Chunk Versus Point Sampling: Visual Imaging in a Small Insect

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The eyes of strepsipteran insects are very unusual among living insects. In their anatomical organization they may form a modern counterpart to the structural plan proposed for the eyes of some trilobites. Externally they differ from the usual "insect plan" by presenting far fewer but much larger lenses. Beneath each lens is its own independent retina. Anatomical and optical measurements indicate that each of these units is image-forming, so that the visual field is subdivided into and represented by "chunks," unlike the conventional insect compound eye that decomposes the visual image in a pointwise manner. This results in profound changes in the neural centers for vision and implies major evolutionary changes.

Xenos peckii is a strepsipteran insect and an endoparasite of paper wasps. The males burrow out of their host, fly away, and in their few hours of life must find a mate. The females remain within the wasp's body, where mating occurs. The faceted eye of a male *X. peckii* appears as a cluster of large convex lenses, giving it a raspberry-like appearance (Fig. 1A). In previous, primarily morphological studies of the strepsipteran eye (*1*) the unusual design has been noted, but the mode of function and the underlying neuronal substrate have not been investigated.

In *X. peckii* (*2*) there are about 50 lenses, an order of magnitude fewer than the more than 700 facets in another small but representative insect, *Drosophila melanogaster*. Moreover, a typical lens in *X. peckii* is about 65 μm in diameter and covers about the same area as do 15 lenses of *D. melanogaster* (Fig. 1B). The large lenses in *X. peckii* are separated from each other by rows of prominent brush-like microtrichia (Fig. 1C; Fig. 2, A and C), which may restrict the entrance of off-axis light. Beneath each lens lies its own retina, containing more than 100 photoreceptors (*3*) surrounded by a pigmented cup (Fig. 2, A and C), which we believe defines a self-contained functional unit capable of processing the portion of the visual field projected onto the retina by its overlying lens. This organization differs from that of conventional compound eyes, where each optic unit (facet) has 8 to 10 photoreceptors (*4*) and contributes to only one sample point. To distinguish the optical units of *X. peckii* from those in conventional compound eyes, we refer to them as eyelets.

One implication of the strepsipteran organization is that each eyelet should be capable of bringing an object in the visual field into sharp focus (Fig. 3, A and B). Our optical measurements (*5*) of the plane of focus of the inverted image indeed corresponded well to histological measurements of the distance between retina and lens (12 to 13 μm). Because of its large size, the eyelet does not operate at the diffraction limit; the spatial cutoff frequency would allow a resolution of several thousand sample points.

In compound eyes, the light-gathering power of individual lenses can be characterized by the product of the facet diameter D and the interommatidial angle $\Delta\Phi$ (*6*). In diurnal insects $D\Delta\Phi$ rarely exceeds 1 μm (*7, 8*). If the eye of *X. peckii* were a compound eye, its $D\Delta\Phi$ of 31 μm (*9*) would far exceed the light requirement for diurnal insects. Because it is unusual that such a high $D\Delta\Phi$ value is found in a compound eye, a similar calculation has been used for schizochroal trilobites (with $D\Delta\Phi$ values ranging from 70 to 130 μm) to suggest that they also had an eyelet-like organization (*10*). In addition, it is possible to calculate the absolute sensitivity, S (*8*), which indicates whether sufficient light is captured for individual receptors to resolve image points. We calculated for *X. peckii* that $S = 0.22$ (*11*), which indeed is consistent with an image-forming eye, at least for an insect that (like *X. peckii*) is active in bright light (*12*).

The consequences of the eyelet organization in *X. peckii* give rise to differences in the neuroanatomical organization of the visual system beneath the compound eyes. One difference is the presence of multiple additional optic chiasmata in the Strepsiptera. The projections of the receptor cells from each eyelet form a nerve that terminates in the lamina (Fig. 2A) and twists around its axis (Fig. 2B), so that the spatial representation of each retina onto the lamina is rotated about 180°. This organization was demonstrated by anterograde staining of the receptor axons with a fluorescent dye (*13*) (Fig. 2, D and E). The relationship between two adjacent eyelets was revealed in a neighboring pair that had been stained and photographed using confocal microscopy (Fig. 2E), and independently by examination of osmium-stained tissue. In both cases, it is seen that bundles of receptor terminals from a pair of neighboring eyelets project into adjacent areas. This reinforces our supposition that eyelets are independent visual units that process neighboring regions of visual space.

One of the most striking neuroanatomical features of insect visual systems is the occurrence of linear arrays of "optic cartridges" that stand out with lattice-like regularity throughout the optic ganglia (*14*). In *X. peckii*, there is a conspicuous absence of anything like optic cartridges, at least at the level of the lamina, where many projections run obliquely (Fig. 2, D and E). Surprisingly, the medulla also lacks clear periodicity (Fig. 2F). Nevertheless, the size and fiber density of the medulla and lobula indicate the importance of vision for Strepsiptera: The optic neuropils constitute about 75% (*15*) of the entire brain and are constructed of a dense meshwork of fibers. It seems that a large amount of optic processing is taking place at these levels, such as would be necessary if each eyelet contributes more than one image point. For the processing of roughly 50 image points on each side of the head, less elaborate and smaller optic neuropils would surely be expected.

On the basis of these anatomical data, we propose a model for visual processing in the eye of *X. peckii* (Fig. 4B) that is substantially different from conventional compound eyes (Fig. 4A). In Strepsiptera each eyelet forms a

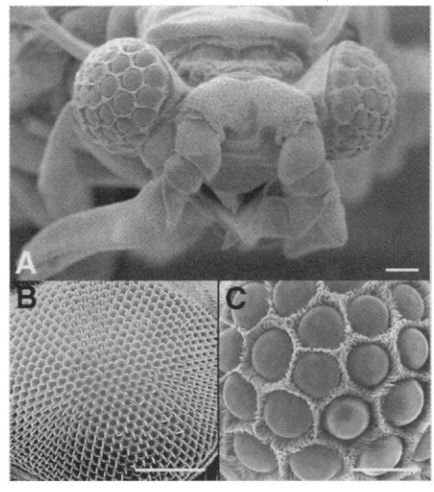

Fig. 1. (**A**) Head of a male *X. peckii*. Note the relatively few but large lenses in each eye. (**B** and **C**) Comparison of lens size for (B) the fly *D. melanogaster* and (C) the strepsipteran *X. peckii*. The lenses of *X. peckii* are noticeably larger (65 ± 4 μm) and well separated. Each lens is fringed by brush-like microtrichia. Scale bars, 100 μm.

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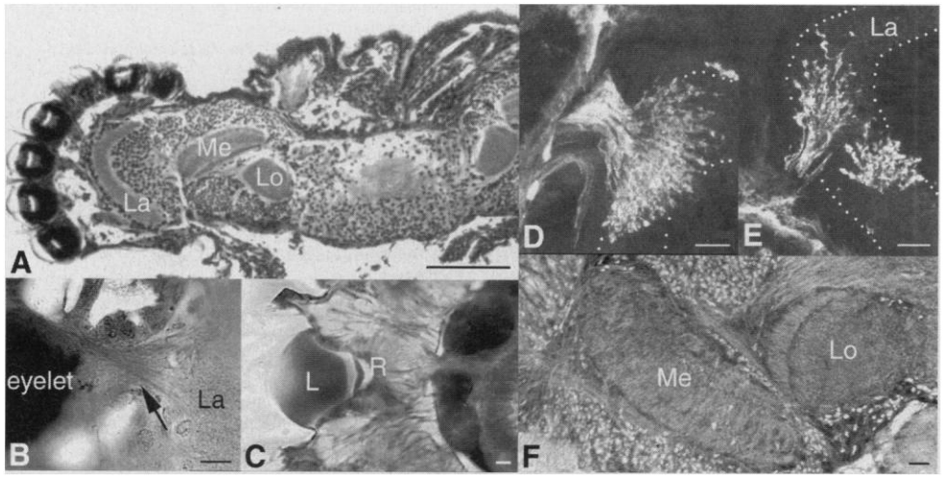

Fig. 2. (**A**) Histological frontal section through the head of *X. peckii*. Individual eyelets are evident (left). The deeper optic neuropils (La, lamina; Me, medulla; Lo, lobula) have a similar shape to those in other insects. Each eyelet is connected to the lamina through a chiasm. (**B**) A close-up of an optic chiasm (arrow). (**C**) Ethyl gallate–stained section of one optic unit of the strepsipteran eye. The thick lens (L) and underlying cup-shaped retina (R) are 12.3 ± 2.1 μm apart. Dextran-fluorescein applied to the retina fills bundles of receptor cells that terminate in the lamina. (**D**) Staining from projections of one optic unit. Note the presence of oblique projection within the lamina (area outlined in dots). (**E**) Fill of two neighboring optic units. As the projection area of one eyelet is not circular within the lamina, these projections appear less wide than in (D), but more arborizations are found in adjacent planes of sections. No overlap is seen between the two projections. (**F**) Fluorescent staining of the medulla and lobula, illustrating their dense fiber architecture and large extent. Scale bars, 100 μm (A), 10 μm (B to F).

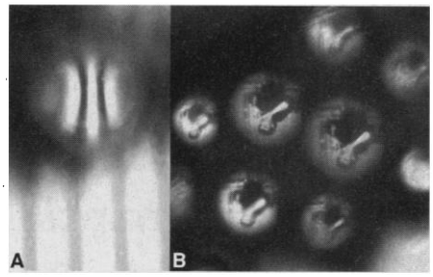

Fig. 3. Optical properties of the lens of *X. peckii*. (**A**) The image of a grating, which illustrates the optical quality of the lens, is in focus 12.4 ± 1.5 μm behind the lens. The central lens region yields a sharp image, whereas the peripheral portion is focused on a different plane. Such a curvature of the image may correspond to the curvature of the retina. (**B**) View of an image through a number of lenses to illustrate the quality of the lens in terms of spatial resolution. Although the curvature of the corneas of the individual lenses was preserved, the eye was flattened during the dissection. This and the removal of screening pigment results in a larger overlap of the fields of view than is present in the insect.

partial image, which has been inverted by the lens, upon its associated retina. To reconstruct the entire image in register at the level of the lamina (as is the case in other insects), the eye must re-invert each neurally encoded image so that points adjacent in optical space are adjacent in their neural representation. This re-inversion is accomplished by the crossing of receptor fibers in the chiasmata behind each of the eyelets (Figs. 2B and 4B). The advantage of such a composite-lens eye could lie in a combined high light-gathering ability and image resolution that otherwise would be difficult to achieve in small insects (*16*). Each strepsipteran lens potentially resolves many more points in visual space than do the 15 facets that cover the same surface area, for example, in *D. melanogaster*.

There are other arthropods that have single-chambered, image-forming eyes (*8, 17*), but in contrast to Strepsiptera, they never have more than a few lenses and each lens generally forms a unit with its own neuropil (*18, 19*). The eyes of *X. peckii* are different because many eyelets are integrated into a single set of optic neuropils. In the periphery there is some similarity between the morphology of a single eyelet in *X. peckii* and, for example, the entire simple eyes of spiders (*20*) or insect larvae (*21*). In its fine structure, the strepsipteran rhabdom forms a dense meshwork that resembles, at least superficial-

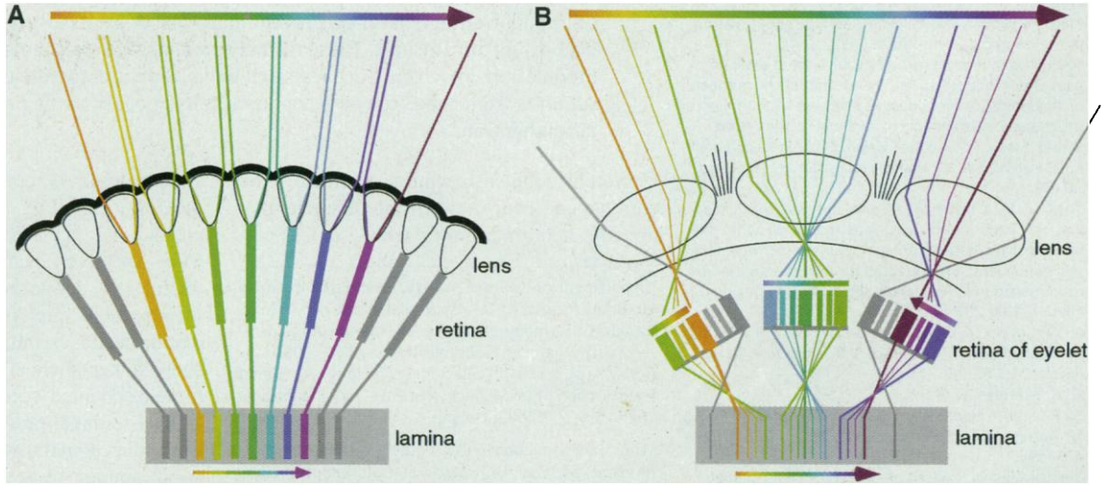

Fig. 4. Schematic comparison of an apposition-type insect eye and a proposed model for the function of the eye of *X. peckii*. Color is used to depict the representation of an image and does not imply functional chromatic differences. (**A**) In the apposition eye (a common eye type in insects), each optic unit represents only one sample point. Neighboring points of an image (arrow) are represented next to each other, at the level of the retina as well as of the lamina. [Modified after (*4*).] (**B**) In the Strepsiptera, the image is viewed through many eyelets. The model assumes the most simple case of no overlap between the visual fields of neighboring units. Because each lens is image-reversing and only captures a partial image, the coherence of the entire image is lost at the level of the retinae, but is restored by chiasmata between retinae and lamina.

ly, that of the highly visual tiger beetle larvae (*18*). However, in the strepsipteran eye no screening pigment appears to be present (*22*). The effective image resolution within each eyelet of *X. peckii* therefore depends not only on the number of photoreceptors but also on the extent of optical pooling, which remains to be further investigated. The image resolution would also be influenced by the degree of overlap between the visual fields of neighboring eyelets. The acceptance angle of an individual eyelet can be estimated if the focal length of the lens is known. On the basis of measurements of the image magnification (*23*), a focal length of 44 ± 5 μm (n = 21) and an acceptance angle of 33° ± 6° (n = 10) was calculated. Thus, the values for the acceptance angle are, if at all, only slightly greater than those of the inter-eyelet angle of 27° ± 6°. Our model (Fig. 4) assumes no overlap, but a small amount of overlap is conceivable and could be consistent with the unusual absence of clearly definable cartridges of the medulla.

For more than a century the arthropod eye has been extensively studied in structure and function, and many common features are conserved throughout this group. Although the detailed modes of function of arthropod eyes vary considerably (*8*), it is remarkable how profoundly the structural features of the eye of Strepsiptera have changed. The course of its evolution is unclear, but it is certainly noteworthy; after all, its organization may be a living counterpart to the eyes of some of the long-extinct trilobites.

References and Notes

1. R. K. Kinzelbach, in *Handbuch der Zoologie IV/2/2*, J. G. Helmcke, D. Starck, H. Wermuth, Eds. (de Gruyter, Berlin, ed. 2, 1971), pp. 1–68; K. Strohm, *Zool. Anz.* **36**, 156 (1910); P. Rösch, *Zeitschr. Naturwiss.* **50**, 97 (1913); H. F. Paulus, in *Arthropod Phylogeny*, A. P. Gupta, Ed. (Van Nostrand Reinhold, New York, 1979), pp. 299–383; H. R. MacCarthy, *J. Entomol. Soc. Brit. Columbia* **88**, 27 (1991).
2. Male *X. peckii* were collected as late pupae within their wasp hosts near Ithaca, NY.
3. The heads of late pupae and adults were stained with osmium or reduced silver. We estimated the number of rhabdoms (or functional receptor units) from light microscopy examination of sections of the retina.
4. D.-E. Nilsson, in *Facets of Vision*, D. G. Stavenga and R. C. Hardie, Eds. (Springer-Verlag, Berlin, 1989), pp. 30–73.
5. The back focal distance (distance between focal plane and the back of the lens) was measured with the hanging drop method, using a cover slip–corrected oil immersion lens, and corrected for the refractive index of immersion oil. For details see M. Wilson, *J. Comp. Physiol.* **124**, 297 (1978).
6. A. W. Snyder, *J. Comp. Physiol. A* **116**, 161 (1977); A. W. Snyder, D. G. Stavenga, S. B. Laughlin, *J. Comp. Physiol. A* **116**, 183 (1977).
7. G. A. Horridge, *Endeavour* **1**, 7 (1977).
8. M. F. Land, in *Handbook of Sensory Physiology VII/6B*, H. Autrum, Ed. (Springer-Verlag, Berlin, 1981), pp. 471–592.
9. The calculation is based on an average lens diameter of 65 μm and average inter-eyelet angle of 27°. Because there are on average 11 receptors across the diameter of the retina, the value for each receptor would be 2.8 (31 μm/11 receptors), a value that is comparable to $D\Delta\Phi$ values of conventional compound eyes.
10. D. Fordyce and T. W. Cronin, *Paleobiology* **19**, 288 (1993).
11. The sensitivity [from (*8*)] was calculated as $S = (\pi/4)^2(A/f)^2d^2[1 - \exp(-kx)]$, where A is aperture (65 μm, measured), f is focal length (44 μm, calculated), d is receptor diameter (1.5 μm, estimated), x is receptor length (11 μm, measured), and k is the absorption coefficient. We used for k [like (*8*); see all invertebrates in table 5] the estimate of 0.0067 μm^{-1} based on measurements by M. S. Bruno, S. N. Barnes, T. H. Goldsmith, *J. Comp Physiol. A* **120**, 123 (1977).
12. J. Meixner, in *Handbuch der Zoologie IV/2/2*, T. Krumbach, Ed. (de Gruyter, Berlin, ed. 1, 1936), pp. 1349–1382.
13. Retinal receptor terminals were stained by applying dextran molecules conjugated to fluorescein (molecular weight 3000, lysine-fixable; Molecular Probes) to individual eyelets and were viewed with a Bio-Rad 600 confocal microscope.
14. S. R. Cajal and D. Sánchez, *Trab. Lab. Invest. Biol. Univ. Madrid* **13**, 1 (1915).
15. Volumes were estimated by serial reconstruction. Outlines of neuropils were scanned in, measured in Adobe Photoshop, multiplied by section thickness, and the relative proportion of the summed volumes was calculated.
16. If each photoreceptor of *X. peckii* required its own lens, then the diameter of each lens would only be 6 μm (65 μm/11 receptors), resulting in a $D\Delta\Phi$ value of 0.24, and therefore diffraction would be limiting (*8*). A single-lens eye with a wide field of view, however, requires a very short focal length for a small insect and thus a very thick lens, which usually has pronounced spherical and chromatic aberrations. Thus, pooling the information of a number of simple-lens eyes with a restricted field of view ("chunk sampling") may be a good compromise.
17. E. J. Warrant and P. D. McIntyre, *Prog. Neurobiol.* **40**, 413 (1993).
18. Y. Toh and A. Mizutani, *Cell Tissue Res.* **278**, 125 (1994).
19. P. Duelli, *Cell Tissue Res.* **187**, 417 (1994); N. J. Strausfeld and F. G. Barth, *J. Comp. Neurol.* **328**, 43 (1993); N. J. Strausfeld, P. Weltzien, F. G. Barth, *J. Comp. Neurol.* **328**, 63 (1993).
20. M. F. Land, *J. Exp. Biol.* **51**, 443 (1969).
21. C. Gilbert, *Annu. Rev. Entomol.* **39**, 23 (1994).
22. E. Wachmann, *Z. Zellforsch.* **123**, 411 (1972).
23. The setup for image magnification measurements was as described in (*5*). The focal length f was calculated using the lens formula $O/U = I/f$, where O is object size, U is object distance, and I is image size. The acceptance angle is determined by f and the extension of the retina behind the lens.

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Calmodulin Dependence of Presynaptic Metabotropic Glutamate Receptor Signaling

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Glutamatergic neurotransmission is controlled by presynaptic metabotropic glutamate receptors (mGluRs). A subdomain in the intracellular carboxyl-terminal tail of group III mGluRs binds calmodulin and heterotrimeric guanosine triphosphate–binding protein (G protein) βγ subunits in a mutually exclusive manner. Mutations interfering with calmodulin binding and calmodulin antagonists inhibit G protein–mediated modulation of ionic currents by mGluR 7. Calmodulin antagonists also prevent inhibition of excitatory neurotransmission via presynaptic mGluRs. These results reveal a novel mechanism of presynaptic modulation in which Ca^{2+}-calmodulin is required to release G protein βγ subunits from the C-tail of group III mGluRs in order to mediate glutamatergic autoinhibition.

G protein–coupled receptors modulate ionic currents and exocytotic fusion reactions that underlie neurotransmitter release (*1*). Glutamate release is controlled by presynaptic mGluRs inhibiting voltage-activated Ca^{2+} channels (*2, 3*) via G protein βγ subunits (*4*). The selective localization of group III mGluRs at active zones is consistent with their predominant role as autoreceptors mediating feedback inhibition (*5*). The mGluRs show a heptahelical structure typical of G protein–coupled receptors (*2, 6*), and their COOH-terminal tails represent the major intracellular domain, which exhibits high variation among receptor subclasses (*2*). We chose this region to investigate interacting proteins that might contribute to the functional diversity of mGluRs.

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References and Notes

[10] **Trilobite Vision: A Comparison of Schizochroal and Holochroal Eyes with the Compound Eyes of Modern Arthropods**
David Fordyce; Thomas W. Cronin
Paleobiology, Vol. 19, No. 3. (Summer, 1993), pp. 288-303.
Stable URL:
http://links.jstor.org/sici?sici=0094-8373%28199322%2919%3A3%3C288%3ATVACOS%3E2.0.CO%3B2-M