Molecular evolution of visual pigments in the wandering spider *Cupiennius salei*

Project report for the Centre for Excellence in Enquiry-based Learning

Tom Ellis June 2010

During my placement year from the University of Manchester I spent twelve months researching the visual pigments of the central American spider *Cupiennius salei* at the Dept. for Evolutionary Biology at the University of Vienna. This work was the first to examine the visual system of an **arthropod**, but this work unfortunately ended somewhat inconclusively, and so during the Easter break of 2010 I was awarded a CEEBL grant to return to the lab to tie up these loose ends. This report will attempt to summarise this work in non-technical language, but where academic terms are used, they are given in bold and explained in a glossary.

What are visual pigments?

Visual pigments, more commonly referred to as opsin proteins, absorb **photons** and cause the brain to recognise light, and are an essential component of animal visual systems. They consist of one or more **GPCR** proteins with 7 cell membrane-spanning helix structures, which are bound to a chromophore molecule at its centre (see figure 2; for a more detailed account of opsin biology see Terakita 2005). When a photon strikes the chromophore it twists and alters the shape of the opsin, causing the nerve cell to fire. Where the sequence of amino acids in the protein differs (see box 1), the protein will have a slightly different shape, affecting its interaction with the chromophore, and thus the **peak spectral sensitivity** of light of the opsin.

Box 1. Proteins, DNA and mRNA

These three molecules are vital to the workings of cell machinery.

Proteins are the molecules that perform the actual work of a cell, such as the haemoglobin in blood which binds oxygen. They are composed of building blocks of amino acids in a specific order, which gives them a specific 3D structure which is crucial to their function. Amino acids bind to form a backbone, and each amino acid has an R-group, whose chemical properties vary and interact with other amino acids. Altering the sequence of amino acids alters the shape of the protein, and therefore its function.

DNA is the master copy of instructions that tell the cell how to build proteins, when, and how much. It is composed of a linear sequence of four kinds of nucleotides, referred to as C, G, A or T, whose sequence tells the cell machinery what order to stick amino acids together to form proteins.

Messenger RNA (mRNA) is chemically very similar to DNA except that it lacks a stabilising oxygen atom, making it far more fragile than DNA. mRNA is the intermediate molecule between DNA and proteins, and is rather like a photocopy of a page from a cookbook: you can make a cheap copy of your instructions that can get damaged and destroyed without ruining the vital master copy.

Numerous arthropod opsins have been sequenced, but work has concentrated on crustaceans and insects (e.g. Chang et al. 1996, Kitamoto et al. 1998, Sakamoto et al. 1996, Oakley & Huber 2004). The results point to a pattern of repeated gene duplication and deletion in the arthropods, with significant instability and variation in the number of opsin genes present

(Kayashima et al. 2009, Spaethe & Briscoe 2004). No work has been carried out on **myriapods**, and among **chelicerates** only the visual systems of horseshoe crabs (Clay Smith et al. 1993) and jumping spiders (Koyanagi et al. 2008) have been examined on a molecular level. More work is needed on the molecular evolution of opsins in the basal arthropods to further elucidate the pattern of opsin evolution beyond insects and crustaceans. Spiders represent a particularly interesting model for research because like vertebrates they exhibit simple eyes, rather than the compound eyes found in most other arthropods, and as such provide scope for comparison with ourselves.

Cupiennius salei

C.salei is a large wandering spider from central America, which emerges from its daylight retreat only at dusk to search for prey and mates on bromeliads and other plants during the hours of darkness Like most spiders, it has four pairs of eyes: the anterior-medial (AM), or principal eyes, posterior medial (PM), anterior lateral (AL) and posterior lateral (PL), but both hunting and courtship behaviour are guided primarily by vibratory mechanisms (Barth & Schmitt 1991; Land & Barth 1991). The quality of vision in all eyes is nevertheless good, and plays an important role in substrate identification in locating the bromeliads where it hunts (Schmid 1997).

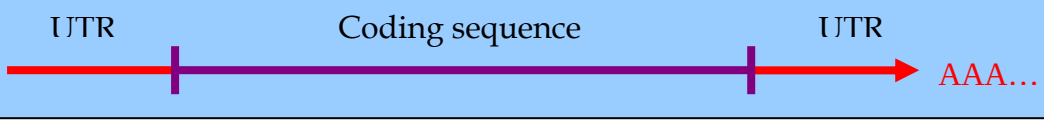
Figure 1. *Cupiennius salei* feeding on a cricket.

Electrophysiological studies have demonstrated that *C. salei* has three types of photoreceptor cell with distinct peak spectral sensitivity curves, which points to the possibility of trichromatic vision via pigments with peak spectral sensitivities of 360nm (in the UV), 480nm (blue) and 520nm (green) (Barth 1993, Walla et al 1996). Although behavioural studies using moving coloured target suggest that *C. salei* is functionally colour-blind (Schmid & Orlando, forthcoming paper), the authors suggest that the three receptors allow the spider to detect light across a broad spectrum, thus increasing overall visual sensitivity even in very low light levels. Furthermore, Koyanagi et al. (2008) successfully sequenced three opsins (Rh1, Rh2, Rh3) from each of the salticid spiders *Hasarius adonsoni* and *Plexippus paykulli*, and inferred peak spectral sensitivities from their amino acid sequences which were similar to those expected for *C. salei*.

Previous work from my placement year was unsuccessful in finding homologs for the Rh1 and Rh3 genes, but identified at least 17 copies of the Rh2 gene. By sequencing a short section in the middle of these genes, I found

Box 2. The structure of mRNA

mRNA can be viewed as having three components: a coding sequence, the section of the molecule which actually codes for a protein, and two buffer regions on either side referred to as untranslated regions (UTRs). The end of the molecule has a 'poly-A tail'. The UTRs do not affect the protein, and are (theoretically) not subject to natural selection, and as a result the sequence of nucleotides can change quickly, which makes them a useful tool for trying to deduce evolutionary histories.



extremely high diversity in the amino acid structure of the genes, which indicates repeated gene duplication events leading to diverse opsins capable of absorbing light over a broad spectrum.

The purpose of this follow-up project was to sequence these genes over almost the entire length of the coding sequence (see box 2), and to plot the amino acids which showed variation on a 3D representation of the molecule. This would allow me to infer which site were likely to have an effect on the spectral sensitivity of the protein, and from that infer the likely functional diversity of long-wave opsins in *C. salei*. Furthermore I planned to sequence the **UTR** after the coding sequence to look for evidence of gene duplication events.

Materials & Methods

I extracted mRNA from the retina of 3 adult *C. salei* and converted this into DNA (see **box**). I needed RNA for the experiment because unlike normal DNA in the cells it does not contain 'junk' information in the form of unwanted **nucleotides**. However RNA is unstable, therefore reverse transcribing it to DNA gives a stable product whilst retaining the nucleotide sequence of the RNA molecule.

Based on sequences obtained during previous work (Ellis 2009), I designed new **primers** which would allow me to amplify a much broader stretch of the genes than I had done previously via a **PCR**. Furthermore I also designed primers which would span the terminal UTR, and used a published primer designed to bind to the poly-A tail of the UTR. I cloned this amplified DNA into colonies of E. Coli before sequencing to ensure that only one gene product was being sequenced at a time.

I generated a 3D model of the *P. paykulli* Rh2 opsin using the SWISS Model application (Guex & Peitsch 1997), and mapped the sites showing variation in the program Chimera (Pettersen et al. 2004)

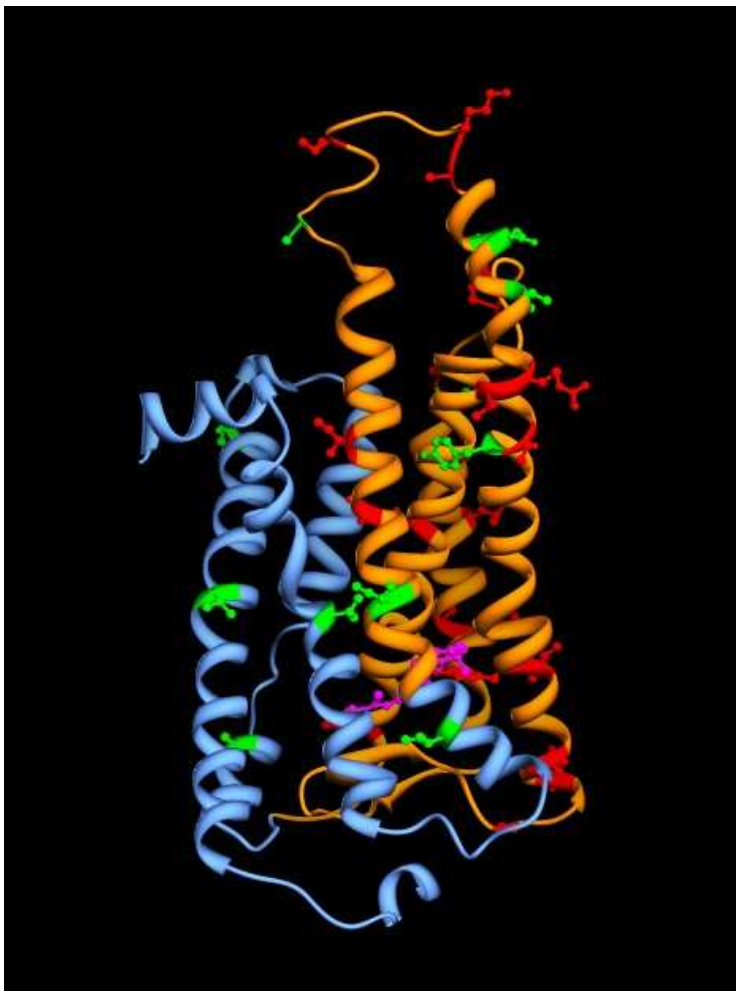


Figure 2. A 3D model of a visual opsin. The structure of a longwave opsin from a jumping spider was plotted onto the crystal structure of the squid opsin. Orange areas indicate those areas previously examined by Ellis (2009) and the red amino acids those sites found to be variable. The blue region is the area examined in this study, with the 14 new variable sites in green. The chromophore is shown in magenta.

Results & Discussion

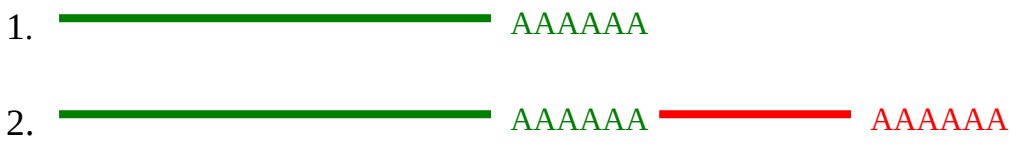
I successfully isolated 51 transcripts for *C. salei* longwave opsin transcripts. Figure 2shows those sites that show variation. 14 new variable sites were identified in this study, including some in the region that had previously been examined (Ellis 2009). This is extremely high level of diversity, comparable with the diversity in homologous opsins seen throughout entire orders of insects. Furthermore, 7 of these mutations brought about changes in the chemistry of the R group. It is very difficult to predict which sites will affect the spectral sensitivity of an opsin, but those sites near the chromophore and those bringing about chemical changes have a high probability of having functional significance (Briscoe 2002).

Sequencing the UTRs was less successful, but still yielded interesting results. In some transcripts, it appeared that there was a second poly-A region in the terminal UTR some distance from the actual end of the molecule (see figure 3). This is further evidence of gene duplication events, whereby a gene

and its poly-A tail is copied and spliced somewhere else in the genome. A new poly-A tail is inserted by the cell machinery at it new location.

These results strongly suggest a diverse toolkit of similar opsins with subtle differences in spectral sensitivity. As Schmid & Orlando (forthcoming)

Figure 3. Evidence for gene duplication events. A: a normal gene with a terminal poly-A tail within the genome. B: the gene is copied and spliced somewhere else, and an additional poly-A tail built on the end.



demonstrated that this spider is colour-blind, this raises the question of why it should have such opsins. I agree with the postulations of Schmid & Orlando that the diversity of opsins allows the spider to absorb light over as broad a spectrum as possible.

Glossary

Arthropod: A highly successful and specious group of invertebrates with a hard external skeleton. Includes insects, crustaceans, centipedes and millipedes, scorpions and spiders among many others.

Chelicerate: An evolutionarily 'primitive' group of arthropods characterised by having fangs (chelicerae) rather than jaws. Includes spiders, scorpions, mites and horseshoe crabs among others.

GPCR: G-protein coupled receptor, an important class of proteins

Myriapoda: Centipedes and millipedes, lit. many legs in Greek.

PCR: Polymerase chain reaction, a very common technique in molecular biology for making many copies of a stretch of DNA. A good description of the procedure can be found on wikipedia: http://en.wikipedia.org/wiki/PCR

Peak spectral sensitivity: Each opsin has a specific pattern of sensitivity to different wavelengths of light. The peak sensitivity is the wavelength most strongly absorbed, and is a figure used to compare sensitivities of different opsins.

Photon: The basic unit of light.

UTR: Untranslated region see figure 3.

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