

*The Julius Silver, Roslyn S. Silver, and Enid Silver Winslow*

**DIALOGUES IN ARTS AND SCIENCE**

**COLOR AND POLARIZED  
LIGHT VISION: THE  
*DROSOPHILA* RETINAL  
MOSAIC**

*Claude Desplan*

*Silver Professor of Biology and Neural Science*



NEW YORK UNIVERSITY

## ***Color and Polarized Light Vision: The Drosophila Retinal mosaic***

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Although the types of eyes found across the animal kingdom vary extensively in general organization and complexity, all visual systems use similar mechanisms to respond to environmental cues. In particular, all animals use related opsin photopigment proteins in their photoreceptor cells to capture photons (for review, see Arendt & Wittbrodt 2001).

The visual system serves several major purposes. At the origin, it likely served as a light detector for photophobic or photophilic behaviors. It then rapidly acquired an additional function as a light detector for entraining the circadian clock. These uses did not require complex optics or sophisticated neural processing, and in fact some of these functions were performed by using non opsin photodetectors. The most important use for the sophisticated eyes of many higher vertebrates (from fish to mammals) and many invertebrates (from the camera-like eye of squids to the compound eye of arthropods), is a dual role in image formation/motion detection but also in the detection of the quality of light, the wavelengths (color) or vector of skylight polarization for navigation. This requires the ability to form images with complex optics (image formation), the specialization of photoreceptors to detect particular types of light (color or polarization detection), as well as the building of a sophisticated processing

machinery in the central nervous system. The different subclasses of photoreceptors exhibit important morphological and molecular differences as well as characteristic distribution patterns through the retina in order to maximize the amount of information extracted from the environment. Development of the retina and correlative connections to the optic lobes must be exquisitely regulated to achieve the impressive mosaic of photoreceptors observed in many retinas.

Patterning of the retina involves a series of highly coordinated and organized processes. Many of the factors involved in the formation of the *Drosophila* eye have now been identified, and interestingly, similar patterning events and genetic regulations often occur in the development of the vertebrate retina. This further implies that the vertebrate single lens eye and the insect compound eye use similar strategies to achieve their function. Rather than reflecting common ancestry of the visual systems (which clearly evolved independently in vertebrates and invertebrates), this must represent convergent mechanisms used to control opsin expression in different photoreceptor subtypes and may provide insight into understanding how the complexity of the retina is created and maintained in different systems.

### ***1. Retinal Mosaics In Humans And Flies***

Humans use rod photoreceptors (“rods”) for detecting objects under lowlight conditions, while cone photoreceptors (“cones”) contribute to color discrimination as well as to high acuity vision. Color vision requires the compar-

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ison of the output of at least two photoreceptors that can detect different wavelengths (colors) of light. To serve these purposes most efficiently, the human retina contains blue, red and green cones that compute their input through “triangulation” of the wavelength of light. These three types of cones are highly concentrated in the center of the retina, the fovea. Interestingly, their distribution appears to be stochastic, resulting in a cone mosaic that can be visualized in vivo (Fig 1A). As the photodetector structure of cones is of small diameter and the cells are very densely packed, this allows the fovea to serve as the color and high acuity center for the eye under bright light conditions. Rods, on the other hand, are concentrated towards the periphery of the eye which specializes in shape and motion vision under low light conditions.

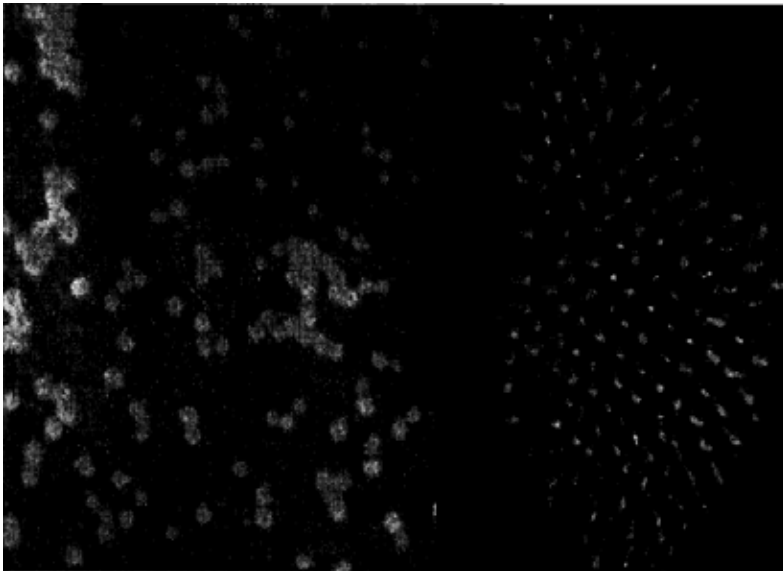


Figure 1: Retinal mosaics in humans and flies. (A) Pseudocolor image of the trichromatic cone mosaic from a living human retina with blue, green and red cones. (B) Visualization of the ommatidial mosaic in R8 photoreceptors of *Drosophila*: staining of blue and green opsin.

Even species as distantly related to humans as flies share important similarities in the organization of their retina. For instance, specialized groups of fly photoreceptors (called inner photoreceptors) sensitive to green, blue and ultraviolet light (UV) are used to discriminate between colors in analogy to cones), whereas other photoreceptors (called outer photoreceptors) have been optimized for the detection of shapes and for motion detection (in analogy to rods). As in the human retina, the different fly photoreceptors also exhibit specific distribution (Fig 1B). For instance, despite the dramatic differences in retinal organization, both fly and human color photoreceptor subtypes show a similar random distribution throughout the retina in flies, or in the fovea in humans. Additionally, another group of fly photoreceptors is only found in the dorsal part of the retina, thereby forming a specialized eye region. The retinal mosaic of *Drosophila* therefore represents an attractive model system to study both stochastic and localized specification events occurring during retinal patterning (for review, see Wernet & Desplan 2004).

## ***2. The Drosophila Compound Eye***

The *Drosophila* compound eye consists of ~800 stereotypical unit eyes (ommatidia), each containing 8 light-sensing photoreceptors (called R1-8) as well as accessory cells involved in forming the lens or in shielding photoreceptors from light coming from other ommatidia (Fig 2A, B and C; for review, see Hardie 1985). According to their morphology, projections to the brain and opsin

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expression, the fly photoreceptors of the adult ommatidium can be grouped into two functional categories: The “outer photoreceptors” (R1-R6) are the fly equivalent of the vertebrate rods and are involved in motion detection and image formation (Fig 3A). Computation of their outputs begins in the first optic lobe of the fly, the lamina (L), where the outer photoreceptors project their axons.

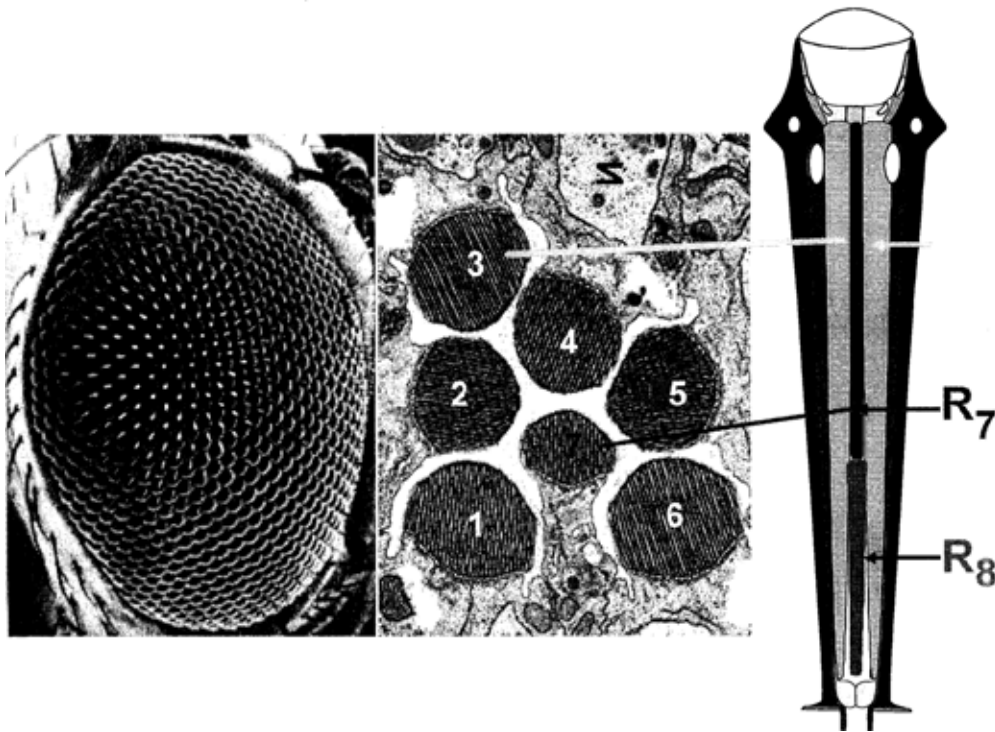


Figure 2: The *Drosophila* compound eye.

(A) Scanning electron micrograph of an adult *Drosophila* eye, composed of ~800 unit eyes (ommatidia).

(B) Cross section through an adult *Drosophila* ommatidium. The light gathering structures (rhabdomeres) of seven photoreceptors are visible (R8 is below focal plane). The Rhabdomere diameter is larger for outer photoreceptors (R1-R6) which are aligned as a chiral trapezoid. R7 has a smaller rhabdomere diameter and is located in the center of the trapezoid.

(C) Schematic representation of an adult ommatidium. In the center of the ommatidium, R7 (blue) is located distally on top of R8 (green) in the same path of light. Outer photoreceptors (grey) span the entire retina from the apical to the basal side. Pigment cells (red) shield the ommatidium from light received by neighboring ommatidia, while cone cells (yellow) secrete the lens.

The light gathering structures of these outer photoreceptors (rhabdomeres) are organized in a trapezoid whose center is occupied by the second type of photoreceptors, the two inner photoreceptors R7 and R8. The rhabdomere diameter of these photoreceptors is significantly smaller than those of outer photoreceptors and they span only half of the retina, with the R7 rhabdomere located distally on top of that of R8, just below the lens: they are therefore in the same path of light, providing the ideal configuration to compare their outputs. This is absolutely required for the two functions of inner photoreceptors, color vision and detection of the vector of polarized light. Inner photoreceptors project to the second optic lobe, the medulla (M), where the neuronal processing for both color and polarized light vision begins (for review, see Meinertzhagen 1993; Meinertzhagen & Hanson 1993; Morante & Desplan 2004). The outer photoreceptors are molecularly and morphologically identical in all ommatidia. They capture photons with high efficiency, due to the expression of their broad spectrum rhodopsin Rh1, as well as the large diameter of their rhabdomeres which extend from the basal to the apical side of the retina (Zuker et al. 1985) and can therefore function in dim light.

Different techniques allow the characterization of the adult *Drosophila* visual system. Cloning of the *rh1* gene allowed the visualization of outer photoreceptors with antibodies generated against the Rh1 protein. Autofluorescence of Rh1 in outer photoreceptors can also be used (Pichaud & Desplan 2001).

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Visualization of the ommatidal mosaic was revolutionized by the development of multi-colored fluorescent antibodies and Green Fluorescent Protein reporter (GFP) that allows visualization of live preparations. In particular, projections of the outer photoreceptor axons to the lamina can be visualized by fusing the rh1 promoter to a reporter gene (e.g. GFP) that can diffuse down the photoreceptor axons (Sheng et al. 1997).

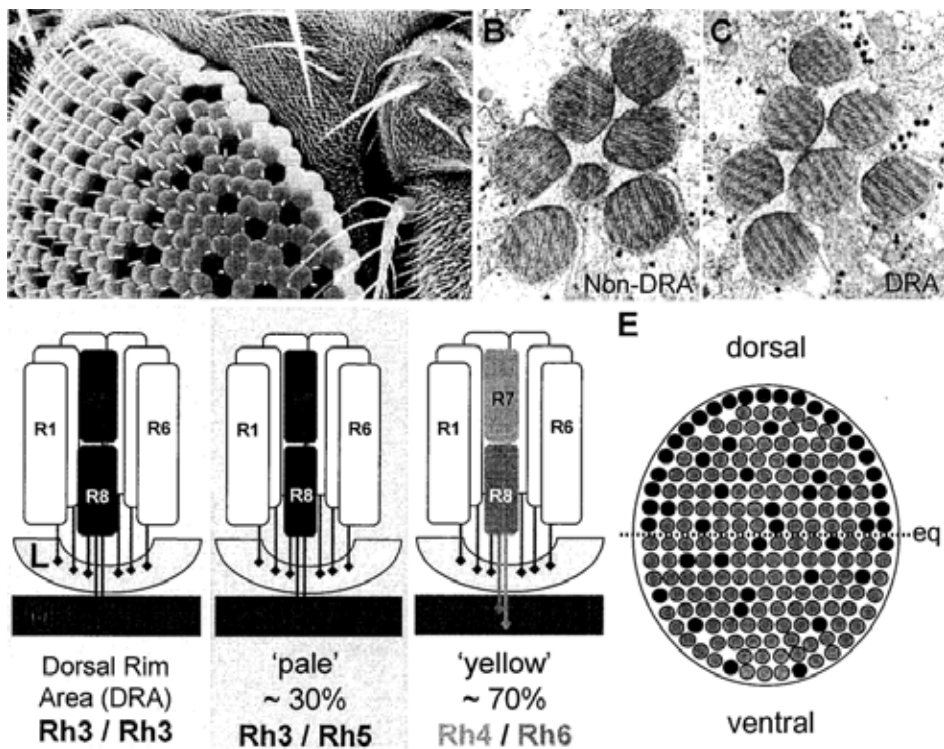


Figure 3

- (A) Scanning electron micrograph pseudo-colored for the different types of ommatidia, DRA (pink), pale (blue) or yellow.
- (B-C) Electron micrograph of a color (B) or polarized light ommatidium (C).
- (D) Expression of various rhodopsin genes in the three types of ommatidia.
- (E) Distribution of polarized and color ommatidia.



### ***3. The Dorsal Rim Area: Polarization Detectors***

Although the general external morphology of the fly eye does not indicate heterogeneity among ommatidia, three ommatidial subtypes have been described in *Drosophila* (Fig 3A). In all three cases, the inner photoreceptors exhibit distinct molecular and sometimes morphological features. A subset of ommatidia always found as one row at the dorsal rim of the fly eye, called the “dorsal rim area” (DRA, shown in pink in Fig 3A) (Wada 1971, 1974; Hardie 1984) exhibits an enlarged rhabdomere diameter as well as highly regular rhabdomeric membrane microvilli, making them strongly polarization sensitive (Fig 3B, C).

The rhodopsin photopigment expressed in the various types of photoreceptors are specialized in their specific tasks. Inner photoreceptors in DRA ommatidia are monochromatic as they express the Rh3 opsin in both R7 and R8 (Fig 3D left) (Fryxell & Meyerowitz 1987, 1991; Fortini & Rubin 1990, 1991). As R7 and R8 express the same opsin, they cannot compare their output with regard to wavelength; however, the rhabdomeric membranes in R7 and R8 form two orthogonal polarizing filters: the comparison of the light detected by these two polarizers allows the fly to precisely assess the vector of light polarization, and therefore the position of the sun, even when it is hidden behind clouds (Wolf et al. 1980; for review, see Labhart & Meyer 1999; 2002). By computing this information with the time of the day indicated by the central clock, the fly knows exactly the orientation of its flight pattern. As the polarized sunlight reflected by the sky is very rich in UV, this explains the utilization of UV-sensitive Rh3 as

well as the dorsal position of the DRA. It must be noted that it is important that R7 and R8 express the same opsin since the comparison between the two cells could be confused if they were sensitive not only to different vectors of light polarization but also to different wavelengths.

#### ***4. The Drosophila Retinal Mosaic: Color Discrimination***

The remaining ommatidial subtypes have first been characterized in elegant studies by Franceschini and Kirschfeld (Franceschini et al. 1981) who identified two separate classes of ommatidia interspersed randomly: inner photoreceptors appeared either *pale* (*p*) or *yellow* (*y*), with 30% being *p* and the remaining 70% being *y* (Fig. 3E). The *p* ommatidia always contain the UV-sensitive photopigment Rh3 in R7 associated with the blue-sensitive Rh5 in R8 (Fig. 3, middle). A different UV-sensitive Rh4, is found in the R7 of *y* ommatidia, which always contain the green-sensitive Rh6 in R8 (Chou et al. 1996; Huber et al. 1997; Papatsenko et al. 1997) (Fig. 3D, right). Therefore, the four rhodopsins are always coupled between R7 and R8, leading to the formation of only two ommatidial subtypes (*p* and *y*) in the main part of the retina (for review, see (Cook & Desplan 2001). The differences in opsin expression play a crucial role for the fly's ability to discriminate between colors, with the *p* ommatidia better discriminating among shorter wavelengths (UV to blue) while the *y* ommatidia are specialized in the perception of longer wavelengths, reaching into the green part of the spectrum. Interestingly, the human cones, which express blue-,

red- or green-specific opsins are also distributed stochastically in the fovea, but there is no tight coupling of the opsin expression between different cells (for review, see Nathans 1999). Taken together, the retinal mosaic of *Drosophila* is composed of three ommatidial subtypes, which are found either localized (DRA) or randomly distributed (*p* and *y*) throughout the retina (Fig 3E). While humans are trichromats, flies contain two apposed dichromatic systems, making it likely that they have good color vision, which extends to the UV to which humans are not sensitive.

Cloning of the genes encoding the four *Drosophila* inner photoreceptor Rhodopsins (*rh3-rh6*) and antibodies against the Rh3 and Rh4 proteins have allowed a precise characterization of *p* and *y* ommatidia. Fusion of the *rh* promoters with GFP or lacZ allowed the visualization of the *p* and *y* subtypes in R7 cells): *rh3-lacZ* is restricted to the DRA inner photoreceptors as well as to the *p*R7 cells, whereas *rh4-lacZ* expression is specific to the *y*R7 cells. Due to the cytoplasmic localization of the protein encoded by the reporter gene, these transgenes can also be used to visualize the axonal projections of inner photoreceptors to the medulla (M) while outer photoreceptors can be seen to project to the lamina with *rh1-GFP*.

### **5. Development Of Motion Detection vs. Color Photoreceptors**

An important first step towards understanding the formation of the complex fly eye which can achieve multiple specialized functions came with

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the description of the genetic pathways that lead to the formation of the retinal mosaic during development of the organism. After the initial steps of eye formation involving the recruitment of eight photoreceptors per ommatidium, a cascade of genetic events leads to the specialization of sub-groups of photoreceptors. One particularly critical gene serves as a binary switch between the fates of color (i.e. R7 or R8) vs. motion detection (i.e. R1-R6) photoreceptors. This gene, called *spalt* (Kuhnlein et al. 1994) is specifically activated in inner R7 and R8 and loss of *spalt* leads to a loss of inner photoreceptors. Instead, R7 and R8 gain outer photoreceptor markers, like rhabdomere morphology and *rh1* expression. However, the axonal projections of these transformed inner photoreceptors to the medulla are maintained (Mollereau et al. 2001; Domingos et al. 2004). Therefore inner photoreceptors are initially properly specified in *spalt* mutants, but then lose their identity and instead terminally differentiate into outer photoreceptors. *Spalt* is therefore necessary to distinguish differentiating color photoreceptors from an otherwise motion detection outer photoreceptor-like “ground state” toward which all photoreceptors tend to develop (right). The presence of two distinct genetic programs, specification, followed by differentiation illustrates the dual function of *Drosophila* photoreceptors: They are first specified as neurons that must find their appropriate target in the optic lobes. Subsequently, they differentiate as light sensing cells. In contrast, the vertebrate retina has two cell types to perform these roles: rods and cones detect light, but do not have neural extensions, while retinal ganglion cells project out of the retina and carry visual information to the brain.

## **6. Distinguishing Between R7 And R8 Cell Fates**

Both inner color photoreceptors require spalt to adopt their appropriate cell fate. Nevertheless, R7 or R8 represent different photoreceptors, both morphologically (position within the retina) and functionally (different *rhodopsins*): Other factors are therefore necessary to further distinguish between the two inner R7 or R8 photoreceptor cell fates. The gene *prospero* appears to be responsible for distinguishing R7 from R8 (Cook et al. 2003). *Prospero* is a regulatory gene that is known to play other important roles during development of the nervous system (Kauffmann et al. 1996). It is expressed specifically in R7 cells where it represses the expression of R8 opsin genes (*rh5* and *rh6*). Loss of *prospero* indeed leads to a de-repression of *rh5* and *rh6* in adult R7 cells, creating a second R8-like cell per ommatidium. Interestingly, loss of *prospero* also results in repression of the *rhodopsins* normally expressed in R7, most likely to avoid coexpression of opsin genes, a situation that is generally not observed in sensory receptor cells (Mazzoni et al. 2004). Although R7 cells mis-express R8 rhodopsins in *prospero* mutants, they do not gain all R8 markers and their rhabdomeres are still positioned correctly in the distal part of the retina, which could be interpreted as a reversion of R7 back to a generic inner photoreceptor fate. This suggests that another function is necessary to push the generic inner photoreceptor fate toward the R8 cell type. This gene is likely to be *senseless*, which also encodes a transcription factor (*sens*; Nolo et al. 2000): It is specifically expressed in R8 cells throughout eye development and photoreceptors

are misspecified in *sens* mutants (Domingos et al. 2004). As expression of both Pros and Sens is lost in *spalt* mutant photoreceptors, this suggests that inner photoreceptors undergo a series of consecutive determination steps by sequentially gaining expression of different combinations of transcription factors. R7 cells form after expression of Spalt and Prospero and R8 cells after expression of Spalt and Senseless.

### ***7. Model For Ommatidial Subtype Specification***

Outside of the DRA, *p* and *y* ommatidia are distributed randomly, with a ratio of 30:70, and similarly blue, green and red cones are distributed stochastically in the human retina with variable ratios. Although an elegant model has been proposed to explain the distribution of green and red cones (Nathans 1999), it draws on principles that are unlikely to be general to other systems, and is still not clear how stochastic choices are made between different photoreceptor cell fates in humans, or in flies.

However, a mechanistic model can now be proposed to describe the instructive signals specifying *p* and *y* ommatidia in *Drosophila*. In the absence of R7 cells (*sevenless* mutant), R8 cells always express the *y*R8 opsin *rh6*, which therefore appears to represent the “ground state opsin” expressed in R8 cells. This supposes that a signal from the R7 cells that have chosen to express the *p* opsin *rh3* (R7) is necessary for R8 to acquire the *p* fate (*rh5*). A model for stochastic specification of *p* and *y* ommatidia can therefore be divided into

two steps (Fig 4): First, the stochastic, but biased choice between  $p$  and  $y$  fates appears to be made in R7 cells (left), which then impose the corresponding fate onto the underlying R8 cells. The result is the fly color vision system: a mosaic of two ommatidial subsets with the R8 cells exhibiting highest spectral sensitivity in the blue ( $p$ ) or green ( $y$ ) part of the spectrum, comparing their inputs with UV-sensitive R7 cells.

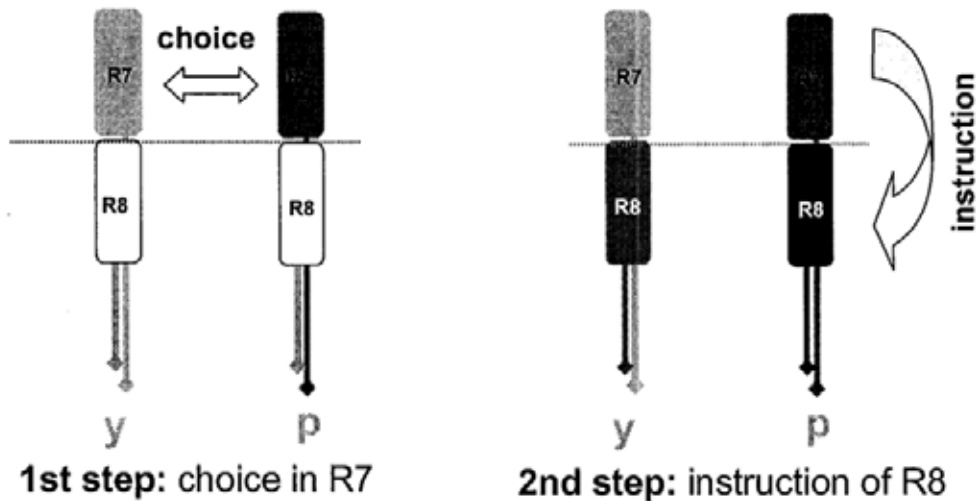


Figure 5: Model for ommatidial subtype specification.

In a first step,  $p$  and  $y$  subtypes get specified in R7 cells (choice). In a second step, this choice gets transmitted to the underlying R8 cell via an unknown signal transduction pathway (instruction).

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