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Molecular Evolution of Spider Vision: New Opportunities, Familiar Players

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Abstract. Spiders are among the world's most species-rich animal lineages, and their visual systems are likewise highly diverse. These modular visual systems, composed of four pairs of image-forming "camera" eyes, have taken on a huge variety of forms, exhibiting variation in eye size, eye placement, image resolution, and field of view, as well as sensitivity to color, polarization, light levels, and motion cues. However, despite this conspicuous diversity, our understanding of the genetic underpinnings of these visual systems remains shallow. Here, we review the current literature, analyze publicly available transcriptomic data, and discuss hypotheses about the origins and development of spider eyes. Our efforts highlight that there are many new things to discover from spider eyes, and yet these opportunities are set against a backdrop of deep homology with other arthropod lineages. For example, many (but not all) of the genes that appear important for early eye development in spiders are familiar players known from the developmental networks of other model systems (*e.g.*, *Drosophila*). Similarly, our analyses of opsins and related phototransduction genes suggest that spider photoreceptors employ many of the same genes and molecular mechanisms known from other arthropods, with a hypothesized ancestral spider set of four visual and four nonvisual opsins. This deep homology provides a number of useful footholds into new work on spider vision and the molecular basis of its extant variety. We therefore discuss what some of these first steps might be in the hopes of convincing others to join us in studying the vision of these fascinating creatures.

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Abbreviations: AL, anterior lateral; AM, anterior median; BLAST, Basic Local Alignment Search Tool; CNS, central nervous system; KAAS, KEGG Automatic Annotation Server; KEGG, Kyoto Encyclopedia of Genes and Genomes; LWS, long wavelength sensitive; MAFFT, Multiple Alignment using Fast Fourier Transform; MWS, middle wavelength sensitive; PL, posterior lateral; PM, posterior median; RAxML, Randomized Axelerated Maximum Likelihood; UVS, ultraviolet sensitive.

Online enhancements: supplemental material.

Introduction

Spiders are a large and ancient group of animals. The earliest spiders may have arisen as early as the Devonian (~400 million years ago; Foelix, 2011), and since that time, their numbers have grown to an estimated 80,000 extant species (Raven and Yeates, 2007), with only a little over half of these species described (46,433 species described to date; Platnick, 2017). These diverse animals are found in every biome and on every continent, save Antarctica (Turnbull, 1973). They are voracious predators (Jackson and Pollard, 1996), often playing a role in the control of prey populations (Riechert and Lockley, 1984) and the dynamics of trophic cascades (Schmitz *et al.*, 1997; Schmitz, 2008). Several large families of hunting spiders are also highly visual (Land, 1985; Foelix, 2011), including the wolf spiders (Lycosidae), lynx spiders (Oxyopidae), and jumping spiders (Salticidae). Others, such as the orb weavers (Araneidae), rely less on their eyes for prey capture yet still retain visual functions that play an important role in their daily lives (Foelix, 2011).

Despite their taxonomic diversity and often-visual habits, our understanding of the molecular and genetic basis of spider visual systems lags significantly behind that of other major animal groups. This is unfortunate, because spider visual systems are highly diverse and hence present many useful opportunities to probe the genetic bases of visual specializations. Their phylogenetic position within the Arthropoda also provides a critical opportunity for deepening our understanding of the conservation and/or retooling of ancient developmental pathways. Efforts along these lines promise to pro-

vide important new insights into how and why visual systems evolve in the way they do.

Despite the paucity of genetic information on vision in this group, several recent studies (Eriksson *et al.*, 2013; Samadi *et al.*, 2015; Schomburg *et al.*, 2015), along with the increasing availability of transcriptomic data, are providing new opportunities to evaluate the molecular underpinnings of spider vision. In this review, we highlight some of these opportunities in the hopes of convincing researchers interested in the molecular basis of vision to give spiders "another look."

We begin by describing some of the general features of spider vision and visual ecology. This is a large topic. Thus, we seek to survey some of the more salient aspects of the diversity available in this group. We then move to discuss hypotheses regarding the origins of the arachnid eye. These hypotheses are perhaps best resolved by looking at the genetic basis of spider eye development, the topic we tackle next. Research on spider eye development and its evolution is almost nonexistent, but the few studies that do exist allow us to highlight promising candidate genes and gene networks, many of which are already familiar from other groups. We then turn our gaze from development to core genes of the phototransduction cascade, with a particular focus on opsins. Available transcriptomic data provide us with some initial glimpses into spider opsin evolution. Finally, we conclude with a discussion of next steps and critical needs. There is much to be done. We hope to convince you to join us.

Spider Vision: Versatility Through Modularity

Spiders have eight simple eyes, with the exception of a few unusual taxa that have only six (Land, 1985). These eyes comprise four eye pairs named after their relative positions on the head: the anterior median (AM) eyes, anterior lateral (AL) eyes, posterior median (PM) eyes, and posterior lateral (PL) eyes (Fig. 1A). The four eye pairs can be divided into two fundamental types: the AM eyes are called "principal" eyes, whereas the AL, PM, and PL eyes are all "secondary" eyes. While the principal and secondary eyes often appear similar from the outside, they differ in their internal morphology, developmental basis, functional capabilities, and neural connectivity to the brain (Fig. 1D; Land, 1985; Barth, 2002). These differences suggest distinct evolutionary histories, a topic we approach later in this review.

The differences between principal and secondary eyes offer a good starting point for understanding the visual system of spiders. These begin with differences in retinal morphology (Homann, 1971). The retinas of principal eyes are everted, which is to say that the light-sensitive rhabdomeres of their photoreceptors face toward incoming light, with the cell bodies positioned below (Fig. 1D). This is in contrast to the secondary eyes, whose retinas are inverted, with their rhabdomeres positioned below their photoreceptor cell bodies (Fig. 1D; Homann, 1971). This morphological distinction suggests major differences in the development of these retinas (Homann, 1971; Paulus, 1979). It also poses challenges for the sensitivity of the secondary eyes, because their photoreceptor cell bodies often lie in the light path and are therefore prone to absorbing or scattering photons before they can reach the light-sensitive visual pigments in the underlying rhabdomeres (Blest, 1985a; Walla *et al.*, 1996). Several families, including the Salticidae and Sparassidae, have relocated these cell bodies lateral to the rhabdomal region and therefore outside of the light path (Blest, 1985a). Where this is not the case, this challenge appears to have been addressed by increased transparency of the cell bodies in the secondary eyes, increased size of the rhabdoms, and presence of an underlying reflective tapetum that approximately doubles the light path available to incident photons (Fig. 1D; Homann, 1971). Tapeta are entirely lacking in the principal eyes, consistent with the greater sensitivity offered by their everted retinas (Homann, 1971).

These inherent differences in photoreceptor sensitivity may also help to explain other common differences between principal and secondary retinas, such as their acuity and spectral sensitivity. Principal eyes often exhibit higher spatial acuity than secondary eyes as the result of denser photoreceptor packing and/or advanced optics, like the dual lens systems of jumping spiders (Williams and McIntyre, 1980; Land, 1985), adaptations that may have been enabled by the sensitivity of these photoreceptors. In addition, when color vision exists in spiders, it is often provided by the principal eyes through the expression of multiple photoreceptor types that differ in their peak spectral sensitivity (Yamashita, 1985; Zurek *et al.*, 2015). Color vision comes at an inherent sensitivity cost, because it requires the comparison of inputs from photoreceptors with restricted yet distinct spectral sensitivities (Cronin *et al.*, 2014). Moreover, color vision requires multiple receptors to have overlapping visual fields, leading to costly redundancies in spatial sampling. Thus, it is not surprising that it is the frequently layered, everted retinas of the principal eyes that tend to be used for color vision (Yamashita, 1985; Zurek *et al.*, 2015). However, several notable exceptions exist, including the presence of ultraviolet (UV)-, blue-, and green-sensitive photoreceptor cells in the secondary eyes of the ctenid spider *Cupiennius salei* (Keyserling, 1877) (Walla *et al.*, 1996) and orb weavers in the genus *Argiope* (Yamashita and Tateda, 1978; Yamashita, 1985). Diversity in the retinal location and spectral range of color vision remains critically understudied in spiders. Thus, it is difficult to know what arrangements are the "rule" *versus* the "exception" and whether more sophisticated color vision is widespread or has evolved only occasionally in derived taxa (*e.g.*, Zurek *et al.*, 2015).

Finally, the principal eye retinas are also unusual in that they can be moved behind their fixed lenses by dedicated muscles attached to the retinal envelope (Land, 1969a). The sophistication of this musculature and the resulting retinal movements differ across taxa. Some spider groups, such

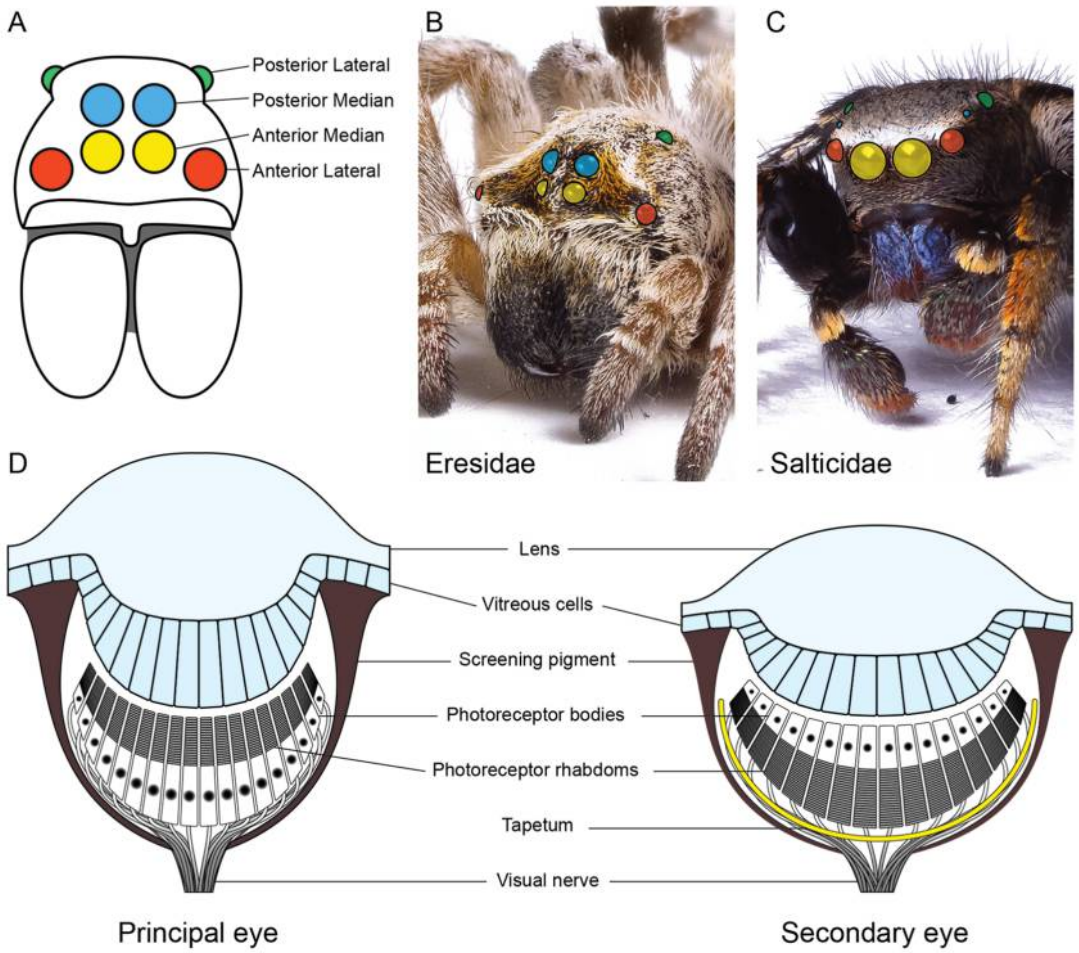


Figure 1. Basic properties of the spider visual system. (A) Spiders have four eye pairs, named after their relative positions on the head, including anterior-posterior and lateral-medial positioning. These names are retained even in the face of dramatic differences in size and exact positioning between families, as indicated by examples of real spiders from the families (B) Eresidae (*Stegodyphus dumicola* Pocock, 1898) and (C) Salticidae (*Habronattus altanus* (Gertsch, 1934)). The anterior median eyes are called "principal" eyes and differ in their internal anatomy and developmental origin from all other eye pairs, called "secondary" eyes. These differences (illustrated in D) include whether the rhabdoms of the photoreceptors are everted (principal) or inverted (secondary), whether the eyes have a reflective tapetum (secondary) or lack one (principal), and whether the retinas can be moved by dedicated muscles (principal; not illustrated) or lack any musculature (secondary).

as the ctenids, lycosids, and thomisids, have only four retinal muscles and exhibit a modest repertoire of retinal movements composed of microsaccadic "twitches" of 2–4° and larger displacements of up to 15° (*e.g.*, *C. salei*; Kaps and Schmid, 1996). The microsaccades are thought to help avoid adaptation of the principal eyes to nonmoving stimuli, whereas the larger gaze movements often preempt and lead body turns (*e.g.*, *C. salei*; Kaps and Schmid, 1996) or track prey without betraying their position by moving their bodies. However, salticids have elaborated both their principal eye musculature and associated retinal movements. These animals have six retinal muscles that enable not only vertical and horizontal displacements in a range of up to 50° but also torsional movements of the retina (Land, 1969a). The result is a sophisticated repertoire of microsaccadic, tracking, and scanning movements that allow these retinas to explore features of complex scenes in ways we are only beginning to understand (Land, 1969a; Canavesi *et al.*, 2011). The secondary eyes universally lack eye musculature or retinal movement.

Taken together, these differences between the principal and secondary eyes lead to functional differences in the quality of visual information that each provide. The principal eyes typically offer higher spatial and spectral resolution, as well as retinal movements that support sustained evaluation of stationary objects and complex scenes. Thus, as their name implies, these eyes often (although not always) provide the "principal" functions we associate with our own foveal vision, including object discrimination, pattern detection, and object tracking. In contrast, the secondary eyes collaborate to provide a wider peripheral field of view that serves to

detect objects or movements of interest and guide reorientation of the visual system to allow for subsequent evaluation by the principal eyes. We note, however, that in some hunting spiders (*e.g.*, ctenids, lycosids), the acuity and sensitivity of the PM eyes can exceed that of the AM eyes, making these "secondary" eyes more prominent contributors to vision in these groups than this generic characterization would imply (Land, 1985; Land and Barth, 1992).

The morphological and functional distinction between principal and secondary eyes continues from the periphery into the brain. Principal and secondary visual inputs are processed in separate pathways with distinct neuropil regions, including separate laminae and medullae (Strausfeld and Barth, 1993; Strausfeld *et al.*, 1993; Long, 2016). These pathways are then integrated in the higher processing centers of the protocerebrum, possibly beginning within or just before the arcuate body (also called the central body; Strausfeld and Barth, 1993; Strausfeld *et al.*, 1993; Long, 2016). This integration remains poorly understood but is essential for both active vision (*i.e.*, inputs from the secondary eyes help to guide principal eye retinal movements; Land, 1969a) and vision-based behavior, including body reorientations and predatory behavior.

Beyond this basic ground plan, it is difficult to generalize regarding spider vision. The modularity of the visual system has yielded extraordinary variety in the solutions different species have evolved to match their behavioral needs. And, in fact, it is this variety that provides such a compelling arena for understanding the underlying molecular evolution of spider visual diversity (Fig. 2). When traversing the spider "tree of life," one observes that every aspect of these eyes is subject to evolutionary tinkering, from their position on the head (Fig. 2A) to their lens optics, fields of view (Fig. 2B), sensitivity, resolution, and capacity to discriminate color and polarization (Land, 1985; Yamashita, 1985; Barth, 2002). Many of these changes are rather straightforwardly connected to the

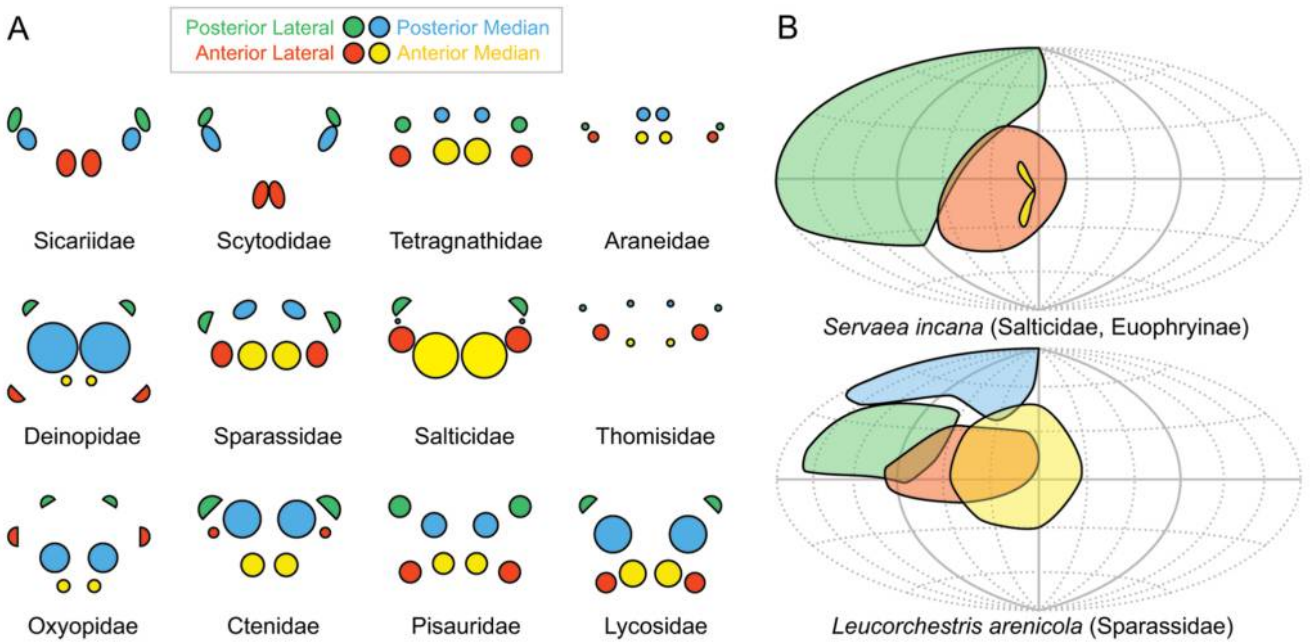


Figure 2. Variation in eye arrangement and field of view across spider families. (A) Typical eye arrangements of 12 spider families, showing the enormous diversity of location and size of the eyes. Color coding of eye types is as follows: anterior median (AM, yellow), anterior lateral (AL, red), posterior median (PM, blue), and posterior lateral (PL, green). Families are listed (left to right, top to bottom) based on phylogenetic relatedness. Predatory lifestyle has a strong influence on visual system arrangement. Web-building spiders include Tetragnathidae (long-jawed spiders) and Araneidae (orb weavers) and tend to have smaller eyes evenly spaced apart. Groups that use vision to actively hunt their prey include the Sparassidae (huntsmen spiders), Salticidae (jumping spiders), Oxyopidae (lynx spiders), Ctenidae (wandering spiders), Pisauridae (nursery web spiders), and Lycosidae (wolf spiders). These groups often have enlarged AM or PM eyes that assist in visually guided predatory tasks. The Sicariidae (recluse spiders), Scytodidae (spitting spiders), Deinopidae (net-casting spiders), and Thomisidae (crab spiders) are sit-and-wait predators whose visual systems, with the exception of the unusual PM eyes of deinopids, more closely resemble those of the web-building groups. Note that in the two groups represented that have only six eyes (Sicariidae and Scytodidae), it is the AM eyes that are lost. This holds true for all six-eyed spiders. (B) Two examples of variation in fields of view of these spider visual systems, with the fields of view of specific eyes color coded as in (A). The unusual "boomerang" shape of the AM eyes in salticids is a characteristic feature of that group. The PM eyes in modern salticids are also often so reduced as to be nearly vestigial.

lifestyles of the animals. For example, the enormous PM eyes of the ogre-faced spiders in the genus *Deinopis* provide the necessary sensitivity for these nocturnal predators to capture flying prey by casting silk "nets," even under starlight conditions (Blest and Land, 1977; Blest, 1978). In contrast, the AM eyes of the diurnal jumping spider *Phidippus johnsonni* (Peckham & Peckham, 1883) are roughly 10,000 times less sensitive (Land, 1969b) but have far better spatial acuity, allowing these animals to identify and stalk prey.

Variation of this magnitude is not unusual when comparing some of the more exaggerated forms these visual systems have adopted, and, indeed, many opportunities exist to consider how spider visual systems have evolved to extremes. However, spiders also offer a number of more continuous axes of visual system differentiation that have the potential for connecting the evolution of vision, including its molecular basis, to specific sources of ecological selection. We briefly highlight a few of these here.

The first ubiquitous source of variation in spider vision involves where spider eyes "look." This may seem a simple variable, but it involves a complex interplay between eye position on the head and the size and relative position of lenses and retinas. The position of eyes on the head dictates how the visual system collects information from the full sphere around an animal. This is highly labile across spiders (Fig. 2A) and is likely driven by the visual requirements of different predatory lifestyles, although these links have yet to be rigorously explored. For example, active-hunting spider groups (*e.g.*, Salticidae, Lycosidae, Sparassidae) often have enlarged forward-facing eyes that provide necessary visual information for prey identification and tracking, combined with nearly 360° of low-resolution peripheral vision for prey detection (Fig. 2; Land, 1985). In contrast, web-building groups (*e.g.*, Tetragnathidae, Araneidae) and sit-and-wait predators (*e.g.*, Thomisidae) often exhibit reduced eyes distributed more evenly around the top of the head (Fig. 2A). How such differences in spatial placement are accomplished during development is not known but is likely to involve genes responsible for patterns of cell growth and proliferation during embryogenesis.

Of course, the position of the eyes on the head is only part of the story. The field of view of each eye is also crucial in determining where a spider sees and how well. Fields of view are highly variable across spider taxa (Fig. 2B), the result of changes to both retinal morphology and the size, position, and optical properties of their associated lenses (Land, 1985; Barth, 2002). In addition, the relative placement of the retina with respect to the focal length of the overlying lens dictates how each eye is focused or, more precisely, the distance at which objects appear in focus (Land, 1981). This focusing distance determines the behavioral utility of an eye. Eyes that can resolve objects only many body lengths away are best suited for long-distance detection and identification, whereas those focused closer to the body can aid in prey handling and intraspecific communication. We note that there is currently no evidence of accommodation in spider vision, through either dynamic changes in lens shape or retinal position, and, thus, focusing distances are likely to remain constant, at least within a developmental stage.

Research on variation in field of view and focusing distance of spider eyes remains sparse, leaving us with a largely anecdotal sense of spider diversity on this front (Land, 1985; Barth, 2002). However, examples range from the narrow fields of view and remote minimal focusing distance (~20 cm) of the AM eyes of the araneophagic jumping spider *Portia fimbriata* (Doleschall, 1859) (Williams and McIntyre, 1980) to the wider fields of view and closer focusing distance (~4 mm) of the AM eyes in *C. salei* (Land and Barth, 1992). Some web-building spiders, including those in the genus *Storena*, even have underfocused and therefore only crudely image-forming eyes (Land, 1985), reminiscent of the optics of the dorsal ocellar system in some insects (Goodman, 1981). Work to understand the molecular, developmental, and physical bases of this variation is much needed.

Lastly, retinal properties show huge variation across spiders, including differences in spectral sensitivity, temporal speed, acuity, and overall light sensitivity (Blest and Price, 1984; Blest, 1985a, b; Yamashita, 1985; Barth, 2002). This variation is likely driven by differences in activity pattern (*i.e.*, nocturnal *vs.* diurnal; Blest, 1978; Laughlin *et al.*, 1980), habitat (*e.g.*, forest *vs.* open habitats; Blest, 1985b), predatory lifestyle, and intraspecific communication (Zurek *et al.*, 2015). It can also help to shape patterns of biodiversity in the behaviors and visual appearance of both conspecifics and prey. For example, jumping spiders in the genus *Habronattus* have evolved a retinal filter in the AM eyes that increases their spectral sensitivities from UV-green dichromacy to UV-green-red trichromacy (Zurek *et al.*, 2015). This shift in visual sensitivity has changed the way that these animals interact with colored prey (Taylor *et al.*, 2014, 2015) and may also help to explain the rapid diversification of color in the courtship displays of males in this group. Investigation of the molecular basis of these changes in visual sensitivity is only just beginning and is likely to be an exciting avenue of research in the years ahead.

The Evolutionary Origins of Spider Vision: A Tale of Two Eye Types

How does this variety in visual function arise? One place to start is to examine the evolutionary origins of spider eyes. As with any trait whose origins date back ~400 million years, reconstructing the evolution of spider eyes requires consulting the molecular basis of eyes in phylogenetic neighbors, including other chelicerates, insects, and crustaceans (Fig. 3).

As mentioned above, the biggest difference between spider eyes is the division between principal and secondary eye types. This division appears to be the result of an ancient evolutionary divergence. Specifically, there is evidence that the principal eyes are homologous to insect ocelli, whereas the secondary

Genes	Principal/Medial Eyes					Secondary/Lateral Eyes				
Pax-6	not detected	*Cs-pax6a*	?	not detected	*toy*	not detected	not detected	?	not detected	*toy & ey*
eyes absent	*Pt-eya*	*Cs-eya*	?	?	*eya*	*Pt-eya*	*Cs-eya*	?	?	*eya*
sine oculis	*Pt-so1*	*Cs-six1b*	?	?	*so*	*Pt-so1* (+*Pt-so2* in AL eyes)	***Cs-six1***	?	?	*so*
optix/six3	not detected	not detected	?	?	*optix*	*Pt-six3.2*	***Cs-six3***	?	?	*optix*
dachshund	not detected	not detected	?	?	*dac*	*Pt-dac2* (+*Pt-dac1* in AL eyes)	*Cs-daca* (only AL & PL eyes)	?	?	*dac*
atonal	?	?	?	not detected	*ato*	?	?	?	not detected	*ato*
orthodenticle	*Pt-otd2*	not detected	?	?	*otd*	not detected	*Cs-otxb*	?	?	*otd*

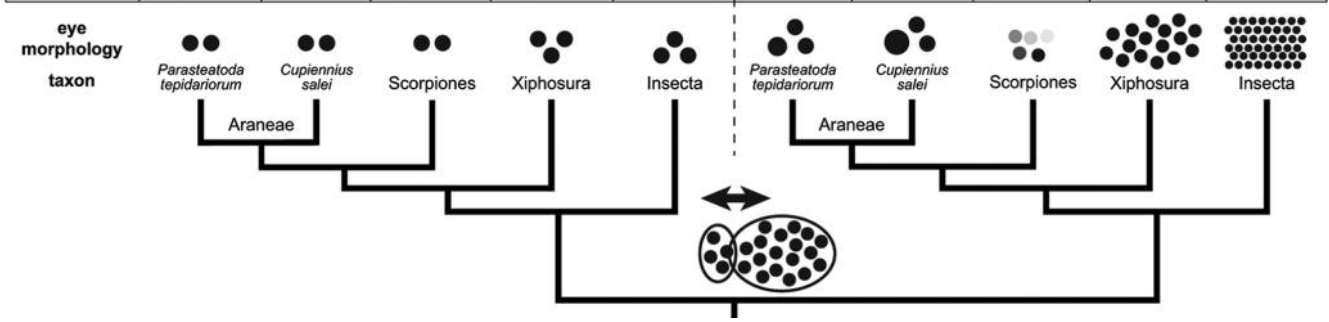


Figure 3. Although the literature is sparse, emerging evidence suggests the existence of an ancestral gene network that underlies all arthropod eyes. These include the key genes highlighted here. Similarities in gene expression support the hypothesis that an ancestral compound eye separated to give rise to both the medial/principal and the lateral/secondary eyes of arthropods (Zhou *et al.*, 2016). These then independently diversified, leading to differences in morphology and visual function. Phylogenetic relationship is based on Shultz (2007). Data on gene expression are from recent studies cited in the text. Genes labeled "not detected" indicate that an ortholog was identified in the focal taxon but its expression was not detected in these eyes. Expression of genes labeled with a question mark has not been investigated in the respective eyes.

eyes likely derived from ancestral compound eyes (Paulus, 1979). The presence of median single eyes and lateral compound eyes is typical for the Euarthropoda, a pattern already apparent in fossils, including those of trilobites and horseshoe crabs (Paulus, 2000; Strausfeld *et al.*, 2016). This division also manifests itself in development: the principal eyes develop from a median ectodermal groove and innervate the protocerebrum, whereas the secondary eyes develop from lateral head ectoderm and innervate the optic lobes that arise from the lateral protocerebrum (Strausfeld and Barth, 1993; Strausfeld *et al.*, 1993). Further evidence for the fundamental difference between these two eye types derives from functional analysis in spiders, such as by Land and Barth (1992), which mirrors similar differences found in scorpions (Loria and Prendini, 2014), and from horseshoe crabs in the genus *Limulus*, in which the lateral eyes develop separately from the medial eyes (Harzsch *et al.*, 2006).

Despite these functional and developmental distinctions between medial and lateral eyes, researchers have begun probing whether these two types of eyes share a common compound-eye origin even deeper in evolutionary time. For example, based on a recent analysis of regulatory gene networks in *Drosophila melanogaster* Meigen, 1830, Zhou *et al.* (2016) proposed that the medial eyes of insects are anciently related to their lateral eyes, presumably having budded off an ancestral photoreceptor organ that was present about ~500 million years ago, prior to the diversification of arthropods. Fossil evidence does support the idea that at least the compound-eye nature of the lateral eyes preceded the diversification of major arthropod lines (Strausfeld *et al.*, 2016). Some debate exists, however, as to whether the relatively large and varied ommatidial units of Xiphosura and Myriapoda are an ancestral trait, from which the more stringently organized insect eyes evolved (Harzsch *et al.*, 2005), or whether they are derived from an ancestor with more precisely regulated ommatidial cell identity (Paulus, 2000), as is known to be the insect-crustacean ancestral state.

This possibility of deep shared ancestry is important because it would be expected to leave its mark on the gene networks involved in eye development. For example, medial (principal) and lateral (secondary) eyes may share a core set of developmental genes (*i.e.*, a proto-arthropod eye developmental network) but with key developmental differences arising between the eye types after their divergence. Such shared ancestry is apparent from the morphological development of arthropods. For example, a study of myriapod eye growth sug-

gests that in this group, as in the much better-studied insect compound eyes, new units are added anteriorly row by row. The same is also the case in horseshoe crabs (Meadors *et al.*, 2001; Harzsch *et al.*, 2006) and is thought to have been the case in trilobites, suggesting that this type of eye growth is the ancestral pattern of arthropod lateral visual system formation (Harzsch *et al.*, 2007).

From an optical point of view, it is worth noting that instead of crystalline cones that often assist refraction in insects and crustaceans, horseshoe crab lenses extend proximally and are the sole source of refractive power (Nilsson and Kelber, 2007). This difference of optical structure raises the possibility that despite shared compound-eye ancestry, optical structures in these two major lineages (myriapod/arachnid *vs.* insect/crustacean) may have evolved independently, hinting that their last common ancestor may have had eyes with little refractive power (Nilsson and Kelber, 2007) or a relatively simple corneal lens (Strausfeld *et al.*, 2016).

How Image-Forming Eyes Evolved From Ommatidial-like Ancestors

Current evidence suggests that image-forming spider eyes may have evolved multiple times (Miether and Dunlop, 2016). In principle, there are two ways in which ancestral compound eyes can lead to image-forming camera-type eyes: fusion of multiple units that together give rise to a single eye, or enlargement of individual units that then each become image forming. Both transitions have been observed within stemmata, the larval eyes of holometabolous insects (for review, see Buschbeck, 2014). Examples of stemmatal formation by combined fusion and expansion also exist, such as in the flour beetle *Tribolium castaneum* (Herbst, 1797) (Liu and Friedrich, 2004). However, it is more likely that arachnid eyes evolved from the expansion of individual ommatidia for the following reasons. First, the photoreceptor arrays of spider secondary eyes (Blest *et al.*, 1980; Land and Barth, 1992) do not show remnants of ommatidial borders, as is often observed for eyes that have evolved from the fusion of multiple ommatidia (Nilsson and Modlin, 1994; Buschbeck, 2014). Second, in scorpions, which also have median (principal) and lateral (secondary) eyes, the latter are highly variable in number, composed of zero to five pairs (Loria and Prendini, 2014). In scorpions, all of the lateral eyes on each side are situated within a patch, which suggests that they developed from a single "eye field." Moreover, in 11% of the scorpions examined by Loria and Prendini (2014), the number of units differed between the 2 sides. Thus, scorpion lateral eyes appear to originate from a variable number of individual "ommatidia" generated within a lateral eye field. This arrangement bears striking morphological resemblance to spider lateral eye development. Finally, preliminary genetic information provides tantalizing support for this model of spider lateral eye development from a primitive compound eye field. In the common house spider *Parasteatoda tepidariorum* (C. L. Koch, 1841), analyses of gene expression during embryonic development suggest that the secondary eyes initially develop from a common eye field that is subsequently split into three separate units, each giving rise to one eye (Schomburg *et al.*, 2015).

If spider secondary eyes evolved from single ommatidia, what might these ancestral ommatidia have looked like? Given the phylogenetic position of horseshoe crabs within chelicerates, it is likely that their eyes most closely resemble those of the last common ancestors of extant chelicerates, and hence the structural organization of their ommatidia provides a good starting point for tackling this question. Compared to insect ommatidia, which are the product of exactly 26 stringently organized cells (Waddington and Perry, 1960), horseshoe crab ommatidia are composed of a much larger (>100) and more variable number of cells (Paulus, 1979). During development, the first ommatidia appear by the end of the first larval stage. From there, ommatidia are added row by row (Smith *et al.*, 2002) but irregularly (Harzsch *et al.*, 2006), such that the adult eye does not have the level of precision that is known from, for example, insect eyes. In addition, the average facet size increases by about sixfold between hatching and adulthood (Waterman, 1954). Thus, development of ommatidia in this ancestral eye field is likely to have been more flexible, and served by a greater number of cells, than is observed in insects. How such a system arrived at the fixed number of secondary eyes found in all spiders is an intriguing question that remains to be investigated.

Sparse but Definitive Overlap in Patterns of Gene Expression During Early Eye Development in Insects and Spiders

If spider secondary eyes indeed evolved from a compound-eye-like ancestor, then we can leverage the extensive knowledge of compound-eye development in insects to identify potential genes and gene networks involved in spider eye development (Fig. 3). Insect compound-eye development begins with the specification of photoreceptors, the molecular underpinnings of which involve the retinal determination network. This gene network is now well described in *Drosophila melanogaster* (for reviews, see Kumar, 2009, 2010). On the top of this network are the *Pax-6* transcription factors eyeless (*ey*) and twin of eyeless (*toy*), which function as eye-field selector genes and are characterized by two highly conserved DNA-binding domains (the paired domain and a homeodomain). Work on cnidarians suggests that their *Pax-6* ortholog may have evolved from an ancestral *PaxB*-like gene that duplicated after the separation of Bilateria from Cnidaria (Kozmik *et al.*, 2003). The importance of this gene for retinal and eye formation is intriguing, since a wealth of earlier studies suggested nearly universal involvement in eye morphogenesis in both vertebrates and invertebrates alike (Gehring and Ikeo, 1999; Gehring, 2002). However, in recent years a number of exam-

ples have emerged in which animal eyes appear to develop without the expression of a *Pax-6* ortholog, including the adult eyes of the annelid worms in the genus *Platynereis* (Arendt *et al.*, 2002) and some of the photoreceptor subtypes of amphioxus (Glardon *et al.*, 1998).

In chelicerates, *Pax-6* ortholog expression has not been found in the developing eyes of the horseshoe crab *Limulus polyphemus* (Linnaeus, 1758) (Blackburn *et al.*, 2008), despite its presence in their lateral sense organ (the function of which remains unclear). This is even more surprising when considering that the *Limulus* Pax-6 (Lp Pax6) paired domain region shares 92% amino acid identity with the corresponding region of *Drosophila*'s Pax-6 version Toy. In addition, even though two *Pax-6* orthologs were isolated from the spider *Parasteatoda tepidariorum*, expression analysis did not reveal the presence of either in any of the spider's eyes (Schomburg *et al.*, 2015). On the other hand, a recent study of gene expression in the spider *Cupiennius salei* provides evidence for *Pax-6* ortholog expression in the principal eyes but not in the secondary eyes (Samadi *et al.*, 2015). Taken together, there are several instances in chelicerates where eye development may not depend on *Pax-6* ortholog expression. However, more work is clearly needed to better define the role of *Pax-6* orthologs in spider eye development (Fig. 3).

In *Drosophila*, downstream of *ey* and *toy* lies the core of the retinal determination network, composed of the *Six* gene family member *sine oculis* (*so*) and its transcriptional coactivator *eyes absent* (*eya*). As is the case for *Pax-6*, these genes and their orthologs have been found to be important in visual system development of both vertebrates and invertebrates (reviewed in Kumar and Moses, 2001; Vopalensky and Kozmik, 2009). Because these genes form a duplex, loss of either of them has been documented to lead to the loss of eye formation in *Drosophila* (Cheyette *et al.*, 1994; Bonini *et al.*, 1997; Pignoni *et al.*, 1997) and *Tribolium castaneum* (Yang *et al.*, 2009), and loss of function of the *so* ortholog prevents eye formation during regeneration in planaria (Pineda *et al.*, 2000). Evidence for a conserved role of these genes more generally in arthropod eyes has been derived from a clever comparative analysis of gene expression between anciently diverged compound eyes and ocelli of *Drosophila* (Friedrich, 2006). In arachnids, we are aware of only two studies in which expression of these two genes was investigated. Remarkably, expression of spider orthologs to both *so* and *eya* was found in the principal and secondary eyes of *C. salei* (Samadi *et al.*, 2015) and *P. tepidariorum* (Schomburg *et al.*, 2015), albeit in each species different members of the *Six* gene family were found expressed in specific eyes (Fig. 3).

A third important retinal differentiation gene in this network is *dachshund* (*dac*). In *Drosophila*, loss of function of *dac* leads to the reduction or loss of compound eyes but not ocelli (Mardon *et al.*, 1994). Interestingly, expression of spider orthologs has been observed in the secondary eyes but not principal eyes of both *C. salei* and *P. tepidariorum* (Samadi *et al.*, 2015; Schomburg *et al.*, 2015). These results hint that *dac* may play distinct roles in the development of compound eyes *versus* ocelli and provide further circumstantial support for the notion that spider secondary eyes may have compound-eye ancestry whereas principal eyes may derive from ocellar predecessors.

At this point we are at the infancy of chelicerate eye development research, and apart from these genes little else is known about what other genes might be involved in spider eye development. There are only two further genes that deserve mentioning here. First, there is the proneural transcription factor *atonal* (*ato*), which lies directly downstream of the determination network and in *Drosophila* is associated with photoreceptor differentiation (Jarman *et al.*, 1994); *ato* was also predicted to be part of an ancestral network (Arendt *et al.*, 2002), presumably conserved between compound eyes and ocelli (Friedrich, 2006). Recent findings highlight the shared use of the same *cis*-regulatory elements in these two organs, further supporting a deep evolutionary connection (Zhou *et al.*, 2016). However, while the presence of its respective ortholog was documented in the lateral sense organ of the horseshoe crab *L. polyphemus*, *ato* ortholog expression was not found in developing *L. polyphemus* eyes (Blackburn *et al.*, 2008). Likewise, two *ato* orthologs were identified in *C. salei*, but they were not found to be expressed in the spider's eyes (Fig. 3; Samadi *et al.*, 2015).

The last gene to mention here is *orthodenticle* (*otd*), which in *Drosophila* is essential for the proper development of photoreceptors (McDonald *et al.*, 2010; Terrell *et al.*, 2012). This gene is also predicted to be part of the ancestral regulatory network (Friedrich, 2006; Friedrich *et al.*, 2016). Interestingly, orthologs have been identified in the secondary but not principal eyes of *C. salei* (Samadi *et al.*, 2015) and, rather curiously, in the principal but not secondary eyes of *P. tepidariorum* (Schomburg *et al.*, 2015). It is worth noting here, however, that expression analyses are always somewhat tricky, and the absence of noted expression always leaves the possibility that expression levels are below the detection threshold or that expression is too transient to be detected.

Taken together, while we are just beginning to understand what genes might be involved in spider eye development, an intriguing picture is emerging that many of the genes responsible for eye development in other arthropods are also expressed in spider eyes. Thus, it is becoming increasingly plausible that much of the ancestral gene network that gave rise to *Drosophila* compound-eye development also underlies eye development in spiders (Fig. 3).

Spider Phototransduction: Preliminary Clues into Variation

Studies of spider phototransduction cascades and opsin proteins began relatively recently. The first opsin sequences characterized from spiders were from the jumping spiders

Hasarius adansoni (Audouin, 1826) and *Plexippus paykulli* (Audouin, 1826) (Koyanagi *et al.*, 2008). There is still relatively little known about the molecular aspects of spider phototransduction, with only a few additional species such as *Cupiennius salei* (Eriksson *et al.*, 2013; Zopf *et al.*, 2013) and *Parasteatoda tepidariorum* (Schomburg *et al.*, 2015) receiving in-depth study. Thus, there is almost no literature to review, in the more traditional sense, on this topic. Instead, we have chosen to analyze publicly available data for preliminary clues into the molecular basis of spider phototransduction.

We assembled a set of previously published spider transcriptomes (Bond *et al.*, 2014; Garrison *et al.*, 2016). RNA-seq data from selected lineages representing differences in eye arrangement, visual behaviors, and ecologies (Table 1) were assembled using Trinity, version 2.0.6 (Grabherr *et al*., 2011; Haas *et al*., 2013), on the National Center for Genome Analysis Support (NC-GAS) mason server; and we searched for phototransduction genes using phylogenetically informed annotation (Speiser *et al.*, 2014). Each assembled Araneae RNA-seq data set was first entered into the KEGG Automatic Annotation Server (KAAS; Moriya *et al.*, 2007; Kanehisa *et al.*, 2017), where it was run against data from the genomes of *Drosophila melanogaster* and *Ixodes scapularis* Say, 1821. BLAST hits were then mapped onto the *D. melanogaster* phototransduction pathway map (KEGG pathway 04745) using KAAS (Kanehisa *et al.*, 2016). Although no single transcriptome contained genes similar to all elements of the *Drosophila* phototransduction pathway (likely the result of the low sequence coverage of the source transcriptomes), when considering all transcriptomes as one large data set, we found evidence for Araneae versions of all key genes in the *Drosophila* phototransduction cascade (Fig. 4). This indicates that the canonical phototransduction elements known from insects may also be expressed in spider eyes.

Because of their importance in photon capture and initiation of the phototransduction cascade, we focused more deeply on analyses of spider opsins. Opsin transcripts identified in our source transcriptomes were converted to amino acid sequences and aligned with the data set from Battelle *et al.* (2016) using MAFFT (Katoh *et al.* 2002; Katoh and Standley 2013) (see supplemental opsin alignment data, available online, for full alignment). The Battelle *et al.* (2016) data set already contained opsin sequences from several arachnids as well as those identified from the genomes of three xiphosuran horseshoe crabs for comparison. To phylogenetically place each transcript within known opsin evolutionary diversity, the resulting alignment of 565 in-group opsin amino acid sequences, 12 out-group sequences from *Trichoplax adhaerens* Schultze, 1883 and closely related nonopsin G protein-coupled receptors (*e.g.*, mouse melatonin receptor and human thyroid-stimulating hormone receptor) were used to estimate phylogenetic relationships and node confidence as bootstrap values using RAxML (Stamatakis 2006, 2014; Stamatakis *et al.* 2008; Pattengale *et al.* 2010; Liu *et al.* 2012). Our phylogeny confirms earlier studies that the Araneae spiders contain opsins from three of the nine major opsin lineages present in the bilaterian ancestor (Ramirez *et al.*, 2016): canonical r-opsins, canonical c-opsins, and peropsins (Fig. 5; Koyanagi *et al.* 2008; Nagata *et al.* 2010; Eriksson

Table 1

Species included in analyses of spider opsin evolution and phototransduction, including information on taxonomy, data source, and reference

Order	Family	Species	Data type	Accession numbers	Reference
Ixodida	Ixodidae	*Ixodes scapularis*	Genome	XM_002408275.1, XP_002408319	Gulia-Nuss *et al.*, 2016
Scorpiones	Buthidae	*Mesobuthus martensii*	Genome	Genes mined as in Battelle *et al.* 2016	Cao *et al.*, 2013
Araneae	Agelenidae	*Agelenopsis emertoni*	RNA-seq	PRJNA254752, SRX652511	Bond *et al.*, 2014
Araneae	Antrodiaetidae	*Aliatypus coylei*	RNA-seq	PRJNA254752, SRX652492	Bond *et al.*, 2014
Araneae	Aranaeidae	*Micrathena gracilis*	RNA-seq	PRJNA254752, SRX652498	Bond *et al.*, 2014
Araneae	Ctenidae	*Cupiennius salei*	RNA-seq	CCP46949–CCP46951, CCO61973–CCO61975	Eriksson *et al.*, 2013; Zopf *et al.*, 2013
Araneae	Deinopidae	*Deinopis longipes*	RNA-seq	PRJNA254752, SRX652495	Bond *et al.*, 2014
Araneae	Dictynidae	*Cicurina vibora*	RNA-seq	PRJNA254752, SRX652499	Bond *et al.*, 2014
Araneae	Eresidae	*Stegodyphus mimosarum*	Genome	KFM62776, KFM67509, KFM75836, KFM77099, KFM77100, KFM77102	Sanggaard *et al.*, 2014
Araneae	Euctenizidae	*Aptostichus stephencolberti*	RNA-seq	PRJNA254752, SRX652490	Bond *et al.*, 2014
Araneae	Salticidae	*Habronattus ustulatus*	RNA-seq	PRJNA267594, SRX763246	
Araneae	Salticidae	*Hasarius adansoni*	cDNA	AB251846–AB251848, AB506462, AB525082	Koyanagi *et al.*, 2008; Nagata *et al.*, 2010, 2012,
Araneae	Salticidae	*Plexippus paykulli*	cDNA	AB251849–AB251851	Koyanagi *et al.*, 2008
Araneae	Tetragnathidae	*Leucauge venusta*	RNA-seq	PRJNA236497, SRX451005	Garrison *et al.*, 2016
Araneae	Theraphosidae	*Aphonopelma iviei*	RNA-seq	PRJNA254752, SRX652487	Bond *et al.*, 2014
Araneae	Theridiidae	*Parasteatoda tepidariorum*	Genome assembly	GCF_000365465.1	Schomburg *et al.*, 2015
Araneae	Thomisidae	*Misumenoides formosipes*	RNA-seq	PRJNA306047, SRX1560150	Garrison *et al.*, 2016

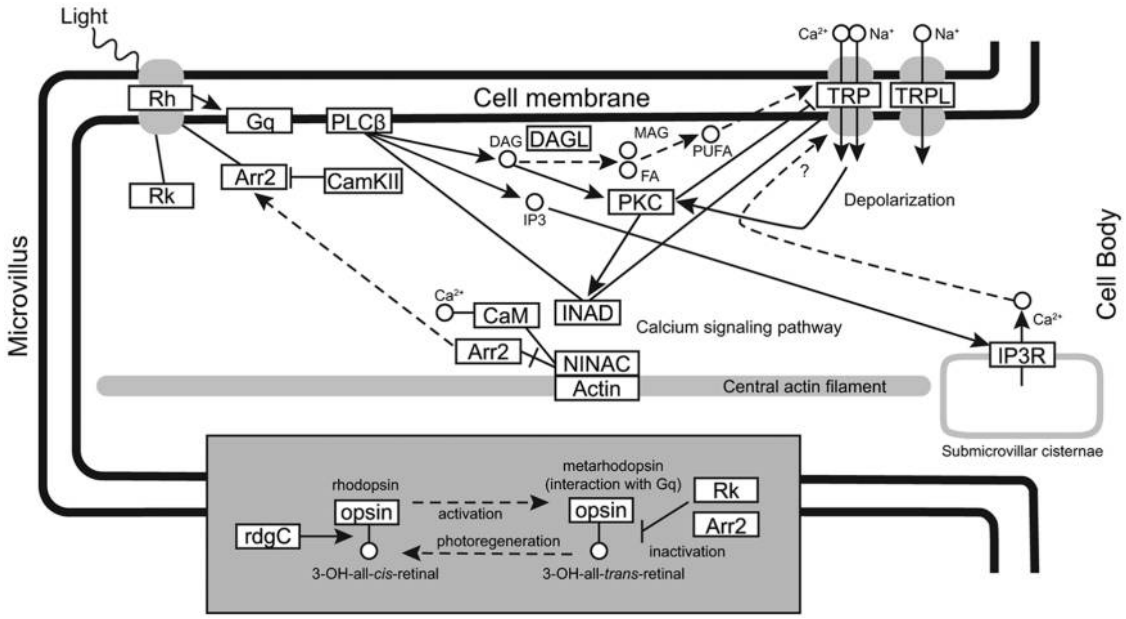


Figure 4. Consensus rhabdomeric phototransduction pathway for Araneae, using transcriptome assemblies from transcriptomic data published by Garrison *et al.* (2016). When considering all of the Araneae assemblies as a set of information, genes similar to all of the known *Drosophila melanogaster* phototransduction pathway genes were identified in spiders, indicating that the full phototransduction pathway represented here is likely to be intact in the Araneae. Genes illustrated, for which homologs were identified in the Araneae, are as follows: *Arr2*, arrestin 2; *CaM*, calmodulin; *CamKII*, calmodulin-dependent protein kinase II; *DAG*, diacylglycerol; *DAGL*, DAG lipase; *FA*, fatty acid; *Gq*, heterotrimeric Gq protein; *INAD*, inactivation no afterpotential D; *IP3*, inositol triphosphate; *IP3R*, IP3 receptor; *MAG*, monoacylglycerol; *NINAC*, neither inactivation nor afterpotential C; *PKC*, protein kinase C; *PLCβ*, phospholipase Cβ; *PUFA*, polyunsaturated fatty acids; *rdgC*, retinal degeneration C; *Rh*, rhodopsin; *Rk*, rhodopsin kinase; *TRP*, transient receptor potential channel; *TRPL*, TRP-like channel.

et al. 2013; Schomburg *et al.* 2015). We discuss each of these in more depth below.

Gq-opsin lineage: canonical r-opsins

Within the canonical r-opsins, spiders generally have two groups of opsins associated with spatial vision: long-wavelength-sensitive (LWS) and ultraviolet-sensitive (UVS) opsins. Based on our sampling, spiders in general appear to have very little variation in the numbers and types of expressed visual opsins (Fig. 6). The majority of the species investigated here have two LWS opsins and one UVS opsin. The involvement in spatial vision of the visual pigments formed from these opsins is confirmed in tissue expression studies in the salticid *Hasarius adansoni*. In *H. adansoni*, Nagata *et al.* (2012) identified four visual opsins (two LWS and two UVS) and found gene expression differences both among the eye types (AM, AL, PM, and PL) and among the four-tiered photoreceptor layers within the principal (AM) eyes. Within the principal eyes, the LWS opsin *Rh1* was expressed in the two deepest (proximal) retinal layers, while the UVS opsin *Rh3* was expressed in the distal two retinal layers (Nagata *et al.*, 2012). The *Rh1* opsin was also expressed in the AL and PL eyes. The remaining two opsins (LWS *Rh2* and UVS *Rh4*) were mostly expressed in the PM eyes (Nagata *et al.*, 2012).

Although there is little variation in the number of visual opsins among the species we investigated, the phylogenetic relationships among species and gene copies suggest a more complicated evolutionary history of lineage-specific duplication and loss. In the spider LWS opsins there are three main clades (Fig. 5). Two of the clades are well supported (LWS *Rh1* and LWS *Rh2A* in Fig. 5) and correspond to the salticid *Rh1* and *Rh2* genes described in Koyanagi *et al.* (2008). Of the three identified LWS spider opsin clades, only the *Rh2A* clade has evidence of species-specific gene duplications, with multiple copies identified in *Stegodyphus mimosarum* Pavesi, 1883, *Leucauge venusta* (Walckenaer, 1842), and *Cicurina vibora* Gertsch, 1992. The third clade (LWS *Rh2B* in Fig. 5) contains sequences from only two spider species (*Aptostichus stephencolberti* Bond, 2008, and *Aliatypus coylei* Hedin & Carlson, 2011), the tick *Ixodes scapularis*, and the bark scorpion *Mesobuthus martensii* (Karsch, 1879). Although the low support values for this clade suggest that the evolutionary placement is still tenuous, the fact that the only spider species that we found with this opsin are from the basal Megalomorphae lineage suggests that this gene may have been lost in more

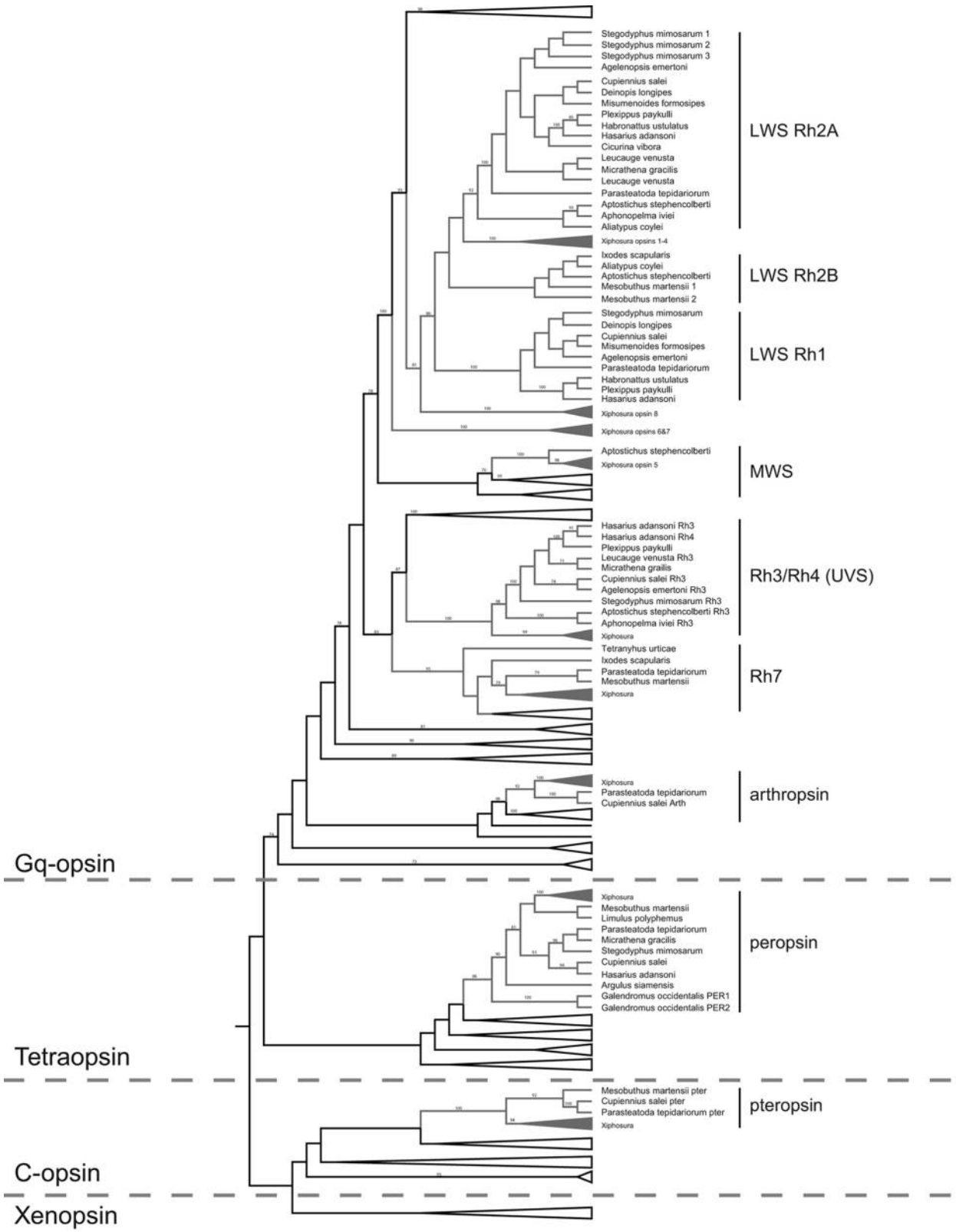
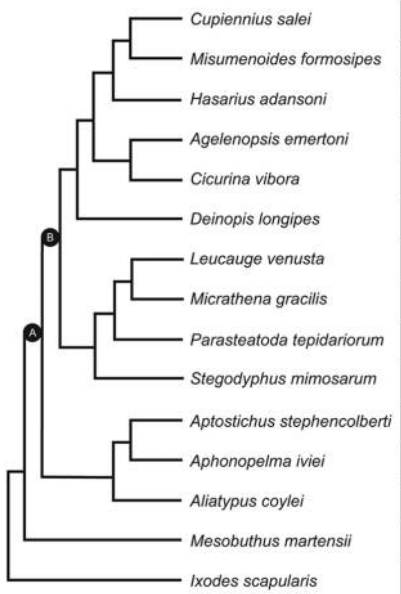


Figure 5. Phylogenetic relationships of known Araneae opsins (see Table 1 for Araneae species included in analyses; see also the sequence database and opsin alignment data, both available online). The four major clades of opsins (Gq-opsin, c-opsin, Xenopsin, and Tetraopsin; Porter *et al.*, 2012; Ramirez *et al.*, 2016) have been indicated by dashed lines. Within each major clade, only chelicerate opsins have been colored and labeled to indicate specific opsin lineages, including three long-wavelength-sensitive (LWS; *Rh1*, *Rh2A*, and *Rh2B*) lineages; middle-wavelength-sensitive (MWS), ultraviolet-sensitive (UVS *Rh3* and *Rh4*), and *Rh7* lineages; and arthropsin, peropsin, and pteropsin lineages.



	Gq-opsins						C-opsins	Tetra-opsins	Total
	LWS Rh1	LWS Rh2A	LWS Rh2B	MWS	UVS	ARTH	PTER	PER	
Cupiennius salei	1	1			1	1	1	1	6
Misumenoides formosipes	1	1							2
Hasarius adansoni	1	1			2			1	5
Agelenopsis emertoni	1	1			1				3
Cicurina vibora	1*								1
Deinopis longipes	1	1							2
Leucauge venusta		2			1				3
Micrathena gracilis	1				1			1	3
Parasteatoda tepidariorum	1	1			1	1	1	1	6
Stegodyphus mimosarum	1	3*			1			1	6
Aptostichus stephencolberti		1*	1	1	1				4
Aphonopelma iviei		1			1*				2
Aliatypus coylei		1	1						2
Mesobuthus martensii			2		1		1	1	5
Ixodes scapularis			1		1				2

Figure 6. Phylogenetic relationships among Araneae species, with the scorpion *Mesobuthus martensii* and the tick *Ixodes scapularis* for out-group comparison (relationships taken from Garrison *et al.*, 2016). For each species, the number of opsin sequences that were identified from available genomic, cDNA, or assembled RNA-seq data is indicated for each of the eight identified lineages of spider opsins. Asterisks indicate where identified transcripts represented closely related, nonoverlapping fragments that were counted as a single transcript to represent a conservative estimate of expressed opsin transcripts for each species. Lettered nodes indicate hypotheses of gene duplication events among long-wavelength-sensitive (LWS) genes. For brevity, we include only one species per spider family. For the Salticidae, we had information for three species but represent results from only *Hasarius adansoni*, the salticid species with the most comprehensive genomic coverage. ARTH, arthropsin; LWS, long-wavelength-sensitive; MWS, middle-wavelength-sensitive; PER, peropsin; PTER, pteropsin; UVS, ultraviolet-sensitive.

derived spider lineages. This pattern leads to a clear difference in opsin gene expression between the Megalomorphae, which express opsins *Rh2A* and *Rh2B*, and the Araneomorphae, which express opsins *Rh1* and *Rh2A*. The fact that most spider species investigated express two LWS opsins, but that there is a difference in which two opsins are expressed between the Megalomorphae and Araneomorphae, is an interesting pattern that warrants further investigation, particularly of opsin expression patterns among eyes and retinal layers. This hypothesis also suggests that we have potentially missed identifying the *Rh2B* opsin from the transcriptome of the tarantula *Aphonopelma iviei* Smith, 1995. Additional investigations of species from both the Megalomorphae and the Araneomorphae will be needed to better characterize this potential difference in opsin expression and usage.

Another anomaly of opsin expression relative to the species studied here is the identification of a middle-wavelength opsin from the Megalomorphae species *Aptostichus stephencolberti* (Figs. 5, 6). Although opsin genes from this clade have been identified from several xiphosuran horseshoe crab species (Battelle *et al.*, 2016), this is the first characterization of a middle-wavelength-sensitive (MWS) opsin from a spider. Finding a MWS gene in *A. stephencolberti* suggests either that there has been massive gene loss of this opsin in other spider lineages or that it is expressed in a very small subset of cells and has been missed in most transcriptomic studies. As more spider genomes become available, testing these alternative hypotheses will become feasible.

In the short-wavelength-sensitive clade of opsins, there are two groups of chelicerate opsins (Fig. 5). The first clade consists of all of the UVS opsins identified in our set of species, including both UVS opsins previously described from *Hasarius adansoni* (Nagata *et al.*, 2012). The latter implies that these UVS opsins are likely to be expressed in the eyes of these other species rather than extraocularly. However, we found evidence for UVS opsins in only 9 of the 13 spider species included here. Whether the remaining four species have lost this gene or it is just difficult to characterize requires further study. The identification of a second UVS opsin in the well-studied *H. adansoni* illustrates the difficulty in identifying transcripts from this clade when expressed in a subset of spider eyes and/or retinal layers (Nagata *et al.*,

2012) and suggests that with further studies UVS opsins will most likely be identified in these species. However, the dim or dark habitats of three of the four species (nocturnal *Deinopis longipes* F. O. Pickard-Cambridge, 1902, cave-obligate *Cicurina vibora*, and burrow-dwelling *Aliatypus coylei*) where UVS opsins were not detected suggest the possibility of a tantalizing link between visual ecology and UVS opsin expression in spiders. A recent investigation of similar links between nocturnality and crepuscularity and opsin complement in insects revealed that UVS opsins are often retained but experience strong purifying selection in nocturnal and crepuscular insect lineages (Feuda *et al.*, 2016). Further work on this topic would help to resolve potential differences and/or similarities between insects and spiders as to how their visual systems respond to changes in visual niche.

The second clade of chelicerate short-wavelength-sensitive opsins is the *Rh7* clade, which are poorly characterized but have been found expressed in neural tissues in *Drosophila* (Kistenpfennig, 2012) and at low levels in dragonfly eyes (Futahashi *et al.*, 2015). This clade is notable for the fact that while genes have been found in horseshoe crabs (Battelle *et al.*, 2016) and in the genomes of scorpions (*Mesobuthus martensii*) and ticks (*Ixodes scapularis*), there has not yet been an *Rh7* opsin identified from an Araneae species. Here, we report evidence of *Rh7* opsin in the *Parasteatoda tepidariorum* genome (Fig. 5). Much work is needed across the arthropods, including within spiders, to characterize the expression patterns and functions of this enigmatic group of opsin genes.

The final clade of arachnid opsins found in the canonical r-type opsin lineages are the arthropsins (Fig. 5). Similar to the *Rh7* clade, the arthropsins are poorly characterized in terms of function, tissue expression patterns, and evolutionary history. A recent study in onychophorans found arthropsin expression in the neural tissues of the central nervous system (CNS) but not in the eyes, suggesting that arthropsins may be involved in extraocular photoreception (Eriksson *et al.*, 2013). Similar expression patterns were found in the chelicerate *Limulus polyphemus*, with arthropsin expression found in three of four neural tissues tested, as well as in the ventral eye. It should be noted, however, that there were two copies of arthropsin identified in the *L. polyphemus* genome, but expression of only one was found in the retinal and neural tissues investigated (Battelle *et al.*, 2016). In our data set, arthropsin expression was found only in *Cupiennius salei* and *Parasteatoda tepidariorum*, although we predict that arthropsin expression will be identified in more species as studies of spider opsins continue.

Tetraopsin clade: peropsins

The peropsins are members of the larger Tetraopsin lineage, which includes neuropsins, Go-opsins, retinal G protein-coupled receptor opsins, and retinochromes (Ramirez *et al.*, 2016). The first arthropod peropsin identified was from the jumping spider *Hasarius adansoni* (Nagata *et al.*, 2010). Subsequently, peropsins have been identified in *Cupiennius salei* (Eriksson *et al.*, 2013), *Parasteatoda tepidariorum* (Schomburg *et al.*, 2015), and *Stegodyphus mimosarum* (Henze and Oakley, 2015). We also identified a peropsin from one additional species, *Micrathena gracilis* (Walckenaer, 1805). The lack of peropsins in the other spider species investigated suggests that peropsins are in general expressed at low levels, at different developmental stages (see below), or, alternatively, that the assembled transcriptomic data were not of sufficient coverage to detect peropsin transcripts.

A number of studies have looked at peropsin tissue expression patterns in chelicerates. The most comprehensive study was in the horseshoe crab *Limulus polyphemus*, where peropsin had ubiquitous expression in all three eye types (median, ventral, and lateral) as well as four regions in the nervous system (Battelle *et al.*, 2016). In the Araneae, expression studies have found slightly different patterns. Tissue-specific transcriptomes in *Cupiennius salei* found peropsin expressed in the CNS and all of the secondary eyes but not in the principal eyes (Eriksson *et al.*, 2013). In contrast, *in situ* hybridization studies in *H. adansoni* found peropsin expressed in nonvisual cells bordering the distal region of the retina in the principal eye (Nagata *et al.*, 2010). Interestingly, developmental expression studies of eyes in a third species, *P. tepidariorum*, found that peropsin was the only opsin transcript present during embryonic stages, particularly associated with the embryonic anlagen of both principal and secondary eyes, suggesting a developmental role as well as a function in the retina (Schomburg *et al.*, 2015).

c-type opsin clade: canonical c-opsins

Canonical c-opsins belong to the c-type opsin clade that is composed mainly of vertebrate opsins found in visual systems and neural tissues (Porter *et al.*, 2012; Ramirez *et al.*, 2016). Arthropod c-type opsins, termed "pteropsins," have been identified from insects (Hill *et al.*, 2002; Velarde *et al.*, 2005), crustaceans (Colbourne *et al.*, 2011), and chelicerates (Eriksson *et al.*, 2013; Battelle *et al.*, 2016), with tissue and transcriptomic expression studies finding pteropsins expressed in the CNS but not the retinas of the honeybee *Apis mellifera* Linnaeus, 1758 (Velarde *et al.*, 2005), the horseshoe crab *Limulus polyphemus* (Battelle *et al.*, 2016), and the spider *Cupiennius salei* (Eriksson *et al.*, 2013). The fact that we identified only one additional spider pteropsin transcript from *Parasteatoda tepidariorum* highlights the difficulty in characterizing this opsin group from RNA-seq data alone.

Ancestral Araneae opsins

When comparing the opsins identified in the Araneae with the opsin complement found in Xiphosura genomes, the difference in copy number is striking. The *L. polyphemus* genome contains 18 opsin genes, a pattern confirmed by the ge-

nome assemblies of 2 additional horseshoe crab species (Battelle *et al.*, 2016). In contrast, there are many fewer identified opsin genes in the Araneae genomes of *Parasteatoda tepidariorum* and *Stegodyphus mimosarum* included here. The overall view from both genomic and transcriptomic data suggests that spider visual systems utilize extremely conserved numbers of visual opsins (LWS and UVS) among species. In particular, despite evidence for gene duplication events producing three main clades of spider LWS opsins (*Rh1*, *Rh2A*, and *Rh2B*), as well as some additional duplication events within particular species (*e.g.*, *Leucauge venusta*, *Stegodyphus mimosarum*), most species seem to express only two main LWS opsins, although which two genes are expressed varies by taxon (Megalomorphae and Araneomorphae). Based on the opsin data set assembled here, the ancestral Araneae opsin complement consisted of five canonical r-opsins: four visual r-opsins (two LWS opsins, one MWS opsin, and one UVS opsin) and one extraocular arthropsin. Evidence of an *Rh7* opsin gene from the *P. tepidariorum* genome suggests that this additional opsin type was also included in the ancestral set, but more data are needed to confirm the presence of an *Rh7* in other spider taxa. Additionally, the ancestral Araneae genome also contained one c-type pteropsin and one peropsin from the Tetraopsin lineage, for a total genome opsin complement of eight opsins from three major opsin lineages. Whether these numbers hold up to deeper transcriptomic and genomic scrutiny remains to be seen, but clearly there is a need to study opsin evolution in this group. This should involve not only additional sequencing but also efforts to characterize the timing and location of opsin expression in both ocular and extraocular tissues.

Conclusions and Future Directions

In this review, we have highlighted some of the tremendous structural and functional diversity offered by spider visual systems. Spiders arguably represent the most diverse group of image-forming "camera" eyes within arthropods, and their variety rivals even that found in the compound eyes of other better-studied groups such as insects and crustaceans. However, while their variety is impressive, we are still in the early days of understanding the genetic and molecular basis that underlies this diversity. Nevertheless, in synthesizing and probing available information, our efforts here have revealed that many of the genetic players implicated in eye development and phototransduction in spiders are likely to be familiar to those studying vision in other systems. This is, on one hand, not so surprising given what we know about other chelicerate and arthropod lineages but, on the other hand, quite exciting because it offers tractable inroads into substantial questions such as: How do spider eyes develop? What genes underlie adaptive changes to spider vision? When did medial and lateral eyes diverge evolutionarily, and how? And, what did the original arthropod eye look like?

Beyond simply gene-by-gene orthology, the potential for shared developmental machinery between spiders and other arthropod groups opens up many new opportunities to probe the molecular basis for variation in spider vision. For example, we might begin to predict how spiders initiate eye formation. In insect compound eyes, it is well understood that photoreceptors are the initiators of a complicated cascade of signals that mediate the development of the rest of the eye (for review, see Kumar, 2012). Here the initial photoreceptor recruits other photoreceptors, which then, in a precise order, recruit multiple layers of support cells, some of which ultimately produce the overlying cuticular lens. Based on the genetic data available thus far, it seems likely that the same general pattern of events also applies to spider eyes. If true, there are several predictions that follow from this developmental paradigm. First, if one were to knock out one of the key photoreceptor determination genes, one would expect that in spiders, just as in insects, this would lead to a loss of the entire eye, not just the loss of photoreceptors. Based on differences in gene expression observed in spiders so far, loss of *dac* might specifically affect the secondary eyes but not the principal eyes (Fig. 3). This line of experiments could be relatively easily performed and would verify that in spiders the development of other ocular structures is likewise initiated by photoreceptors.

A second implication of this shared developmental paradigm is that all photoreceptor populations are expected to be fully differentiated in early development and that photoreceptors cannot be added after other eye structures have developed. This means that the eyes of tiny spiderlings should already contain their full set of photoreceptors, a prediction that has received preliminary support from studies of the principal eyes (Blest and Carter, 1987) but could stand to be tested for the secondary eyes as well.

A third consequence is that one might find spiders that have photoreceptors without lenses, but it is less likely that there are spiders with lenses that lack underlying photoreceptors. This is because if photoreceptors fail to initiate the recruitment of the cuticular lens secreting cells, then the developmental plan has no mechanism to start forming a lens. On the other hand, photoreceptors could start to develop, but the differentiation or recruitment of lens-producing cells may be prevented, thus leading to a visual organ forming without a lens, perhaps similar to some of the simpler and more ancestral visual organs (Nilsson, 2009). To test this hypothesis, one could first target those spiders with vestigial eyes (*e.g.*, some jumping spiders; Land, 1985) and/or spider groups that have lost their principal eyes (Land, 1985). One would expect that as eyes become vestigial or are lost, it is the lens that is lost ahead of the photoreceptors.

Given the parallels between early eye development genes in insects and spiders, it seems reasonable to ask whether later stages of eye development likewise show strong parallels between these groups. This consideration is particularly inter-

esting in light of extant diversity in eye placement on the spider head (Fig. 2A). Because all secondary eyes likely develop from a pair of "eye fields," one on each side of the head, differences in their placement must be the result of migration or separation by subsequently dividing intervening cells. It is possible, then, that transcription factors regulating the development of interommatidial cells in insects could play an important evolutionary role in the diversification of spider eye placement.

Another related question is how spider eyes are focused during postembryonic development. The issue is particularly timely in the light of the recent human epidemic of myopia (Dolgin, 2015). Perhaps study of the naturally underfocused eyes of orb weavers (Land, 1985) could provide some insights into the fundamental mechanisms responsible for the development of refractive errors in camera eyes. More generally, spiders could be valuable subjects in the study of how proper focusing is established in invertebrates, a topic that thus far has largely escaped scientific investigations.

Our initial forays into phototransduction and opsin genetics in spiders also reveal many opportunities. Initial efforts should focus on better describing the temporal and spatial (*e.g.*, ocular *vs.* extraocular) expression of the opsins identified here, as well as deeper or more targeted sequencing of opsins to establish the basic opsin repertoire of spiders. Of particular interest is how opsin expression segregates among eye types and how these expression patterns fit with hypotheses about eye development. Such efforts would then serve as an excellent basis for investigations of the ecological and evolutionary drivers of opsin gains, losses, and modifications. For example, jumping spiders appear to exhibit remarkable diversity in the spectral sensitivities of their principal eyes, ranging from UV-green dichromacy through trichromacy to tetrachromacy (Blest *et al.*, 1981; Yamashita, 1985; Zurek *et al.*, 2015). The molecular basis and evolutionary consequences of transitions between these different states, in jumping spiders and spiders more generally, remains an underexplored but exciting area for future work.

Of course, there are many additional questions that remain unanswered in spiders, ranging from the evolutionary basis of variation in field of view to the developmental control of rhabdom size and shape. Our hope is that more researchers will decide to acquaint themselves with these fascinating animals, whose visual systems have deep homology to more familiar eyes such as those of insects, crustaceans, and even vertebrates. Initial pathways into the study of their vision are provided by our knowledge of arthropod eye genes, but we expect that researchers who look more closely will be rewarded with new and exciting insights into the evolution of seeing.

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