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Primer

Spider vision

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A colleague recently asked me “Why do spiders have so many eyes?”, the implication being that the eight eyes typical of most spiders seemed a bit over-much for an animal that already had “so many legs!” My first instinct was to respond à la the Big Bad Wolf with “All the better to see you with, my dear!”, and indeed, this cheeky answer is not far from the truth. In certain spider groups, most notably the Salticidae or jumping spiders, these eight eyes provide unrivaled visual capabilities, especially when accounting for how small these animals (and their eyes) are. Most jumping spiders, for example, see pattern and detail in the world as well as an elephant can, and some see the colors of the rainbow as well as a peacock. However, there are many ways to answer this seemingly simple question. I articulate a few of these answers below, starting with a description of how spider eyes are constructed and how differences in their construction have resulted in differences in function. I then discuss how such differences arise from distinct developmental pathways and even deeper divisions in their evolutionary history. Finally, I provide a few examples that highlight the versatility and peculiar originality of these modular visual systems. One thing is for sure: spiders, which are one of the most diverse and successful groups of animals on the planet, have benefited immensely from their ability to see the world.

The first thing to know about spider eyes is that they come in two varieties: ‘principal eyes’ and ‘secondary eyes’. By far the most typical arrangement is for a spider to have eight eyes composed of a single pair of ‘principal’ eyes, also called the anterior median eyes for their relative position on the cephalothorax, and three pairs of ‘secondary’ eyes, named the anterior lateral eyes, posterior lateral eyes, and posterior median eyes (Figure 1A). Some spider groups have lost eye pairs over evolutionary time. The most common pair to lose are the principal eyes (e.g., Sicariidae and Scytodidae), but sometimes one of the secondary eye pairs is lost as well (e.g., some members of the family Uloboridae).

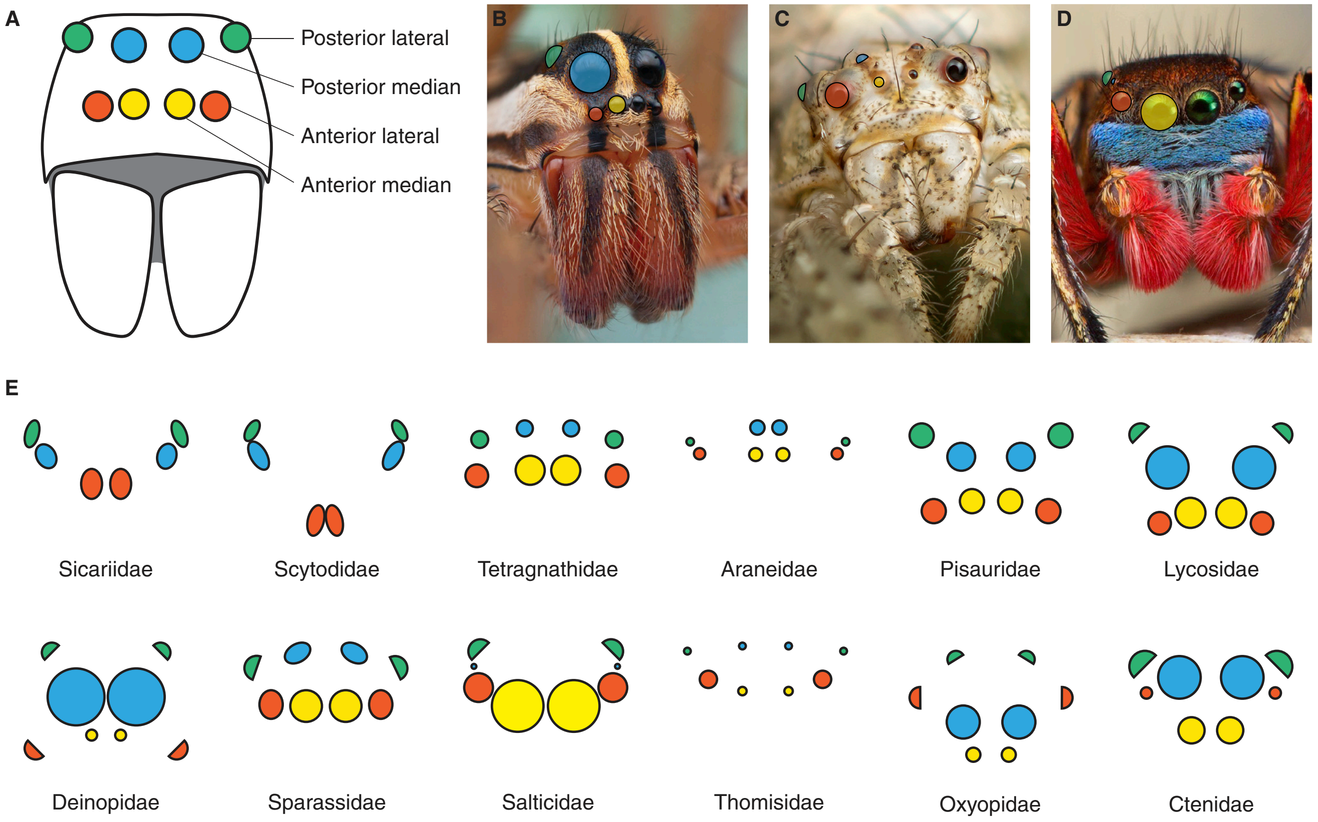

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Figure 1. Typical arrangements of spider eyes and their modification across spider families.
Most spider families have eight eyes, named after their relative position on the cephalothorax (A), including the anterior median (also called ‘principal eyes’), anterior lateral, posterior median and posterior lateral eyes, the latter three often referred to as the ‘secondary eyes’. Spider families exhibit characteristic arrangements and relative sizes of eyes, from the enlarged posterior median eyes of the Lycosidae (B, *Rabidosa rabida*) to the uniformly small constellation of eyes of the Thomisidae (C, *Tmarus* sp.), to the enlarged anterior median eyes of the Salticidae (D, *Habronattus americanus*). A sampling of the eye arrangements of common spider families (E) gives some sense for the extraordinary diversity in position and relative size across this animal group. Photos (B–D) courtesy of Thomas Shahan.

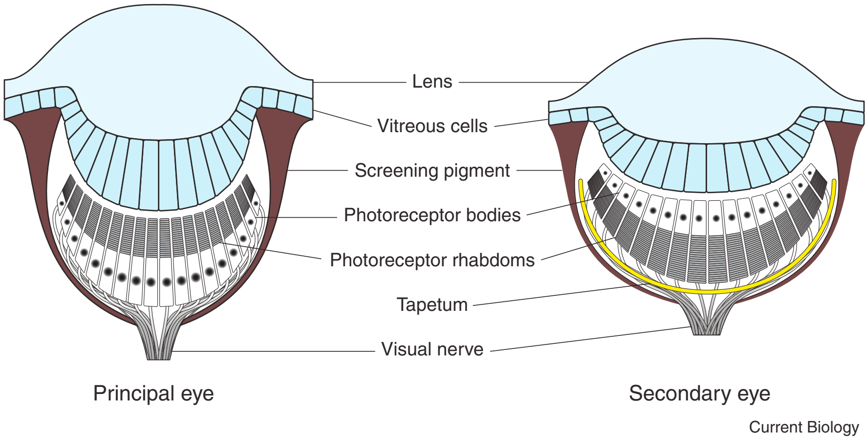

Figure 2. Anatomy of principal and secondary eyes in spiders.
Schematic representations of the two types of spider eyes, illustrating key differences. These include the everted retina of the principal eyes versus the inverted retina of the secondary eyes, the presence of the tapetum below the secondary eye retina (absent in the principal eyes), and the presence of retinal musculature (principal eyes only, not illustrated).

Whether a spider is working with a full set of eight eyes, or a reduced subset, the modularity of this distributed visual system offers a world of possibilities, from where these eyes 'look' to what they are specialized to 'see'. Regardless of which group of spiders one considers, the properties of their eyes are uniquely tuned to their behavioral ecology and the selective pressures it imposes on their visual function. This evolutionary diversification is apparent first at the level of eye arrangement. Different spider families can often be discriminated from each other strictly on the basis of the relative size and positioning of their eyes on the head (Figure 1E), from the oversized, forward-facing principal eyes of jumping spiders (Salticidae, Figure 1D) to the diadem of diminutive eyes characteristic of crab spiders (Thomisidae, Figure 1C).

Modular vision: a tale of two eye types

To better understand the versatility of spider vision, one must first become acquainted with the differences in form and function between the principal and secondary eyes. While both eye types are simple 'camera' eyes, they exhibit key differences in their construction (Figure 2). One of the most important distinctions lies in their retinal morphology. The retinas of the principal eyes are everted, which means that the light-sensitive rhabdomeres of their photoreceptors are positioned distally, facing incoming light, with the photoreceptor cell bodies and axons below. In contrast, the photoreceptors of the secondary eyes are inverted; their rhabdomeres lie below the cell body. While this may seem a minor difference, it has major implications for visual sensitivity and associated visual functions.

All else being equal, the everted photoreceptors of the principal eyes provide greater sensitivity because light can be focused by the lens optics directly onto the rhabdomeres. The secondary eyes, however, must contend with light absorption and scattering by the photoreceptor cell bodies, which lie ahead of the rhabdomeres in the path of incoming light. This is not an unsurmountable difficulty. Indeed, our own retinas also have inverted photoreceptors and provide us with excellent vision (albeit with many tradeoffs and optical adaptations). Nevertheless, this difference has shaped the evolution and function of these two eye types in ways that reflect this inherent difference in light capture efficiency.

A number of adaptations have arisen to address the inverted construction of the secondary eye retinas. In some spider families, including the Salticidae and Sparassidae, the cell bodies of the secondary eye photoreceptors have been shifted laterally to lie beside the rhabdom, bringing them outside of the light path. This improves light sensitivity, but at a spatial sampling cost: the retinal mosaic must now accommodate the width of both the rhabdoms and their attendant cell bodies, necessarily reducing photoreceptor density and associated visual acuity. In other spider families, improved sensitivity has been achieved through increased transparency of the cell bodies (with unknown impacts on photoreceptor physiology) and/or with increases in rhabdomere size.

Another widespread adaptation is the use of light-reflecting tapeta at the base of the retina (Figure 2). These mirror-like structures, composed of guanine crystals, reflect unabsorbed light back through the retina, offering photons a second chance to be absorbed by the photoreceptor. This arrangement nearly doubles the effective path of incident light, thereby improving absorption. Tapeta are absent from all principal eyes, but have evolved a variety of morphologies in the secondary eyes depending on spider family, from a primitive sheet-like morphology to more derived grate-shaped and canoe-shaped tapeta. However, despite the obvious sensitivity benefits of tapeta, they are absent in a variety of spider families, including the Philodromidae, Salticidae, Dinopidae, and Eresidae, perhaps because they lead to reductions in spatial resolution due to increased stray light scattering into nearby photoreceptor units.

Despite these adaptations for improving the sensitivity of the secondary eyes, a number of widespread differences between principal and secondary eyes follow from this underlying difference in retinal morphology. These are general patterns for which there are often exceptions, but nevertheless help to set the stage for the evolutionary trajectories of these two eye types. The first is related to spatial acuity. In general, principal eye retinas exhibit denser photoreceptor packing, often achieving remarkable spatial acuity as a consequence. For example, the interrhabdomal angles found in salticid principal eyes (0.04–0.07°) are an order of magnitude smaller than those of the most densely packed regions of the secondary eyes (0.4–0.7°). Although realized spatial acuity is the result of the combined effects of photoreceptor density and eye optics, principal eyes often achieve higher acuity.

Another key visual function that benefits from high photoreceptor sensitivity is color vision. To perceive differences in the spectral quality of incoming light, eyes (and associated neural tissues) rely on comparisons between photoreceptors with distinct wavelength-specific sensitivities. However, this comes at an inherent sensitivity cost, because photoreceptors useful to color vision must be sensitive to a subset of the wavelengths of available light. The result is a decrease in the overall light sensitivity of a given photoreceptor type. It is perhaps not surprising, then, that color vision, when it occurs in spiders, is often provided by the principal eyes, thereby taking advantage of the superior sensitivity of these retinas. This is best illustrated by color vision in jumping spiders. These diurnal predators express multiple photoreceptor types with distinct peak sensitivities in their principal eye retinas; some species express as many as four spectral classes of photoreceptors ranging from ultraviolet (UV)-sensitive to long-wavelength sensitive (i.e., maximally sensitive to orange or red). However, there are a number of exceptions to this pattern that are worth noting, including the UV-, blue-, and green-sensitive photoreceptors found in the secondary eyes of the ctenid spider *Cupiennius salei*, and orb weavers in the genus *Argiope*.

One additional point regarding color vision is worth remarking on here: for color information to be extracted from a given portion of a visual scene, the visual system must compare the inputs of multiple photoreceptor types. For retinas composed of a sheet of contiguous photoreceptors, as is the typical case in spiders, this results in a reduction in spatial sampling because the visual system must pool information from adjacent photoreceptors. However, jumping spider principal eyes elegantly sidestep this issue through retinal tiering and tier-specific expression of photoreceptor types. For portions of their principal eye retinas, there are four tiers of photoreceptor cells viewing a particular region of space, and thus the inputs of the photoreceptor types from these retinal tiers can be compared to extract color information without any loss in spatial acuity. In contrast, spiders recruiting their secondary eyes in service of color vision must deal with both issues of sensitivity and the additional loss of spatial resolution in order to extract chromatic information from their visual worlds.

The last and most unusual characteristic of principal eyes is their movable retinas. In a number of spider families, the principal eye retinas can be moved behind their fixed lenses inside the head, thanks to a set of dedicated retinal muscles. This allows their resulting fields of view to be dynamically shifted to ‘look’ around the world without the need to move the head or body. The number of muscles responsible for these movements, and therefore the complexity of the resulting gaze movements, differs amongst spider families. The ctenids, lycosids, and thomisids, for example, have four retinal muscles attached to each retina which animate 2–4° microsaccadic ‘twitches’ as well as larger displacements of up to 15°. The smaller microsaccades are thought to help to reduce visual adaptation to non-moving stimuli, whereas the larger displacements are often used to lead body turns and track moving prey. This system of retinal movements has been further elaborated in the Salticidae, which have six muscles attached to each principal eye retina. This affords more complex control over principal eye gaze, including not only horizontal and vertical displacements of up to 50°, but also torsional movements which may assist in edge and shape detection. Although the existence of these retinal movements has been known since the late 1920s, their function, control, and neural integration remains an area of active investigation.

When integrated in a modular visual system, these features of principal and secondary eyes lead to divisions of labor amongst key visual functions. For example, the principal eyes often provide the functions we most associate with our own foveal vision, including pattern and shape recognition, color vision, and object tracking. In contrast, the secondary eyes often collaborate to provide a broad peripheral field of view primarily sensitive to motion, serving to guide body and gaze reorientations that refocus visual attention on objects of interest.

The exact nature of this parsing of visual function differs amongst spider groups, but it is clear that the modularity of spider vision offers enormous versatility for emergent visual capabilities. In addition, some opportunities available to spider vision are unique to this group of animals. For example, the movable retinas of the principal eyes provide a number of unusual advantages. The most obvious might be that this system of gaze control allows spiders to evaluate their visual surrounds without betraying their position to potential predators via head movements. However, closer inspection reveals another unique advantage: movement of the principal retinas does not come at the cost of motion blur in the secondary eyes because the latter remain stationary during gaze inspection of a scene. Conspecifics may also gain some information about a viewer’s visual engagement during communication from the flickering of the retinal movements sometimes visible when looking directly into the principal eyes, a veritable ‘twinkling of an eye’. Much remains to be explored about how spiders take advantage of the unique opportunities provided by their modular visual systems.

Developmental and neural integration

One might reasonably wonder how these differences in eye form and function arise. An initial answer to this query comes at the proximate level of development and neural integration. The principal eyes develop from a median ectodermal groove, whereas the secondary eyes develop from the lateral head ectoderm. This developmental division persists in the neural pathways responsible for processing of their visual inputs: the principal eyes and the secondary eyes separately innervate the protocerebrum and lateral protocerebrum, respectively, employing distinct neuropil regions including separate laminae and medullae. Subsequent neural integration occurs in the higher processing centers of the protocerebrum, either within or just ahead of the arcuate body (also called the central body). Although the exact location and neural anatomy of this integration remains a mystery, recent work has revealed the presence of neurons that respond to inputs from multiple eye types, indicating that spider brains stitch together the input from various eyes to create a unified view of their visual surroundings.

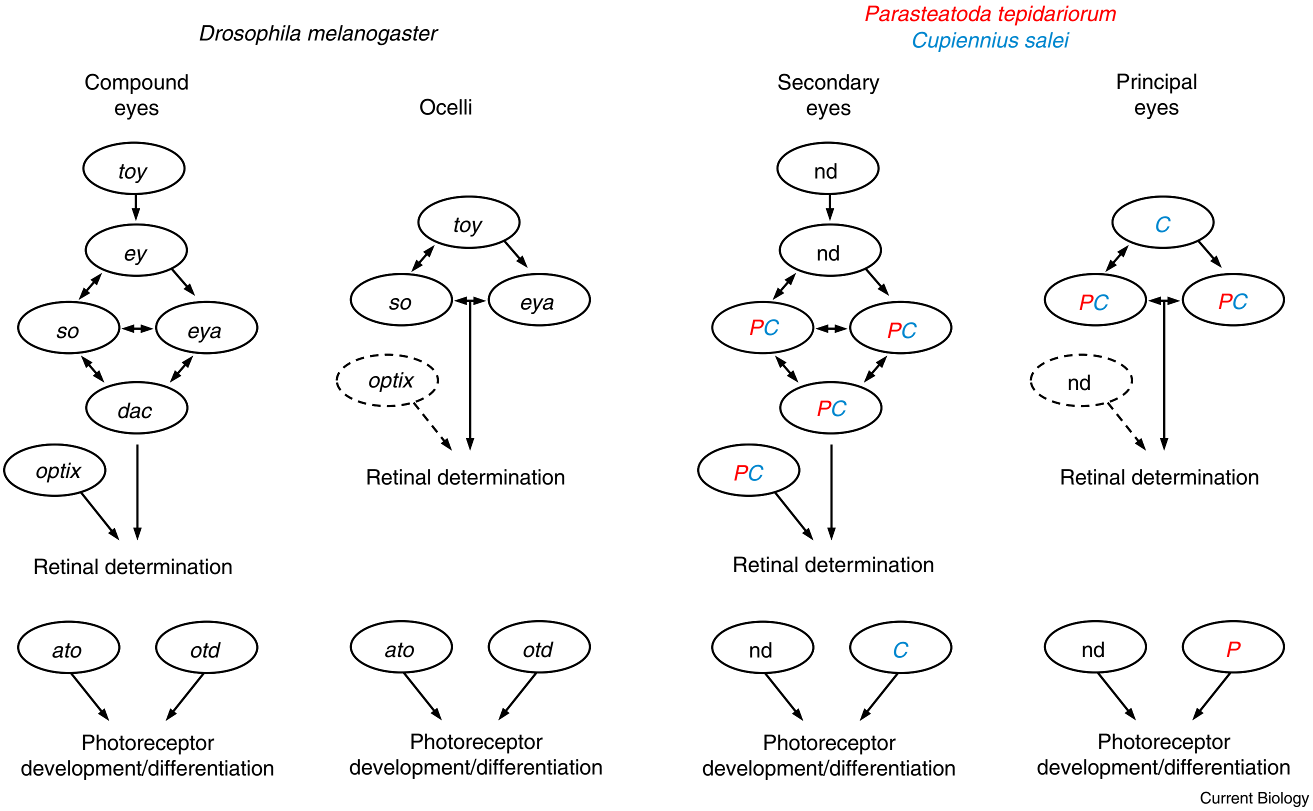

Figure 3. Conservation of gene networks controlling spider eye development.
Comparison of key genes responsible for eye development in *Drosophila melanogaster* and the presence of orthologs identified from gene expression studies in two spider species (*Parasteatoda tepidariorum*, P, and *Cupiennius salei*, C), suggesting deep evolutionary conservation of gene networks for secondary/compound eyes and ocelli/principal eyes. Genes are labeled in their established positions within the gene networks in *Drosophila* on the left, and the detection of spider orthologs indicated in the same position in the network diagrams on the right. These include key members of the retinal determination network such as the *Pax-6* transcription factors *eyeless* (*ey*) and *twin of eyeless* (*toy*), the *Six* gene family member *sine oculis* (*so*) and its transcriptional co-activator *eyes absent* (*eya*), *dachshund* (*dac*), *optix* (*optix*), as well as genes involved in photoreceptor development and differentiation, including the proneural transcription factor *atonal* (*ato*), and *orthodenticle* (*otd*). Genes for which a spider ortholog has yet to be detected in a particular eye type during gene expression studies are labeled 'nd'.

Evolutionary history

These developmental, morphological, and functional differences between principal and secondary eyes hint at a deeper evolutionary divide between these eyes. Indeed, mounting evidence supports the hypothesis that these two eye types hail from distinct evolutionary lineages, the principal eyes deriving from ancestral medial eyes and the secondary eyes from ancient lateral compound eyes. Fossil evidence from extinct arthropods and morphological comparison of extant lineages suggest an ancient visual system bauplan, arising in the Cambrian or even earlier, that included a set of medial eyes and a pair of lateral compound eyes. These distinct eye lineages have been retained in various arthropod groups over time, resulting in familiar examples such as the medial ocelli and lateral compound eyes of insects and horseshoe crabs. Although the secondary eyes of spiders do not superficially resemble compound eyes, the argument is that these eyes represent the dispersed remnants of a modified pair of compound eyes.

One prediction arising from this hypothesis is that the gene regulatory networks underlying the development of these two eye types should also exhibit some deep conservation. Although work on the genetic basis of eye development in spiders lags behind that of other arthropod groups, most notably insects, the sparse evidence that is available hints at such evolutionary conservation (Figure 3). Comparison between the well-characterized gene regulatory networks responsible for *Drosophila* ocelli and compound eye development and emerging transcriptomic work in several spider species reveals spider-specific orthologs for many key players in the retinal determination network of insect eyes. The increasing availability of genetic tools in non-model systems will be essential in the quest to better understand conservation and innovation across arthropod eye lineages.

Diversity in form and function

To dwell only on the commonalities across spider eyes would, however, pay these incredible animals a great disservice. The power that this group offers to understand basic principles of visual ecology lies in their immense diversity. For nearly all of the 'rules' described above, spiders present just as many exceptions. This diversity has evolved hand-in-hand (or rather foot-in-foot) with the myriad ways in which these small creatures make their living, from web-based prey capture to cursorial prey pursuit, and from nocturnal navigation to diurnal courtship displays that would give a bird of paradise pause. The selective pressures arising from this variety have shaped every facet of eye form and function.

Some of this diversity is evident from the external morphology of spider eyes, whether it be the placement of the eyes on the cephalothorax (Figure 1) or the size of the corneal lenses used to focus incoming light. Even these basic alterations have major impacts on how spiders see their world, for example changing their fields of view in response to their ecological needs (Figure 4). Opportunities abound to investigate both the proximate and ultimate causes of eye placement, eye size, and related visual specializations. And while the fields of view of different spider groups remain poorly understood, even less well-characterized are differences in how their visual optics are focused to provide information about the world at depth. Estimates of minimal focusing distance range from less than a body length to dozens of body lengths away, suggesting that different eyes may be tuned to provide information for different tasks based on distance (e.g., initial prey detection versus prey handling during capture).

Perhaps one of the best ways to convey this diversity is to provide a few brief vignettes that highlight the extremes achieved by spider vision. In the ‘Olympics’ of spider vision, jumping spiders take the gold, silver, and bronze for best visual acuity. Jumping spider principal eyes can resolve points in space that are only 0.04–0.1° apart, a range of visual acuity that they share with much larger vertebrates like pigeons, elephants, and lap dogs. This ability is provided by both a densely packed photoreceptor array and a unique set of lens optics, including a large converging lens fixed to the body wall and a smaller diverging pit lens. The latter lies at the base of a long, transparent ‘eye tube’, and serves to magnify the focused image on the retina below. But while this optical innovation provides unrivaled acuity in a small footprint, it has one significant defect: pronounced linear chromatic aberration. In other words, the lens system focuses different wavelengths of light at different depths within the eye. This liability is addressed by the retinal tiering mentioned above such that photoreceptors sensitive to different wavelengths of light are positioned approximately where the photons they are most sensitive to will be in best focus. However, this alignment of course depends on the depth of the object being imaged, an optical arrangement that jumping spiders capitalize on to judge depth via ‘image defocus’, a mechanism of depth perception that relies on comparison of how in focus an image is in neighboring photoreceptor tiers. Combine this with a set of three-to-four distinct spectral classes of photoreceptor and you have a pair of eyes that each individually provide high quality information about pattern, color, and depth of objects in the world around. No other spider, and indeed, no other animal of its size does it better.

Such extremes of visual function, however, rely on ample photons to work, and although many spiders, jumping spiders included, are diurnal predators, quite a few spiders only hunt at night. These nocturnal creatures place a premium on sensitivity, and the kings of low-light spider foraging are hands down the net-casting spiders in the genus *Deinopis*. Deinopids use their enlarged posterior median eyes (Figure 1E) to detect and capture terrestrial insects on the dim forest floor by casting silken nets, retaining this ability even under starlight. Measurements of their specialized eyes indicate low light sensitivity akin to mid-water and some deep sea animals, and an order of magnitude better than our own. This extraordinary sensitivity arises from a combination of oversized rhabdoms and the largest single lenses known from arthropods (1.4 mm in diameter, and with an astounding *F*-number of 0.58). The latter take up so much of their head that they have earned these animals the nickname ‘ogre-faced’ spiders.

In addition to perceptual capabilities familiar from our own visual experience, a number of spiders are also sensitive to the polarization of light in their environments, a property of light which we can’t perceive without technological assistance. For example, responses to polarized light have been reported from lycosids and agelenids, both of which appear to employ their principal eyes in the task. However, the best characterized example of polarization vision comes from the specialized posterior median eyes of the gnaphosid spider *Drassodes cupreus*. This pair of secondary eyes collaborates to form a unified compass organ that takes advantage of sky polarization patterns at dawn and dusk to guide homeward navigation following foraging trips. These highly modified eyes utilize the polarizing properties of their canoe-shaped tapetal reflectors, oriented at right angles to their other eye counterpart, to extract polarization information from the sky. Rather cleverly, the lenses of these eyes have been all but eliminated, removing their image forming capacity, thus simplifying their responses to only polarization-related differences in brightness. Whether other spider groups have evolved similar or entirely different mechanisms to detect polarization remains an area open for study.

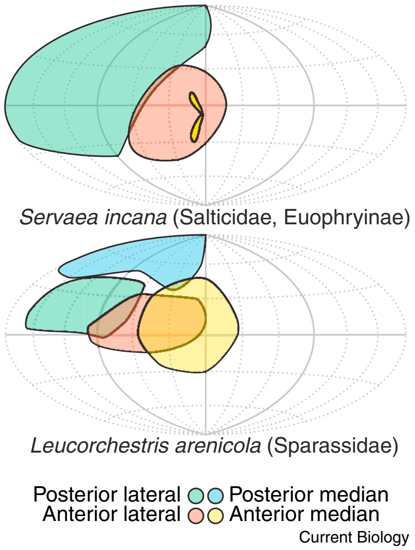

Figure 4. Examples of diversity in the visual fields of spider eyes.
The fields of view of different eye types are color coded (see legend). The jumping spider *Servaea incana* (above) exhibits the characteristic ‘boomerang’ shape of the salticid principal eye retina (in yellow), whose direction of view can be altered with dedicated retinal muscles to rotate torsionally and to travel up to 50° along the vertical and horizontal axes. The fields of view of the posterior median eyes have not been mapped, but face skyward, similar to that represented for the same eye pair in *Leucorchestris arenicola* (below).

These examples only scratch the surface of the diversity present in spider visual systems. With spider species found on every continent except Antarctica, and in every major biome, their ecological diversity rivals that of other more intensively studied groups. And with ‘only’ 48,597 described species, but estimates of global diversity nearly doubling this number, it is likely that many unique visual adaptations remain completely unknown. For those of us captivated by these animals, with their “so many eyes”, whole lifetimes of discovery await.

FURTHER READING

Barth, F.G. (2002). A Spider’s World: Senses and Behavior (Berlin: Springer).
Blest, A.D. (1985). The fine structure of spider photoreceptors in relation to function. In Neurobiology of Arachnids, F.G. Barth, ed. (Berlin: Springer), pp. 79–102.
Dacke, M., Doan, T.A., and O’Carroll, D.C. (2001). Polarized light detection in spiders. J. Exp. Biol. *204*, 2481–2490.
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*, R1092–R1093.
Land, M.F. (1985). The morphology and optics of spider eyes. In Neurobiology of Arachnids, F.G. Barth, ed. (Berlin: Springer), pp. 53–78.
Menda, G., Shamble, P.S., Nitzany, E.I., Golden, J.R., and Hoy, R.R. (2014). Visual perception in the brain of a jumping spider. Curr. Biol. *24*, 2580–2585.
Morehouse, N.I., Buschbeck, E.K., Zurek, D.B., Steck, M., and Porter, M.L. (2017). Molecular evolution of spider vision: new opportunities, familiar players. Biol. Bull. *233,* 21–38.
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.
Paulus, H. (1979). Eye structure and the monophyly of the Arthropoda. In Arthropod Phylogeny, A.P. Gupta, ed. (Vam Nostrand Reinhold: New York), pp. 299–383.
Samadi, L., Schmid, A., and Eriksson, B.J. (2015). Differential expression of retinal determination genes in the principal and secondary eyes of *Cupiennius salei* Keyserling (1877). EvoDevo *6*, 16.
Schomburg, C., Turetzek, N., Schacht, M.I., Schneider, J., Kirfel, P., Prpic, N.M., and Posnien, N. (2015). Molecular characterization and embryonic origin of the eyes in the common house spider *Parasteatoda tepidariorum*. EvoDevo *6*, 15.
Steinhoff, P.O.M., Uhl, G., Harzsch, S., and Sombke, A. (2020). Visual pathways in the brain of the jumping spider *Marpissa muscosa*. J. Comp. Neurol. *528*,1883–1902.
Yamashita, S. (1985). Photoreceptor cells in the spider eye: spectral sensitivity and efferent control. In Neurobiology of Arachnids, F.G. Barth, ed. (Berlin: Springer), pp. 103–117.
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 colorful jumping spiders. Curr. Biol. *25*, R403–R404.

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Increased rate of close-kin unions in the central Andes in the half millennium before European contact

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Spanish colonial sources describe how some groups in the central Andes practiced a unique system of social organization based on ancestry, whereby within-group unions were preferred to facilitate sharing of resources beyond the nuclear family. However, these sources do not quantify the prevalence or origin in time of this ‘*ayllu*’ system. Here, we provide new evidence by analyzing genome-wide data from 46 ancient Andean individuals for close kin unions. We detect a substantial increase in the rate of close-kin unions from 9% to 46% after ~1000 CE. This occurred after the decline of Wari and Tiwanaku cultures and at the start of an era of small-scale polities known as the ‘Late Intermediate Period’. Thus, the mating preferences instantiated in *ayllu* were widespread in Andean society and developed centuries before the expansions of the Inca state in the 15th century.

If a person harbors long stretches of DNA lacking variation between the two copies of the genome they inherited from their parents, so called ‘runs of homozygosity’ (ROH), the only plausible explanation is that their parents are closely related, a signal that can be detected with genome-wide DNA sequencing. Applying a method that can use low coverage ancient DNA to make such measurements [1], we analyzed 46 ancient individuals from the Central Andes [2–4]. We detected the presence of long ROH at the level typical for offspring of first or second cousins in 13 of 46 Central Andes individuals (Figure 1A; Data S1A). The rate increased from before 1000 CE, where we observe it in two of 22 individuals (95% binomial confidence interval: 1.1–29.2%), to afterward when it occurred in 11 out of 24 (25.6–67.2%, p = 0.0083; two-sided Fisher’s exact test). The rate is lower in present-day Andeans (Figure 1B; Data S1B; [S1–S7]): we detect long ROH in two of 86 (0.28–8.2%) Peruvians from Lima, and in 11 of 56 (10.2–32.4%) diverse other Andeans, with the latter signal largely driven by Aymara speakers from the Ventilla region of Bolivia [3] where we observe long ROH in 6 of 18 individuals. Without ancient DNA data from intermediate periods, however, we can not discern whether there was a continuously high rate of close kin unions in this region over the last 500 years.

We considered the possibility that the increased rate of close-kin unions in the five centuries before European contact could be an artifact of uneven sampling. However, the instances of consanguinity are widespread, occurring in 8 out of 11 Late Intermediate Period and Late Horizon sites (1–4 individuals each), and four large regions (Figure 1B). No close relatives were detected within the sample analyzed here [2], showing that the signal is not influenced by clusters of close kin. The signal is also not driven by urban elites: the individuals we analyzed were almost entirely rural (35 of the 37 individuals for which there is an archaeological assignment [2], and were largely commoners (as only three individuals from a single site are archaeologically assigned as elites; Data S1). Close-kin unions were known in the highest strata of Inca society, but our results could not be predicted by this as mating practices are often very different across social strata [5] and our signal dates to centuries before the Inca.

The onset of the period of increased close kin unions coincides with the decline of two major Middle Horizon societies (the Wari and Tiwanaku; ~700–1050 CE) that covered most of the Central Andes, and the beginning of the Late Intermediate Period (~1050–1440 CE) when there was a transition to smaller scale polities. It was only by the Late Horizon (~1440–1534 CE) that large-scale states arose again with the Inca who spread over large parts of western South America [6,7]. Our findings are notable in light of the *ayllu* social units described by the Spanish, whereby groups defined themselves at least in part through shared ancestry and preferred within-group marriages to keep resources within the community and to facilitate cooperation beyond the nuclear family.