal has largely deflected interest in the investigation of dim light vision in this family. This is despite electrophysiological evidence that sensitivity in the photoreceptors of the secondary eyes is remarkably high for the size of the corneal lens - attributed to having a camera-type eye capable of collecting photons more efficiently than the individual facets of compound eyes (Hardie and Duelli, 1978). Additionally, many salticids frequent the leaf-litter zone in the understory of dense-forest habitats and, for some of these species, morphological evidence suggests a sensitivity-acuity trade-off, whereby the principal eyes have improved sensitivity at a cost to spatial acuity (Blest, 1983, 1985).

Most of our knowledge about low-light vision in spiders comes from research on nocturnal species from other families (e.g., Laughlin et al., 1980; Nørgaard et al., 2007, 2008; Pirhofer-Walzl et al., 2007; Fenk et al., 2010a,b; Fenk and Schmid, 2011, Campione and Schmid, 2014). To begin to redress this gap in our understanding, we tested capacity to implement dim-light vision-based behavior in two salticid species. We chose species from the salticid genus *Cyrba* (*C. algerina* (Lucas) from Portugal and *C. ocellata* (Kroneberg) from Kenya). Spartaeinae, the salticid subfamily to which these species belong, is known for species with unusual predatory behavior. Although most salticids may prey primarily on insects (Richman & Jackson, 1992), many spartaeines, including species from the genus *Cyrba*, are known from laboratory experiments to express an active preference for spiders as prey (Jackson, 1990, 2000; Jackson and Li, 1998; Guseinov et al., 2004).

While carrying out preliminary experiments it was discovered that, unlike other salticids which readily respond in a well-lit laboratory, *C. algerina* and *C. ocellata* become more responsive to prey and mates at low ambient light levels (Cerveira and Jackson, 2011, 2013) and yet *C. algerina* and *C. ocellata* frequent scrubland and desert (Wanless, 1984; Guseinov et al., 2004), habitats that would normally be characterized as well lit. However, the typical microhabitats of *C. algerina* and *C. ocellata* are the dimly lit spaces on the undersides of stones (Jackson, 1990; Jackson and Li, 1998; Guseinov et al., 2004), where they often capture prey and interact with conspecific individuals (Guseinov et al., 2004).

We used two well-established methods by which salticids have been tested for their capacity to make decisions while restricted to using vision alone: mirror tests to determine responsiveness when seeing a conspecific rival and lure tests to determine responsiveness when seeing prey. Mirror tests rely on the salticid's predisposition to respond to its mirror image by initiating the threat displays normally directed at same-sex conspecific rivals (e.g., Harland et al., 1999; Lim and Li, 2006). Lure tests rely on salticids often adopting distinctive prey-choice behavior during encounters with living prey and expressing comparable prey-choice decisions when tested with lures made from dead prey (e.g., Nelson and Jackson, 2012). As a step towards investigating dim-light visual capacity among the Salticidae, here we used mirror and prey-choice testing in order to evaluate the capacity of *C. algerina* and *C. ocellata* for visual identification of prey and rivals under progressively lower light levels.

## **MATERIALS AND METHODS**

### **Maintenance and general testing methods**

All spiders were 2<sup>nd</sup> and 3<sup>rd</sup> generation unmated adults (body length 6.0-7.0 mm) from laboratory cultures, *C. algerina* originating from Tavira in Portugal and *C. ocellata* originating from Mbita Point in Kenya. For details concerning the field sites, laboratory maintenance methods, rearing-cage design, terminology and basic experimental methods, see Cerveira and Jackson (2011). Each test spider matured 2-3 weeks before being used in an experiment and, to standardize hunger level, spiders were kept on a 5-day pre-trial fast. Body lengths of all test spiders and lures were accurate to the nearest 0.5 mm. No individual spider and no individual lure were used more than once. International, national, and institutional guidelines for the care and use of animals were followed.

Experiments were conducted in a lightproof room illuminated by a 20 W halogen lamp (Mickson-Model MF6356, AppN19584, 230V, 50Hz). To reduce the light level, we fastened neutral density filters (Marumi ND4 and ND8) directly below the lamp, leaving no spaces for light to enter the room except through the filters. We used specific combinations of filters (e.g., ND20 = 1 ND4 + 2 ND8) to achieve four light levels: 1) 233.89 cd m<sup>-2</sup> (no filters); 2) 1.35 cd m<sup>-2</sup> (ND20); 3) 0.54 cd m<sup>-2</sup> (ND24); 4) 0.24 cd m<sup>-2</sup> (ND28). Using an International Light radiometer (1L1400) in integrated mode (wavelength range between 450-700 nm), we determined light levels by recording reflected light (calibrated on Spectralon reference standard) over an extended time period to average out noise that would interfere with obtaining accurate short-term recordings under low ambient light. For a perspective on natural lighting conditions corresponding to the light levels used in our experiments, note that dim daylight corresponds to about 100 cd m<sup>-2</sup> (Balkenius et al., 2006), mid to late dusk is normally about 0.01-1 cd m<sup>-2</sup>, while full moon is about 0.1 cd m<sup>-2</sup> and starlight is about 0.001 cd m<sup>-2</sup> (Warrant, 2004).

Before each trial, the spider was placed inside a glass tube (length 20 mm, diameter 8 mm, rubber stopper in each end) and kept for a 60-min acclimation period at the light level under which it would be tested. After acclimation, we transferred the spider to a testing arena (see details below). We achieved this by opening one end of the tube and then positioning the open end flush with an introduction hole in the arena. In most instances, the spider promptly walked out of the tube and into the arena; in the rare instances of a spider failing to walk out within 10 min, we opened the other end of the tube and gently prodded the spider with a paintbrush, after which it always walked into the arena. Each trial began when the spider entered the arena.

We recorded behavior using an infrared-sensitive video camera (Sony DCR-TRV18E). With our goal being to investigate the spider's performance when presented with tasks requiring good spatial acuity, we designed the apparatus so that the distance between the introduction hole and the mirror in mirror-response tests, or the lures in prey-choice tests, was considerably farther than the maximum display distances previously determined (Harland et al., 1999) for *C. algerina* (120 mm) and *C. ocellata* (90 mm) and also farther than casual observations suggest is normal when *C. algerina* and *C. ocellata* begin stalking prey. On this basis, we considered it unlikely that test spiders identified the visual stimuli before moving fully into the testing arena. Once in the arena, the spider always walked about, with frequent pauses and changes in orientation. However, before choosing a lure or displaying at the mirror, spiders always first fixated their gaze on lures or on the mirror (definition of 'fixate': remained stationary for a minimum of 5 s with the corneal lenses of the principal eyes aligned with the mirror or a lure). In successful

mirror tests, the spider fixated on the mirror and either displayed from the same location, or approached the mirror without turning away and then displayed when closer. In successful prey-choice tests, the spider fixated at least once on each lure and then, having made a choice, moved directly toward that lure and into the choice area (defined below) of the arena without turning away before doing so.

We recorded gaze fixation distance and duration, both of which refer to the instance of fixation that immediately preceded displaying at the mirror or choosing a lure. For mirror tests, we also recorded display distance, which could differ from the fixation distance. Fixation distance in prey-choice tests was the distance between the test spider and the lure it chose. In mirror tests, display and fixation distances were defined as twice the distance between the spider and the mirror (i.e., it was the virtual distance between the test spider and its image in the mirror). We measured all distances to the nearest 5 mm from the anterior margin of the spider's carapace, achieving this by placing a sheet of paper with a 5 mm grid under the respective transparent glass or plastic testing arenas.

A trial ended when the spider chose one of the lures or began displaying at the mirror, or when 15 min elapsed without the spider displaying or choosing a lure. Using the software package Prism, distance and duration data were analyzed using one-way analyses of variance (see SI for ANOVA tables) or, if data were not normal, Kruskal-Wallis tests. Prey-choice data were analyzed using binomial tests (null hypothesis, spider equally likely to choose either prey), with Chi-square tests of goodness of fit or, when sample sizes were too low, Fisher exact tests to compare between species.

After each trial, the testing arena was wiped with 80% ethanol followed by distilled water. Knowing that in many salticid species (Jackson and Pollard, 1997), including *C. algerina* and *C. ocellata* (Jackson, 1990), females are more responsive to prey and males are more aggressive in intraspecific same-sex encounters, we used females in prey-choice testing and males in mirror-response testing.

The mirror-test arena was a transparent plastic Petri dish (diameter 140 mm, height 15 mm), inside which a mirror (length 85 mm, height 15 mm) was positioned upright and facing into the wider space within the dish (center of mirror 8 mm from nearest rim of dish). As the height of the mirror was equal to inside height of dish (15 mm), spiders could not move around, over or under the mirror. The introduction hole (diameter 8 mm) was situated in the rim of the dish on the side of the arena opposite to the mirror (i.e., it was 132 mm from the mirror).

## Prey-choice tests

For prey-choice testing, we presented spiders with two lures. The prey used for making the lures were collected from the field (Mbita Point, Kenya) as needed. In each trial, one lure was made from a lycosid spider (*Pardosa messingerae*) and the other was made from a chironomid midge (*Nilodorum brevibuca*). For each trial, lure body lengths matched test-spider body lengths.

To make a lure, we first used carbon dioxide to immobilize the prey individual and then immersed it in 80% ethanol for 60 min, after which we mounted the prey individual in a lifelike posture on the center of a cork disc (diameter 15 mm). An aerosol plastic-adhesive spray was used to secure the prey to the disc and for preservation (for details pertaining to making lures, see Jackson et al., 2005).

The prey-choice testing arena was a rectangular glass box (depth 20 mm, inner dimensions 140 mm long x 115 mm wide; other dimensions in Fig. S1) as used in previous work (Nelson & Jackson, 2012). Equidistant from the two longer sides of the arena, there was an introduction hole (center of hole 14 mm from the nearest side of the arena). In the base of the arena at the opposite end, there was a 'left lure hole' and a 'right lure hole' (each 8 mm in diameter), the center of each being 14 mm from the nearest sides of the arena, opposite the introduction hole. Lures placed on top of the lure holes faced each other, with left versus right positioning of the insect and spider lures being determined at random for each trial.

The arena sat on a 150 mm high Plexiglas™ plastic stand, with the free end of a camera cable-release cord being accessible from beneath the stand. By pressing on the cable release, the two prongs on a metal fork, which was attached to the cork disc bases of the lures, lifted the two lures in unison 5 mm above the floor of the arena. From the time at which the spider entered the arena until tests ended, the cable release was pressed every 30 s, causing the lures to move simultaneously up and then down one time.

Two circles made from thin copper wire were placed under the arena, but on top of the paper grid. One lure hole was at the centre of one circle and the other lure hole was at the centre of the other circle. Part of each copper circle went under the testing area, thereby demarcating a choice area corresponding to that particular lure (Fig. S1). Seeing a spider fixate its gaze on a lure and then enter the choice area corresponding to that lure was our criterion for recording its choice of prey. On the rare occasions when the 15-min test period elapsed with the spider's gaze still fixated on a lure, but with the spider still outside the choice area, we extended the test period until it either made its choice or else turned away (i.e., broke off fixation of gaze on the lure).



## RESULTS

No spiders made prey choices or displayed at the mirror when the light level was  $0.24 \text{ cd m}^{-2}$ . Above this, for each light level, there were no significant differences between *C. algerina* and *C. ocellata* with respect to how many spiders responded by choosing prey versus not making a choice ( $234 \text{ cd m}^{-2}$ ,  $X^2_1 = 0.370$ ,  $p = 0.542$ ;  $1.35 \text{ cd m}^{-2}$ ,  $X^2_1 = 1.154$ ,  $p = 0.283$ ;  $0.54 \text{ cd m}^{-2}$ ,  $X^2_1 = 0.033$ ,  $p = 0.855$ ; Fig. 1a) or between how many chose one lure instead of the other ( $234 \text{ cd m}^{-2}$ ,  $X^2_1 = 1.008$ ,  $p = 0.15$ ;  $1.35 \text{ cd m}^{-2}$ ,  $X^2_1 = 0.574$ ,  $p = 0.449$ ;  $0.54 \text{ cd m}^{-2}$ ,  $p = 1.00$ , Fisher exact test; Fig. 1b). There were also no differences between species with respect to how many spiders displayed at the image in the mirror ( $234 \text{ cd m}^{-2}$ ,  $X^2_1 = 0.7$ ,  $p = 0.403$ ;  $1.35 \text{ cd m}^{-2}$ ,  $X^2_1 = 1.020$ ,  $p = 0.313$ ;  $0.54 \text{ cd m}^{-2}$ ,  $X^2_2 = 0.019$ ,  $p = 0.889$ ; Fig. 2). However, the number of spiders that responded by making a choice of prey (*C. algerina*,  $X^2_2 = 24.2$ ,  $p < 0.001$ ; *C. ocellata*  $X^2_2 = 41.4$ ,  $p < 0.001$ ) or displaying toward their reflection (*C. algerina*,  $X^2_2 = 13.5$ ,  $p = 0.001$ ; *C. ocellata*  $X^2_2 = 24.8$ ,  $p < 0.001$ ) declined sharply at  $0.54 \text{ cd m}^{-2}$  relative to the two higher light levels (Figs. 1a, 2).

In prey identification tests, more spiders of both species chose the spider instead of the midge (Fig. 1b) when the light level was  $234 \text{ cd m}^{-2}$  (Binomial test,  $p = 0.003$  for *C. algerina*,  $p = 0.057$  for *C. ocellata*) and when it was  $1.35 \text{ cd m}^{-2}$  ( $p = 0.039$  for *C. algerina*,  $p = 0.018$  for *C. ocellata*), but not when the light level was  $0.54 \text{ cd m}^{-2}$  ( $p = 1.000$  for *C. algerina* and *C. ocellata*). For those spiders that made a prey choice, we looked for effects of light level on distance from the prey when the spider first fixated its gaze on the prey (Fig. 3a) and on the duration of pre-choosing gazing (i.e., how long spiders kept their gaze fixated on the prey before making a choice) (Fig. 3b). For both species, there were significant treatment effects for distance, with fixation distances being lower at the lowest light level (*C. algerina*  $H_2 = 8.173$ ,  $p = 0.017$ ; *C. ocellata*,  $H_2 = 13.64$ ,  $p = 0.001$ ), and also the duration of gaze fixation, with shorter gaze times at the highest light level (*C. algerina*,  $U_2 = 11.10$ ,  $p = 0.004$ ; *C. ocellata*,  $U_2 = 14.92$ ,  $p < 0.001$ ).

In rival identification tests, the distances from which spiders first displayed at their mirror images (see description below) under the three higher light levels (Fig. 4a) did not differ significantly for either species (*C. algerina*,  $F_{2,19} = 1.236$ ;  $p = 0.313$ ; *C. ocellata*,  $F_{2,32} = 2.568$ ;  $p = 0.092$ ). For those spiders that displayed during mirror tests, we looked for effects of light level on the how long the spider kept its gaze fixated on the mirror continuously before displaying (Fig. 4b), and at what distance gaze fixation on the mirror occurred (Fig. 4c). Generally speaking, spiders first fixated on their reflection from closer to the mirror under the lowest light

condition (Fig. 4c). However, while the distances at which spiders first fixated their gaze at their mirror images under the three light levels differed significantly in *C. ocellata* ( $F_{2,32} = 4.427$ ;  $p = 0.020$ ), revealed in post-hoc tests to be driven primarily between the highest and lowest light levels, this was not the case for *C. algerina* ( $F_{2,19} = 2.560$ ;  $p = 0.104$ ). Nevertheless, light had a significant effect on pre-display fixation duration for both species, with this being markedly lower at the highest light level (*C. algerina*,  $F_{2,19} = 6.153$ ;  $p = 0.009$ ; *C. ocellata*,  $F_{2,32} = 6.660$ ;  $p = 0.004$ ; Fig. 4b), suggesting a quick transition between perception of the viewed image and recognition or identification of a rival.

When spiders displayed in mirror tests, they typically moved towards the mirror, eventually stopping, with their gaze fixated on the image in the mirror. Then, while facing the mirror, spiders adopted distinctive displays in which they kept their legs on the substrate while swaying their bodies from side to side, sometimes punctuated by making truncated leaps (i.e., covering only 1-2 mm) toward the mirror. Swaying and truncated leaping are typical male-male displays adopted in these species, and not the displays they use when interacting with conspecific females. Spiders never adopted quivering waving of forelegs, dancing or stepping from side to side, these being types of display behavior males typically perform only when interacting with conspecific females (for details, see Jackson, 1990).

## DISCUSSION

We presented the salticid spiders *Cyrba algerina* and *C. ocellata* with tasks requiring visual attention to fine detail in two contexts: predation (i.e., detecting and identifying preferred prey) and social encounters (i.e., detecting and identifying conspecific rivals). Most spiders were successful in the performance of both tasks even when the light level was as low as  $1.35 \text{ cd m}^{-2}$ , which is well below the light level typical of dawn and early dusk (c.  $10 \text{ cd m}^{-2}$ ). In contrast to *Cyrba*'s good performance at light levels of  $1.35 \text{ cd m}^{-2}$ , only a few spiders met our criteria for successful performance at  $0.54 \text{ cd m}^{-2}$  (i.e., mid to late dusk,  $1 \text{ cd m}^{-2}$ ) and none succeeded at  $0.24 \text{ cd m}^{-2}$ , the light levels typical of twilight ( $0.3 \text{ cd m}^{-2}$ ).

That cues in any modality other than vision might account for *Cyrba*'s responses in our experiments seems unlikely. In mirror tests, spiders responded by performing the display behavior that is specific to conspecific males interacting with each other (Jackson, 1990); yet here, the rival was the spider's odorless, silent, mirror image. Lures instead of living prey were used the prey-choice experiment, and these lures were outside the arena occupied by the test spider. There were two kinds of lures, one being made from a spider (i.e., more preferred prey) and the other from a midge (i.e., less preferred prey). Significantly more test spiders chose the

lure made from a spider instead of that from a midge when the light level was  $1.35 \text{ cd m}^{-2}$  or brighter, but most spiders failed to approach either lure when the light level was  $0.54 \text{ cd m}^{-2}$  and there was no trend toward choosing the spider lure under this low light level. Moreover, the findings for shorter fixation distance and longer gaze duration among salticids that chose a lure in dim light suggest that identifying prey and rivals was more difficult, or provided more uncertainty, when the ambient light level was lower.

As no test spiders responded when the light level was  $0.24 \text{ cd m}^{-2}$ , twilight ( $0.3 \text{ cd m}^{-2}$ ) and full moon (c.  $0.1 \text{ cd m}^{-2}$ ) seem not to be bright enough for *Cyrrba* to undertake vision-based identification of specific prey and rivals. However, good performance under a light level of  $1.35 \text{ cd m}^{-2}$  and marginal performance at  $0.54 \text{ cd m}^{-2}$  is remarkable considering that salticids are usually considered to be predators that rely on exceptional vision in well-lit habitats (Foelix 2011). This is comparable, or better, than findings on the nocturnal spider *Cupiennius salei*, which has the ability to perceive contrast minimal differences when background luminance is above  $16 \text{ cd m}^{-2}$  (Campione and Schmid, 2014). These results therefore suggest that *C. algerina* and *C. ocellata* may have adaptations that enable them to see well under dim light.

Adaptations that compensate for size constraints have been extensively investigated in insects that specialize at seeing under dim light. For example, Kelber et al., (2006) showed that, compared with diurnal and even crepuscular bees, nocturnal bees have larger ocelli, and larger compound eyes comprised of larger ommatidia; all of these characteristics probably function to improve sensitivity by enhancing the photon-capture capacity of the nocturnal bee's eyes. However, when discussing how salticids might improve photon-capture reliability, there are some unusual characteristics of their boomerang-shaped principal-eye retinæ to consider.

Instead of lying in a single plane, photoreceptors in a salticid's principal-eye retinæ, which lie at the end of long, slender eye tubes (Land, 1969; Williams and McIntyre, 1980), are stacked in four distinct tiers (Land, 1969) and, within the tier most distal to the cornea (layer 1), there is a staircase arrangement of receptor tips (Blest et al., 1990). Consistent with this being the layer with the best capacity for high spatial-acuity vision, layer 1 is also where photoreceptors are most densely packed and have the smallest diameters (Williams and McIntyre, 1980).

A variety of functions have been suggested for the four-tier arrangement of the salticid retina (Blest et al., 1981; Nagata et al., 2012; Zurek et al., 2015), including a role in increasing sensitivity by allowing for more opportunities for photon capture as light passes through the successive layers (Land 1969). This mechanism would be akin to how summation is achieved by insects (Warrant, 1999; Frederiksen et al., 2008). How other salticid species perform under dim light is currently unknown, but previous work on *Trite planiceps*, a species which we might

consider to be representative of more conventional salticids, being diurnal and a member of the subfamily Salticoida, to which the majority of salticids (but not spartaeines like *Cyrba*) belong, suggests that *Cyrba* really is better at seeing detail under dim light. In the laboratory, *T. planiceps* required light intensities of at least 50 lux (similar to ND16) for normal visual hunting behaviour to occur (Forster 1982). We currently cannot disambiguate whether dim light vision is a specialized characteristic of *Cyrba* which is enabled by specialized retinal ultrastructure (Blest et al., 1990), or whether dim light vision is widely expressed among salticids due to their four-tier retinal arrangement. Research in this area is needed and, given the variety of visual adaptations adopted by salticids, is likely to yield interesting results.

Spartaeinae, the subfamily to which *Cyrba* belongs, has the widest range in retinal ultrastructure known for salticids (Su et al., 2007). At one extreme, the spartaeine *Portia fimbriata* has rhabdoms in layer 1 packed in a hexagonal retinal mosaic, with the separation of individual rhabdoms minimizing the potential for crosstalk between neighboring rhabdoms and with inter-receptor spacing (1.4  $\mu\text{m}$ ) approaching the limit set by the wavelength of visible light. Owing to these characteristics, *P. fimbriata*'s principal eyes support extremely good spatial acuity (Williams and McIntyre, 1980; Blest and Price, 1984). *Yaginumanis sexdentatus* is at the other extreme, as this species' layer 1 receptive segments consist of essentially two contiguous rhabdomeres, each from a rhabdom in a different cell (Blest and Sigmund, 1984). As a consequence of this arrangement, there is optical crosstalk between neighbouring receptors which increases photon-capture proficiency in this species' eyes, at the expense of spatial acuity.

*Cyrba*'s retinal arrangement, seemingly intermediate between *Yaginumanis* and *Portia*, may allow for sensitivity superior to *Portia*, but inferior to *Yaginumanis*, and spatial acuity superior to *Yaginumanis* but inferior to *Portia*. Each receptive segment in the central region of *C. algerina*'s principal eye layer 1 is wider than those of *Portia* and bears two rhabdomeres (in contrast to *Portia*'s single rhabdomere); however, when progressing toward the lateral outer edge of the retina, one rhabdomere in each pair becomes gradually shorter, meaning that distally, at the end closest to the corneal lens, receptors 1-3 (out of 13, along a horizontal line) consist of two rhabdomeres, but proximally, deeper into the cephalothorax, only a single rhabdomere exists (schematic in Blest et al., 1990; see also Blest and Price, 1984; Blest and Sigmund, 1985). In practice, this means that only the long rhabdomeres will absorb light in the foveal region (i.e., no optical pooling occurs), supporting better spatial acuity, while the inner side of the retina, bearing two rhabdomeres per receptor, contributes more strongly to sensitivity through optical pooling.

Our results are a step toward linking *Cyrrba*'s retinal ultrastructure to its capacity for making specific vision-based discriminations under low ambient light levels. While it is convenient to characterize salticids in general as predators that rely on eyes designed for good spatial acuity in brightly lit environments, saying this suggests that we know more than is the case. There are more than 5,800 described species in the family Salticidae (Maddison, 2015) and it is for only a tiny fraction of these species that we have an understanding inclusive of natural history, behavior and the functioning of eyes. Instead of making sweeping generalizations, we should be encouraging research aimed at gaining a fuller understanding of salticid diversity. Recent work showing previously unknown mechanisms of salticid vision, such as red-light vision enabled through the use of retinal filters in some species (Zurek et. al., 2015), and the role of the secondary eyes in guiding the movement of the retinae of the principal eyes (Jakob et al., 2018), reminds us of how far we are from a full understanding of the salticid's visual system in its various forms.

## **Acknowledgments**

We thank Duane Harland for help with spectrometry and Aynsley McNab for spider maintenance. We gratefully acknowledge the conscientious assistance of Stephen Abok Aluouch in Kenya.

## **Competing interests**

The authors declare no competing financial interests.

## **Author contributions**

RRJ and AMC conceptualized the study and carried out experiments. XJN analyzed and interpreted the data. All authors actively contributed to the writing of the paper.

## **Funding**

Our research was supported by grants from the Royal Society of New Zealand Marsden Fund (XJN: M1149; RRJ: M1096, M1079), the National Geographic Society (RRJ: 8676-09, WW-146R-17) and the US National Institutes of Health (RRJ: R01-AI077722). We also had financial support from CESAM (UID/AMB/50017 - POCI-01-0145-FEDER-007638), to FCT/MEC through national funds, and co-funding by the FEDER, within the PT2020 Partnership Agreement and Compete 2020. AMC was supported by a Doctoral Scholarship (SFRH/BD/8311/2002) from the Fundação para a Ciência e a Tecnologia through the European Regional Development Fund.

## References

- Balkenius, A., Rosén, W. and Kelber, A.** (2006). The relative importance of olfaction and vision in a diurnal and a nocturnal hawkmoth. *J. Comp. Physiol. A* **192**, 431-437.
- Barlow, H. B.** (1956). Retinal noise and absolute threshold. *J. Opt. Soc. Am.* **46**, 634-639.
- Blest, A. D.** (1983). Ultrastructure of secondary retinæ of primitive and advanced jumping spiders (Araneae, Salticidae). *Zoomorphology* **102**, 125-141.
- Blest, A. D.** (1985). The retinal mosaics of the principal eyes of some neotropical jumping spiders: optical trade-offs between sizes and habitat illuminances. *J. Comp. Physiol. A* **157**, 391-404.
- Blest, A. D. and Price, G. D.** (1984). Retinal mosaics of the principal eyes of some jumping spiders (Salticidae: Araneae): adaptations for high visual acuity. *Protoplasma* **120**, 172-184.
- Blest, A. D. and Sigmund, C.** (1984). Retinal mosaics of the principal eyes of two primitive jumping spiders, *Yaginumanis* and *Lyssomanes*: clues to the evolution of salticid vision. *Proc. Roy. Soc. Lond. B.* **221**, 111-125.
- Blest, A. D. and Sigmund, C.** (1985). Retinal mosaics of a primitive jumping spider, *Spartaeus* (Araneae: Salticidae: Spartaeinae): a phylogenetic transition between low and high visual acuities. *Protoplasma* **125**, 129-139.
- Blest, A. D., O'Carroll, D. C. and Carter, M.** (1990). Comparative ultrastructure of Layer I receptor mosaics in principal eyes of jumping spiders: the evolution of regular arrays of light guides. *Cell Tissue Res.* **262**, 445-460.
- Blest, A. D., Hardie, A. C., McIntyre, P. and Williams, D. S.** (1981). The spectral sensitivities of identified receptors and the function of retinal tiering in the principal eyes of jumping spiders. *J. Comp. Physiol.* **145**, 227-239.
- Campione, E. and Schmid, A.** (2014). Brightness discrimination in the day- and night-adapted hunting spider *Cupiennius salei*. *J. Exp. Biol.* **217**, 2704-2709.
- Carreira, A. M. and Jackson, R. R.** (2011). Interpopulation variation in oecobiid-specific prey-capture behaviour and kairomone use by *Cyrba algerina*, an araneophagic jumping spider from Portugal. *J. Ethol.* **29**, 121-129.
- Carreira, A. M. and Jackson, R. R.** (2013). Love is in the air and on the ground: species and sex identification by *Cyrba algerina* and *C. ocellata*, jumping spiders from Portugal and Kenya. *J. Arachnol.* **41**, 374-380.
- Crane, J.** (1949). Comparative biology of salticid spiders at Rancho Grande, Venezuela. Part IV. An analysis of display. *Zoologica* **34**, 159-214.



- Fenk, L. M. and Schmid, A.** (2010). The orientation-dependent visual spatial cut-off frequency in a spider. *J. Exp. Biol.* **213**, 3111-3117.
- Fenk, L. M. and Schmid, A.** (2011). Flicker-induced eye movements and the behavioural temporal cut off frequency in a nocturnal spider. *J. Exp. Biol.* **214**, 3658-3663.
- Fenk, L. M., Hoinkes, T. and Schmid, A.** (2010a). Vision as a third sensory modality to elicit attack behavior in a nocturnal spider. *J. Comp. Physiol. A* **196**, 957-961.
- Fenk, L. M., Heidlmayr, K., Lindner, P. and Schmid, A.** (2010b). Pupil size in spider eyes is linked to post-ecdysal lens growth. *PLoS ONE* **5**, e15838.
- Foelix, R. F.** (2011). *Biology of Spiders*, 3rd ed. New York: Oxford University Press.
- Forster, L. M.** (1982). Vision and prey-catching behavior in jumping spiders. *Am. Sci.* **70**, 165-175.
- Frederiksen, R., Wcislo, W. T. and Warrant, E. J.** (2008). Visual reliability and information rate in the retina of a nocturnal bee. *Curr. Biol.* **18**, 349-353.
- Guseinov, E. F., Cerveira, A. M. and Jackson, R. R.** (2004). The predatory strategy, natural diet, and life cycle of *Cyrba algerina*, an araneophagic jumping spider (Salticidae: Spartaeinae) from Azerbaijan. *N. Z. J. Zool.* **31**, 291-303.
- Girard, M. B. and Endler, J. A.** (2014). Peacock spiders. *Curr. Biol.* **24**, R588-590.
- Hardie, R. C. & Duelli, P.** (1978). Properties of single cells in posterior lateral eyes of jumping spiders. *Z. Naturforsch. C* **33**, 156-158.
- Harland, D. P., Jackson, R. R., and Macnab, A. M.** (1999). Distances at which jumping spiders (Araneae: Salticidae) distinguish between prey and conspecific rivals. *J. Zool.* **247**, 357-364.
- Harland, D. P., Li, D. and Jackson, R. R.** (2012). How jumping spiders see the world. In *How animals see the world: Comparative Behavior, Biology, and Evolution of Vision* (ed. O. Lazareva, T. Shimizu and E. A. Wasserman), pp. 133-164. Oxford: Oxford University Press.
- Homann, H.** (1928). Beiträge zur Physiologie der Spinnenaugen. I. Untersuchungsmethoden, II. Das Sehvermögen der Salticiden. *Z. Vergl. Physiol.* **7**, 201-268.
- Honkanen, A., Takalo, J., Heimonen, K., Vähäsöyrinki, M. and Weckström, M.** (2014). Cockroach optomotor responses below single photon level. *J. Exp. Biol.* **217**, 4262-4268.
- Jackson, R. R.** (1990). Predatory versatility and intraspecific interactions of *Cyrba algerina* and *C. ocellata*, web-invading spartaeine jumping spiders (Araneae, Salticidae). *N. Z. J. Zool.* **17**, 157-168.
- Jackson, R. R.** (2000). Prey preferences and visual discrimination ability of *Brettus*, *Cocalus* and *Cyrba*, araneophagic jumping spiders (Araneae : Salticidae) from Australia, Kenya and Sri Lanka. *N. Z. J. Zool.* **27**, 29-39.



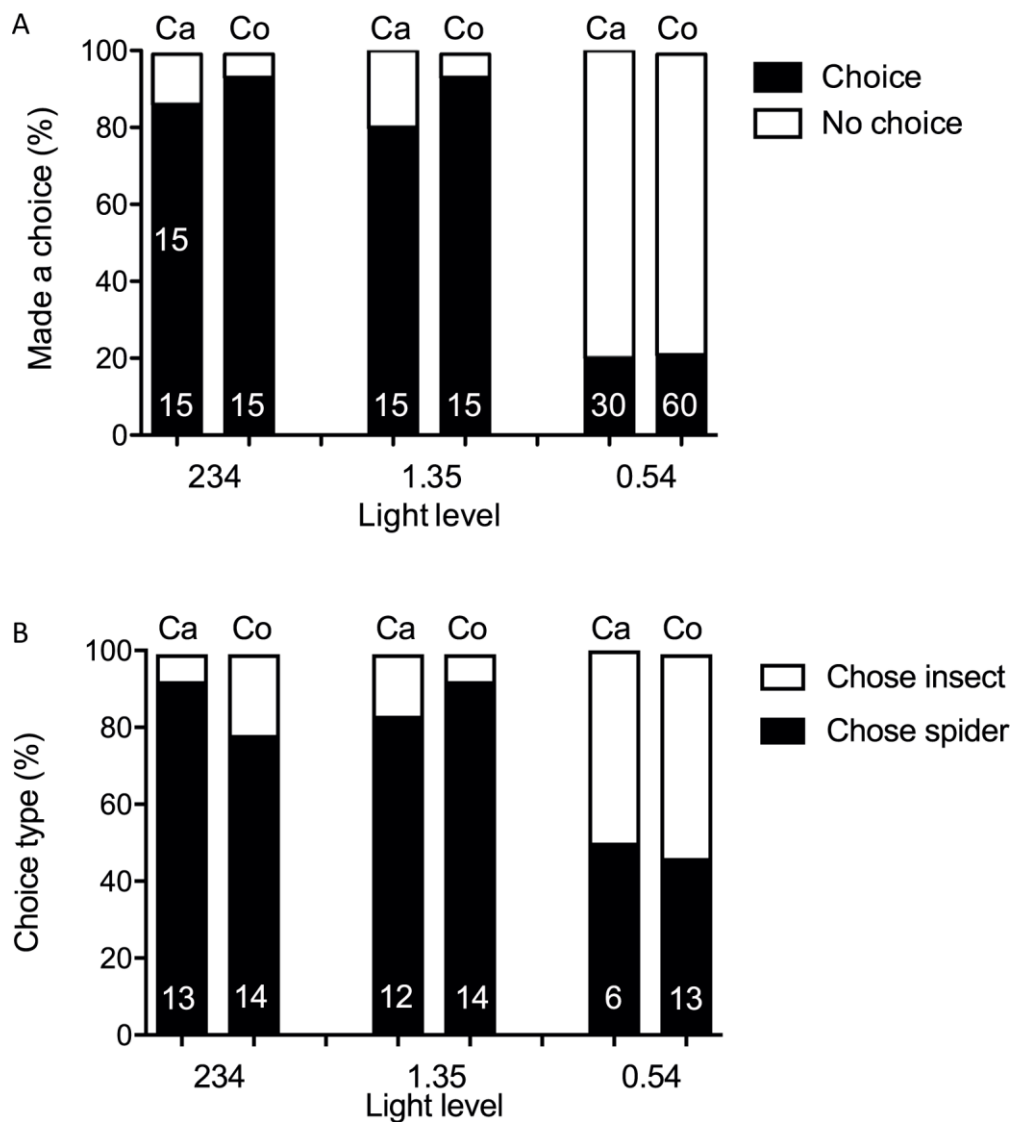
- Jackson, R. R. and Li, D.** (1998). Prey preferences and visual discrimination ability of *Cyrbia algerina*, an araneophagic jumping spider (Araneae: Salticidae) with primitive retinæ. *Israel J. Zool.* **44**, 227-242.
- Jackson, R. R. and Pollard, S. D.** (1997). Jumping spider mating strategies: Sex among cannibals in and out of webs. In *Mating Systems in Insects and Arachnids* (ed. J. Choe and B. Crespi), pp. 340-351. Cambridge: Cambridge University Press.
- Jackson, R. R., Nelson, X. J., and Sune, G. O.** (2005). A spider that feeds indirectly on vertebrate blood by choosing female mosquitoes as prey. *Proc. Natl. Acad. Sci. (USA)* **102**, 15155-15160.
- Jakob, E. M., Long, S. M., Harland, D. P., Jackson, R. R., Carey, A., Searles, M. E., Porter, A. H., Canavesi, C. and Rolland, J. P.** (2018). Lateral eyes direct principal eyes as jumping spiders track objects. *Curr. Biol.* **28**, R1075-1095.
- Kelber, A., Warrant, E. J., Pfaff, M., Wallén, R., Theobald, J. C., Wcislo, W. T. and Raguso, R. R.** (2006). Light intensity limits foraging activity in nocturnal and crepuscular bees. *Behav. Ecol.* **17**, 63-72.
- Land, M. F.** (1969). Structure of retinæ of the principal eyes of jumping spiders (Salticidae: Dendryphantinae) in relation to visual optics. *J. Exp. Biol.* **51**, 443-470.
- Land, M. F.** (1971). Orientation by jumping spiders in the absence of visual feedback. *J. Exp. Biol.* **54**, 119-139.
- Land, M. F.** (1972). Stepping movements made by jumping spiders during turns mediated by the lateral eyes. *J. Exp. Biol.* **57**, 15-40.
- Land, M. F. and Nilsson, D. E.** (2012). *Animal Eyes*. 2<sup>nd</sup> Ed. Oxford: Oxford University Press.
- Laughlin, S., Blest, A. D. and Stowe, S.** (1980). The sensitivity of receptors in the posterior median eye of the nocturnal spider, *Dinopis*. *J. Comp. Physiol.* **141**, 53-65.
- Lim, M. L. M. and Li, D.** (2006). Behavioural evidence of UV sensitivity in jumping spiders (Araneae Salticidae). *J. Comp. Physiol. A* **192**, 871-878.
- Maddison, W. P.** (2015). A phylogenetic classification of jumping spiders (Araneae: Salticidae). *J. Arachnol.* **43**, 231-292.
- Nagata, T., Koyanagi, M., Tsukamoto, H., Saeki, S., Isono, K., Shichida, Y., Tokunaga, F., Kinoshita, M., Arikawa, K. and Terakita, A.** (2012). Depth perception from image defocus in a jumping spider. *Science* **335**, 469-471.
- Nelson, X. J. and Jackson, R. R.** (2011). Flexibility in the foraging strategies of spiders In *Spider behaviour: flexibility and versatility* (ed. M. E. Herberstein), pp. 31-56. Cambridge: Cambridge University Press.

- Nelson, X. J. and Jackson, R. R.** (2012). Fine tuning of vision-based prey-choice decisions by a predator that targets malaria vectors. *J. Arachnol.* **40**, 23-33.
- Nørgaard, T., Henschel, J. R. and Wehner, R.** (2007). Use of local cues in the night-time navigation of the wandering desert spider *Leucorchestris arenicola* (Araneae, Sparassidae). *J. Comp. Physiol. A* **193**, 217-222.
- Nørgaard, T., Nilsson, D. E., Henschel, J. R., Garm, A. and Wehner, R.** (2008). Vision in the nocturnal wandering spider *Leucorchestris arenicola* (Araneae: Sparassidae). *J. Exp. Biol.* **211**, 816-823.
- Pirhofer-Walzl, K., Warrant, E. and Barth, F. G.** (2007). Adaptations for vision in dim light: impulse responses and bumps in nocturnal spider photoreceptor cells (*Cupiennius salei* Keys). *J. Comp. Physiol. A* **193**, 1081-1087.
- Richman, D. B. and Jackson, R. R.** (1992). A review of the ethology of jumping spiders (Araneae, Salticidae). *Bull. Brit. Arach. Soc.* **9**, 33-37.
- Su, K. F. Y., Meier, R., Jackson, R. R., Harland, D. P. and Li, D.** (2007). Convergent evolution of eye ultrastructure and divergent evolution of vision-mediated predatory behaviour in jumping spiders. *J. Evol. Biol.* **20**, 1478-1489.
- Wanless, F. R.** (1984). A review of the spider subfamily Spartaeinae nom.n. (Araneae: Salticidae) with descriptions of six new genera. *Bull. Brit. Mus. Nat. Hist.* **46**, 135-205.
- Warrant, E. J.** (1999). Seeing better at night: life style, eye design and the optimum strategy of spatial and temporal summation. *Vis. Res.* **39**, 1611-1630.
- Warrant, E. J.** (2004). Vision in the dimmest habitats on earth. *J. Comp. Physiol. A* **190**, 765-789.
- Warrant, E. J. and Dacke, M.** (2011). Vision and visual navigation in nocturnal insects. *Annu. Rev. Entomol.* **56**, 239-254.
- Warrant, E. J., Kelber, A., Gislén, A., Greiner, B., Ribi, W. and Wcislo, W. T.** (2004). Nocturnal vision and landmark orientation in a tropical halictid bee. *Curr. Biol.* **14**, 1309-1318.
- Williams, D.S. and McIntyre, P.** (1980). The principal eyes of a jumping spider have a telephoto component. *Nature* **288**, 578-580.
- Zurek, D. B., Taylor, A. J., Evans, C. S. and Nelson, X. J.** (2010). The role of the anterior lateral eyes in the vision-based behaviour of jumping spiders. *J. Exp. Biol.* **213**, 2372-2378.

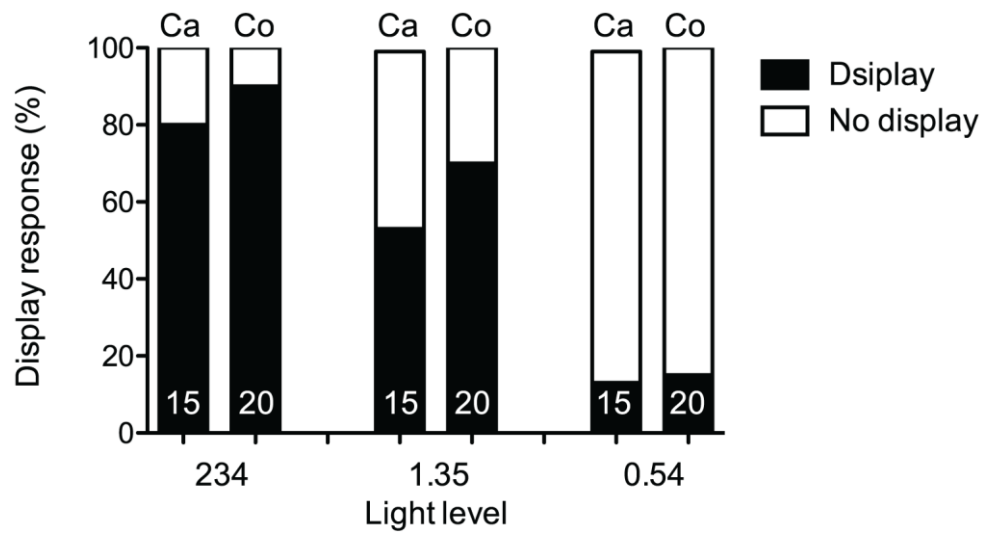
**Zurek, D. B. and Nelson, X. J.** (2012). Saccadic tracking of targets mediated by the anterior-lateral eyes of jumping spiders. *J. Comp. Physiol. A* **198**, 411-417.

**Zurek, D. B., Cronin, T. W., Taylor, L. A., Byrne, K., Sullivan, M. L. G. and Morehouse, N. I.** (2015). Spectral filtering enables trichromatic vision in colourful jumping spiders. *Curr. Biol.* **25**, R403-404.

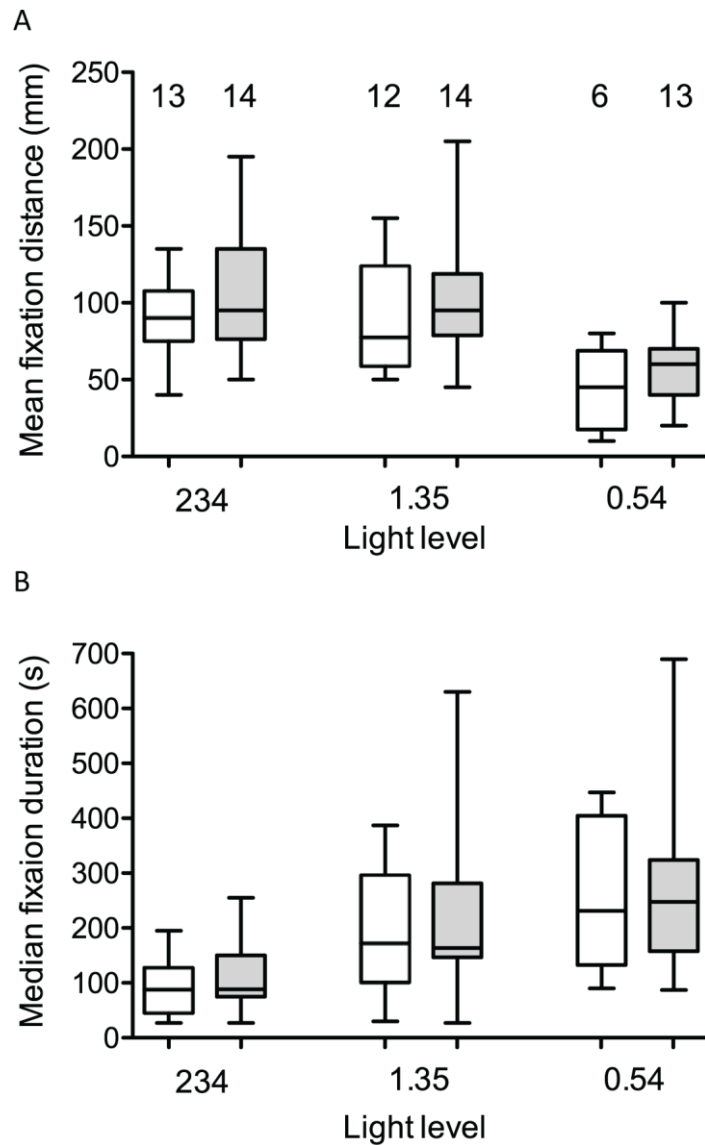
## Figures



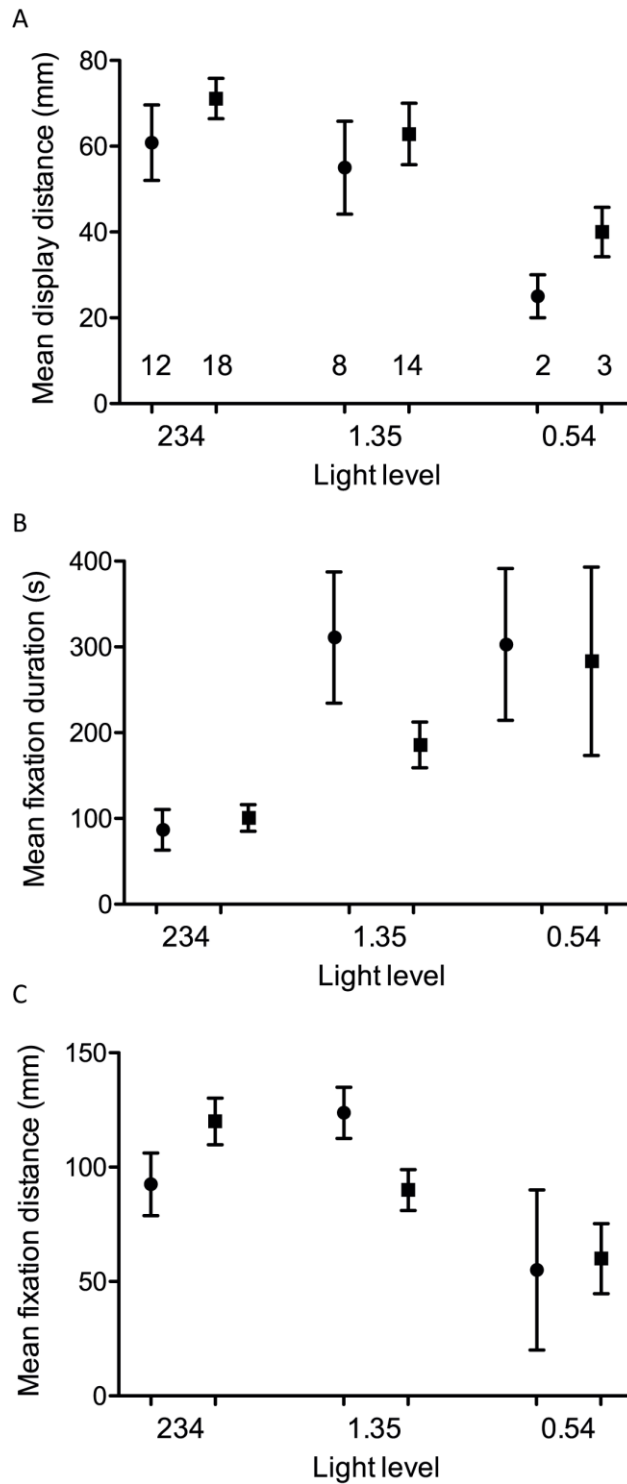
**Fig. 1. Percentages of test spiders that (A) responded during prey-choice tests at each light level (expressed in cd m<sup>-2</sup>) and (B) the prey choices made by test spiders that responded. N shown within each bar in (A); data for (B) are based on black area in (A). N for (B) shown within each bar. Ca: *Cyrrba algerina*. Co: *C. ocellata*.**



**Fig. 2.** Percentages of test spiders that responded (adopted threat display while gaze was fixated on the mirror) during mirror rival-identification tests at each light level (expressed in  $\text{cd m}^{-2}$ ). Ca: *Cyrba algerina*. Co: *C. ocellata*. N shown within each bar.



**Fig. 3. Median (25<sup>th</sup> and 75<sup>th</sup> percentile; whiskers: min to max) pre-prey-choice fixation of gaze on lures by *Cyrrba algerina* (white) and *C. ocellata* (grey) at different light levels (expressed in  $\text{cd m}^{-2}$ ). (A) Distances at which test spiders fixated their gazes on the lures and then, without breaking fixation, chose a lure. (B) Time elapsing between fixating gaze on lures and, without breaking fixation, chose a lure. N shown within panel (A).**



**Fig. 4. Mean ( $\pm$ SEM) responses to mirror image for *Cyrba algerina* (circles) and *C. ocellata* (squares) at different light levels (expressed in  $\text{cd m}^{-2}$ ). (A) Virtual distance from mirror image at which spiders first displayed. (B) Time elapsing between test spider fixating its gaze on its mirror image and then, without breaking fixation, initiating display. (C) Virtual distance (2 x distance) at which test spider first fixated its gaze on its mirror image and then, without breaking fixation, performed threat display. N shown above x-axis within panel (A).**

## Supplementary Material

### ANOVA tables for mirror tests

#### 1. Gaze fixation distance

##### *C. algerina*

	SS	df	MS
Treatment (between columns)	9233	2	4616
Residual (within columns)	34263	19	1803
Total	43495	21	

$F = 2.560$ ;  $p = 0.104$

##### *C. ocellata*

	SS	df	MS
Treatment (between columns)	13114	2	6557
Residual (within columns)	47400	32	1481
Total	60514	34	

$F = 4.427$ ;  $p = 0.020$

#### 2. Gaze fixation duration

##### *C. algerina*

	SS	df	MS
Treatment (between columns)	270386	2	135193
Residual (within columns)	417483	19	21973
Total	687869	21	

$F = 6.153$ ;  $p = 0.009$

##### *C. ocellata*

	SS	df	MS
Treatment (between columns)	115112	2	57556
Residual (within columns)	276528	32	8641
Total	391640	34	

$F = 6.660$ ;  $p = 0.004$

#### 3. Display distance

##### *C. algerina*

	SS	df	MS
Treatment (between columns)	2204	2	1102
Residual (within columns)	16942	19	891.7
Total	19145	21	

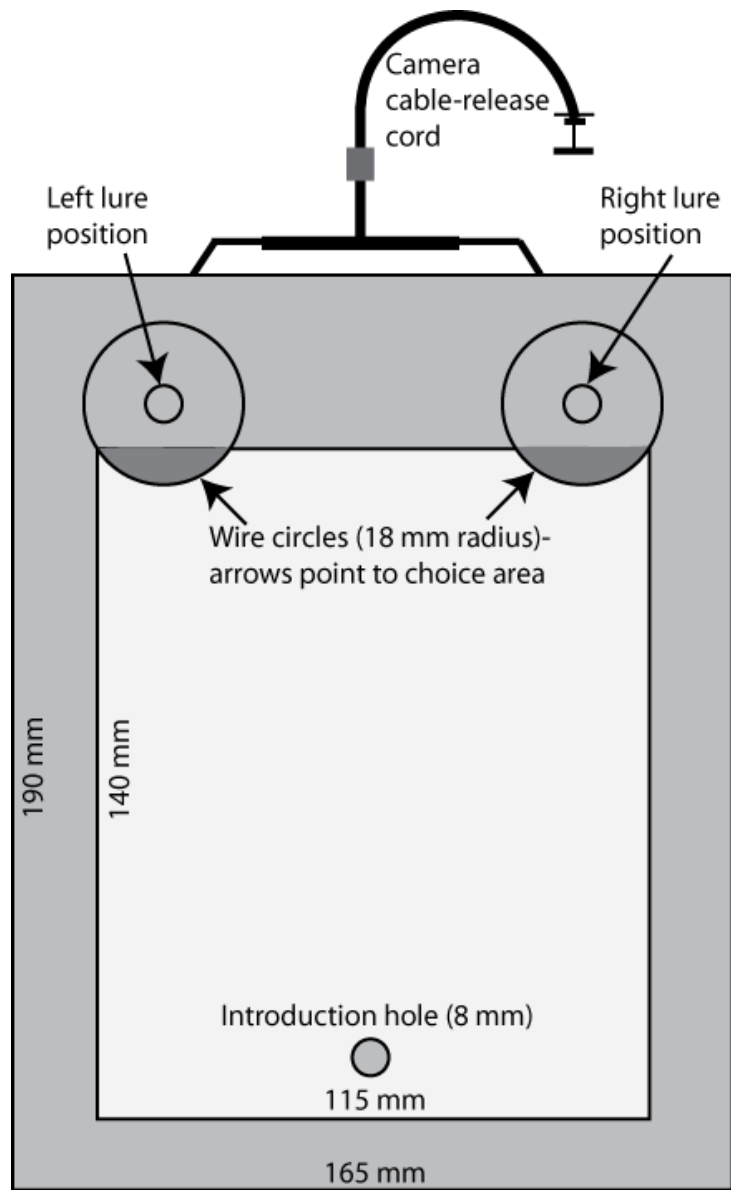
$F = 1.236$ ;  $p = 0.313$

##### *C. ocellata*

	SS	df	MS
Treatment (between columns)	2611	2	1305
Residual (within columns)	16263	32	508.2
Total	18874	34	

$F = 2.568$ ;  $p = 0.092$





**Fig. S1. Prey-choice testing apparatus.** Rectangular glass box (light grey rectangle) with lid, sitting on top of plastic stand (dark grey rectangle). Camera-release cord used to make two lures move up and down together. 'Choice area': dark grey semi-circular area within wire circles.

**Table S1. ANOVA for mirror tests****1. Gaze fixation distance***C. algerina*

	SS	df	MS
Treatment (between columns)	9233	2	4616
Residual (within columns)	34263	19	1803
Total	43495	21	

$F = 2.560$ ;  $p = 0.104$

*C. ocellata*

	SS	df	MS
Treatment (between columns)	13114	2	6557
Residual (within columns)	47400	32	1481
Total	60514	34	

$F = 4.427$ ;  $p = 0.020$

**2. Gaze fixation duration***C. algerina*

	SS	df	MS
Treatment (between columns)	270386	2	135193
Residual (within columns)	417483	19	21973
Total	687869	21	

$F = 6.153$ ;  $p = 0.009$

*C. ocellata*

	SS	df	MS
Treatment (between columns)	115112	2	57556
Residual (within columns)	276528	32	8641
Total	391640	34	

$F = 6.660$ ;  $p = 0.004$

**3. Display distance***C. algerina*

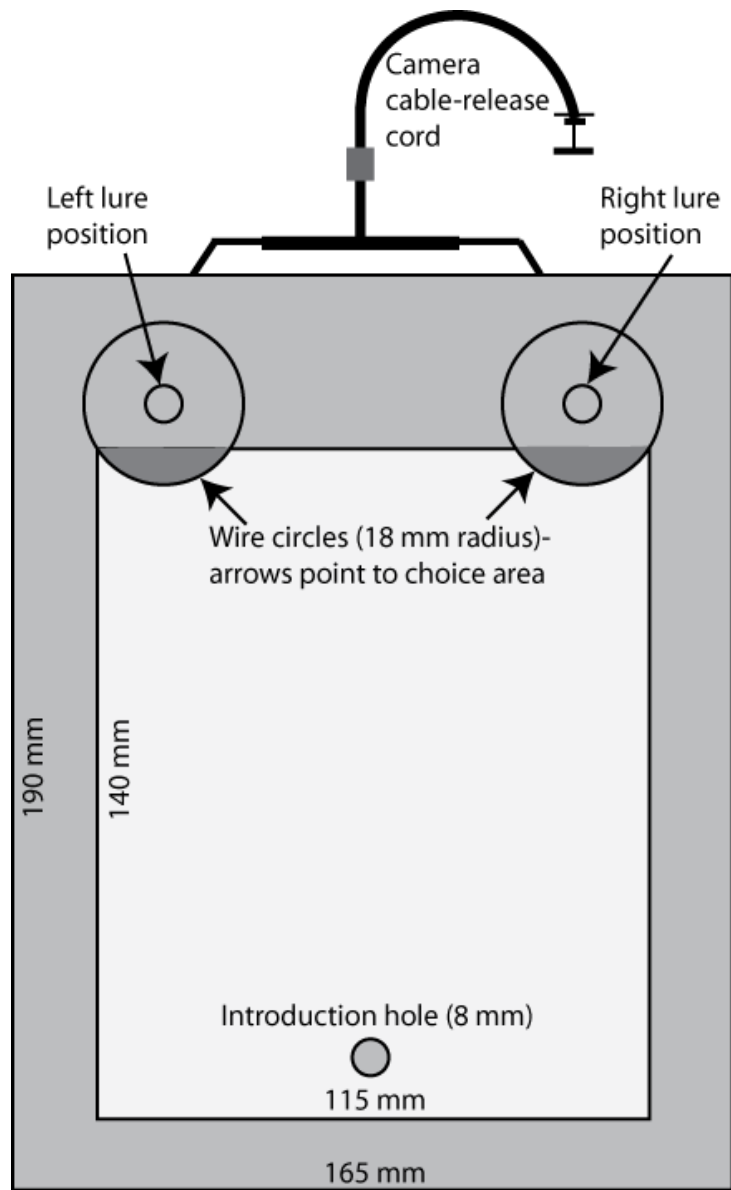
	SS	df	MS
Treatment (between columns)	2204	2	1102
Residual (within columns)	16942	19	891.7
Total	19145	21	

$F = 1.236$ ;  $p = 0.313$

*C. ocellata*

	SS	df	MS
Treatment (between columns)	2611	2	1305
Residual (within columns)	16263	32	508.2
Total	18874	34	

$F = 2.568$ ;  $p = 0.092$



**Fig. S1. Prey-choice testing apparatus.** Rectangular glass box (light grey rectangle) with lid, sitting on top of plastic stand (dark grey rectangle). Camera-release cord used to make two lures move up and down together. 'Choice area': dark grey semi-circular area within wire circles.