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## Can a Flea See? An Investigation of Flea Opsin (Flopsin)

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Visual pigments are responsible for conferring visual perceptual abilities to animals. Various pigments are tuned to absorb light of different wavelengths and to provide contrast and movement perception. All pigments consist of an opsin protein and a chromophore molecule, usually a carotene derivative. Interactions between the chromophore and amino acid residues of opsin fine-tune visual pigments for the absorption of different wavelengths. Opsin variants can tune visual pigments to absorb wavelengths in the ultraviolet, blue, green, and red regions of the spectrum (Briscoe and Chittka 2001).

Insects represent a group of organisms in which visual perception and the associated structural components are highly developed. One of the exceptions to this are Siphonaptera (fleas), which show a transformation or a complete loss of the multifaceted eyes and ommatidia (simple eyes) of most insects. Some fleas, for instance, exhibit a rudimentary eye “spot”, however, SEM and TEM studies show that no ommatidia are present. The extent of visual perception exhibited by these parasites remains unclear. However, behavioral studies have shown that certain circadian rhythms related to sexual behavior and feeding habits are influenced not only by the activity patterns of the prospective hosts but also the environmental photoperiodic cycle (Kheisin and Lavrenko 1956). Thus, light sensitivity appears to be correlated to host-finding behavior. No formal study documenting the presence of opsin genes in Siphonaptera has been attempted.

Whiting (2001) recently demonstrated that Siphonaptera is closely related to Boreidae (snow fleas), and more distantly related to Panorpidae (scorpionflies). Scorpionflies have well developed eyes and are highly visually oriented. Snow fleas, on the other hand, have only limited visual perception. These phylogenetic relationships represent a unique opportunity to study the correlation between opsin gene evolution, eye structure, and visual acuity.

I predicted that with the loss of developed visual organs, some opsin coding genes have lost their functional constraint. Over time, these genes would accumulate random mutations, neutralizing their utility and altering their sequence dramatically. I hypothesized that the transition from fully developed eyes (as in Panorpidae) to weakly developed eyes (as in Boreidae) to eyelessness (as in Siphonaptera) would be characterized by either an absence or a loss of function of opsin variants.

The general procedure for this analysis required several steps. First, DNA from select specimens of fleas, scorpionflies, and snowfleas was extracted and purified. Using a procedure known as the Polymerase Chain Reaction (PCR), the gene encoding the long-wavelength variant of the opsin protein was isolated and amplified. This amplified portion of the gene could then be analyzed to determine the exact DNA sequence for each individual in the study. Once the sequence data were obtained, the sequences were aligned, and I compared both the DNA nucleotide sequences and the amino acid protein sequences encoded by the DNA. Rigorous statistical analyses were performed in order to calculate the degree of similarity and “probability” of relationship. Because of the complexity of the algorithms and the amount of

data generated, the computing power of the Fulton Super Computer, “Mary Lou”, was utilized to perform the analyses. We were then able to construct a phylogenetic “tree” showing the probable relationships across the different groups of insects, and begin to infer evolutionary histories of this gene.

Because this had never been attempted before, there were no published protocols. By modifying studies of distantly related crustaceans, I generated and optimized a new protocol for isolating and amplifying large segments of the opsin gene from fleas. In doing so, I optimized new techniques (for our laboratory) and explored new solutions for overcoming difficulties in working with small quantities of DNA. For example, after initial amplification of the putative opsin gene, I faced two problems in that the product was often too dilute to effectively sequence, and other genes often nonspecifically co-amplified with my target gene. I therefore had to optimize an additional cloning step to separate out the gene of interest and generate sufficient quantities to sequence.

The data showed some surprising results. I was able to successfully amplify and sequence the long-wavelength opsin gene from six individuals of fleas representing five families, and six individuals of scorpionflies. Although specimens were available, I was unable to amplify the opsin gene from any snow fleas. With just a brief comparison of the DNA sequence, it was apparent that the flea sequence was significantly different from that of the scorpionflies. The fleas had several unique long inserts of DNA present in the gene. However, these could easily be explained as introns, segments of DNA removed prior to coding of a new protein. By removing these inserts and translating the DNA code into a protein sequence, the flea opsin showed an open reading frame (no mutations giving a premature stop signal) and a remarkable similarity to the scorpionfly protein sequence. Interestingly, however, when put in the context of a phylogenetic tree along with other major insect lineages, the flea opsin showed less similarity to the scorpionfly opsin than it did other more distantly related insects, such as grasshoppers, bees, and moths.

These data suggest several interesting conclusions that deserve further exploration. First, the presence of an intact open reading frame in the opsin sequence suggests that the opsin has some retained functionality. This leads us to the hypothesis that fleas might possess some visual acuity or at least some photosensitivity. If that is true, we assume that it is most likely functioning in the vestigial eye. However, it is also possible that it may be used elsewhere in some unique photosystem developed by these parasites. This project opens new questions that can now be explored with more in depth investigation of the biochemistry and biology of the flea photosystems.

## References

- Briscoe AD, Chittka L (2001) The evolution of color vision in insects. *Annu. Rev. Entomol.*, 46: 471–510.
- Kheisin IM, Lavrenko LE (1956) Duration of blood-sucking and diurnal rhythm of nutrition and dropping of females of *Ixodes ricinus*. *L. Zool. Zh.*, 35: 379–383.
- Whiting MF (2001) Mecoptera is paraphyletic: multiple genes and phylogeny of Mecoptera and Siphonaptera. *Zoologica Scripta*, 31: 93–104.