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Phototransduction Components in the Visual System of Hard-bodied Ticks

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PHOTOTRANSDUCTION COMPONENTS IN THE VISUAL SYSTEM OF HARD-BODIED TICKS

by

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ABSTRACT

Ticks are terrestrial invertebrate parasites that attach to their hosts to feed on their blood. Ticks are composed of three families: Ixodidae, Argasidae, and Nuttalliellidae. Ixodid ticks include members of the genera *Amblyomma, Ixodes, Haemaphysalis, Hyalomma, Dermacentor,* and *Rhipicephalus*. Ticks can transmit diseases to animals and humans, making them an important organism to study. Current tick-bite mitigation strategies include acaricides (harmful if misused), and CO_2 traps (effective in decreasing tick abundance, but costly to use). Since the visual system of ticks has not been studied extensively, I expect that by studying their visual system, alternative tick-bite mitigation strategies could be developed. In this study, I used the Phylogenetically Informed Annotation (PIA) workflow to analyze the genes present in the phototransduction pathway of hard-bodied ticks from the transcriptome sequences and the whole-genome sequences (WGS). All six of the genera listed above are included. My analyses document the occurrence of opsin proteins, r_opsin, c_opsin, Gq subunits, lark, and ovo genes. These results imply that ticks possess the proteins necessary to sense light, since many of these components enable light-detection in the retinas of animals.

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LIST OF ABBREVIATIONS

CO_2 – carbon dioxide

LIT – Light Interacting Toolkit

NCBI – National Center for Biotechnology Information

TSA – Transcriptome Shotgun Assembly

PIA – Phylogenetically Informed Annotation

INTRODUCTION

Ixodid ticks

Ticks are terrestrial invertebrate parasites that attach to an animal host (including humans), and feed on its blood (de la Fuente, 2003; Charrier et al., 2019). Ticks are classified into three families: i) Ixodidae (hard-bodied ticks), ii) Argasidae (soft-bodied ticks), and iii) Nuttalliellidae, which is monotypic (Charrier et al., 2019). The hard-bodied ticks are divided into the Prostriata, and the Metastriata. Prostriata are characterized by an anal groove that surrounds the anterior part of the anus, which the Metastriata lack. The Prostriata contains the single subfamily Ixodinae, which contains the genus *Ixodes*. The Metastriata comprises the remaining hard-bodied tick subfamilies, which include the Amblyomminae, Haemaphysalinae, Hyalomminae, and the Rhipicephalinae. This study will focus on the following genera of ixodid ticks: *Ixodes*, *Amblyomma*, *Haemaphysalis*, *Hyalomma*, *Dermacentor*, and *Rhipicephalus*.

The hard-bodied tick life cycle

Most ixodid ticks have four life stages – egg, larvae, nymph and adult, and a total lifespan of 1 – 3 years (CDC, 2021). During their lifecycle, ticks feed on one to as many as four different hosts. Larvae and nymphs need one bloodmeal to be able to molt to the subsequent life stage. In black-legged ticks (*Ixodes scapularis*), hosts of larvae and nymphs include small and medium-sized such as white-footed mice, chipmunks, voles, and shrews (CDC, 2021). Female black-legged ticks need a bloodmeal to lay their eggs. Egg laying usually occurs during the spring season, close to where a female detaches from its host following a blood meal (University of Wisconsin, 2012). After laying their eggs, the females die. During the summer months the

larvae start looking for hosts to feed on (CDC, 2021). After feeding, the larvae drop from their chosen host, and over-winter to molt into nymphs. As nymphs, they start seeking hosts during the following spring to feed (CDC, 2021; University of Wisconsin, 2012). Nymphs repeat the same behavior to molt into adults and seek hosts in the fall (CDC, 2021). The black-legged tick has expanded into the northern part of the eastern US (Eisen & Eisen, 2018). During the 1920s this species was known from around Cape Cod, Massachusetts, but later expanded into southeastern states and the Gulf Coast states (Eisen & Eisen, 2018). Black legged ticks are found in New England, Rhode Island, Long Island, NY, northwestern Wisconsin, the upper Midwest, northeast, mid-Atlantic states, and southeastern states (Eisen & Eisen, 2018). Nymphs from the northeastern states are more likely to bite people than the ones in the southeast (Eisen & Eisen, 2018).

The pathogens known to be transmitted from infected black-legged tick females to their offspring are the Powassan virus, and *Borrelia miyamotoi* (CDC, 2021; Eisen & Eisen, 2018). The other pathogens transmitted by *Ixodes scapularis* are transmitted horizontally (where an infected tick transmits a pathogen to a host, and larvae or nymphs feed on the infected host, thus acquiring the pathogen) (CDC, 2021; Eisen & Eisen, 2018). Nymphs may be able to transmit diseases to animals and human hosts, since at this stage they feed primarily on white-footed mice, which are the principal carriers for the organisms causing human babesiosis and human granulocytic ehrlichiosis (University of Wisconsin, 2012). As adults, females look for a host to feed on (usually white-tailed deer) during the fall. After feeding on host, the female ticks detach. The females lay their eggs in the ground following detachment (Patnaude & Mather, 2000). Thereafter, females die (Patnaude & Mather, 2000). Males attach to a host (most likely white-

tailed deer), but rarely feed on their host (Patnaude & Mather, 2000). Instead, they stay attached to the host in the hopes of finding a female to mate with (University of Wisconsin, 2012). *Ixodes scapularis* is an important vector because it transmits zoonotic diseases such as Lyme disease, anaplasmosis, babesiosis, and Powassan virus (Burtis et al., 2021).

Ixodid tick-borne diseases

Currently, tick borne-diseases account for more than 75% of vector-borne diseases in the United States (Eisen & Paddock 2020). Vector-borne diseases are diseases transmitted by arthropods such as fleas, ticks, and mosquitoes (Beard et al., 2016). More than 40% of tick-borne human pathogenic agents have been described after the 1980s (Eisen & Paddock 2020). By 2018, the CDC had recorded around 47,000 cases from the following diseases transmitted by ticks: Lyme disease (the highest number of cases), anaplasmosis, spotted fever rickettsiosis, babesiosis, tularemia, and Powassan virus (CDC, 2019). Tick-borne diseases have not been adequately reduced by using environmental methods such as discontinuing use of plants that attract deer, removing weeds and keeping grass short (Eisen & Gray, 2016). The rate at which humans encounter ticks has not decreased with the methods of intervention described above (Eisen & Paddock, 2020). Furthermore, environmental intervention and other forms of tick-bite mitigation strategies (see below) are associated with high costs of usage, and acceptability (Eisen & Paddock, 2020). Some of the recommendations made to prevent tick-borne diseases include avoiding habitats where ticks are active, using chemicals such as permethrin in one's clothing, applying acaricides to possible tick hosts, and performing daily checks for ticks on oneself (Eisen & Paddock, 2020; Eisen & Gray, 2016).

Tick-bite mitigation strategies

Some of the tick-bite mitigation strategies that exist include the usage of acaricides, carbon dioxide (CO_2)-trapping, and using permethrin (Sonenshine & Roe, 2013; Burtis et al., 2021; Eisen & Eisen, 2018). Acaricides are pesticides or chemicals used to kill mites and ticks (Hoy, 2008). Some popular acaricides used to prevent tick bites include piperidine, permethrin, and bifenthrin. DEET is a popular repellent used to prevent attachment of ticks to clothing and exposed skin (Sonenshine & Roe, 2013). Spraying or using permethrin impregnated clothing has been shown, in military trials, to be more effective than the usage of DEET compounds in repelling *Amblyomma*, and *Ixodes* (Clark & Hu, 2008). It was found that military clothing treated with manufactured permethrin retained its repellent properties for up to 100 washes when the uniforms were already discarded. The various ways of applying permethrin to clothing include polymer-coating, dipping, and spraying. In the polymer coating method, the chemical permethrin was applied to the clothes and then sealed in the clothes to decrease continuous removal of the chemical by constant washing cycles. The polymer coating method is favorable, because this mode enables the clothes to retain its repellent properties through many washing cycles. This contrasts with the dipping method and spraying methods, in which one would have to constantly treat the clothes to ensure its repellent activity. It is also suggested that the application of permethrin impregnated clothing should be a safe and economic option for prospective users, since a high rate of exposure, if permethrin was constantly applied by oneself to one's clothing, could result in an elevated health risk. The various ways permethrin can enter the body include contact with skin (e.g., topical cream for scabies treatment), inhalation (e.g., mosquito nets),

ingestion with food, and water. Depending on the dose administered, permethrin may i) interfere with male fertility in bulls, and rats, ii) cause cardiac hypertension when administered orally in rats, and iii) could affect the immune system of humans (Faulde & Uedelhoven, 2006; Chrustek et al., 2018).

Another, perhaps safer, approach to preventing tick-borne diseases is to reduce tick abundance through CO_2-baited traps. Lone star ticks (*A. americanum*) are easily collected with CO_2 traps in comparison to other tick species, such as *I. scapularis*, because *A. americanum* ticks are more mobile and more aggressive in their questing behavior (Mays et al., 2016). CO_2 trapping is effective, but the effort and money involved in setting up the traps can be prohibitive. When using CO_2-baited traps, one must consider several challenges such as i) the amount of CO_2 needed, which will depend on the sampling area, ii) the maintenance involved in preventing the CO_2 tank and hoses from being damaged, iii) the inherent dangers of working with compressed gas, and iv) and the need to transport the CO_2 gas tanks to the trap sites (Mays et al., 2016). CO_2 trapping can be substituted by dragging when incorporating CO_2 in the tick collection method isn't a viable option, however, it is a more labor-intensive endeavor. Finally, humans can undertake a variety of preventative measures to limit tick attachment. These include wearing light-colored clothing, self-inspection, avoiding walking through vegetation, washing as soon as possible after tick exposure, and putting used clothes in a hot dryer for 20 minutes to kill ticks. It is also suggested that clothes are washed and dried at a temperature of 60 degrees Celsius (Pastula et al., 2014; Rahlenbeck, Fingerle & Doggett 2016). A Lyme disease vaccine used to be available, however in 2002 it was discontinued because it did not yield long-term protection and was not generating enough profits (CDC, 2018).

Unfortunately, the mitigation strategies discussed above have only a mild impact on preventing tick-borne disease. Novel methods that are both cost-efficient and effective are needed to help counteract the expanding distribution and increasing abundance of ticks in North America. In other invertebrate pests, such as insects, numerous strategies that exploit the visual system are being used to mitigate pest damage, prevent disease transmission, and reduce abundance. However, mitigation strategies exploiting the visual system of ticks are currently not possible, as very little is known about their vision relative to their insect counterparts.

If we understand how ticks utilize their vision to find their mates and/or hosts, we could use that information to design interventions that exploit their visual system as a tick bite mitigation strategy and to decrease the rate of disease transmission (Giraldo-Calderón et al., 2017). In order to do so, a more thorough understanding of tick vision is necessary.

Tick Vision

Ticks generally locate their prey by chemosensory organs, such as the Haller's organ, which is situated in the tarsus I of the foreleg (Mitchell III et al., 2017). The Haller's organ is used to detect a variety of substances such as pheromones, host odors, and CO_2, in addition to body heat in the form of infrared radiation (Sonenshine, 1991; Mitchel III et al., 2017). The Haller's organ contains a posterior capsule, and an anterior pit. The anterior pit of the Haller's organ is composed of six to seven sensilla in most ixodid ticks, and is predominantly used for olfaction (Sonenshine, 1991). The different kinds of sensilla have various functions including olfaction, mechanosensilla, a combination of olfacto- and mechanosensilla, gustatory sensilla, and sound perception. Others serve as thermosensory sensilla (Sonenshine, 1991). Recently,

there has been evidence that the Haller's organ is light-sensitive; used by dog ticks (*Dermacentor variabilis*) adults to detect infrared light which might aid in host location (Mitchell III et al., 2017). Using infrared lighting to trap ticks could be another method used to decrease tick bites to hosts (Mitchell III et al., 2017).

Kaltenrieder et al. (1989) used electroretinography (ERG) to determine that the spectral sensitivity of *Amblyomma variegatum*, a congeneric of *A. americanum*, falls within the blue range (450 nm - 485 nm) of the electromagnetic spectrum. When comparing the spectral sensitivity, absolute threshold, and visual system of *A. variegatum* with that of *Hyalomma dromedarii*, Kaltenrieder et al. (1989) found that both exhibit positive phototaxis to horizontal light, where *H. dromedarii* had two sensitivity peaks (near 380 nm, and 500 nm), and *A. variegatum* had one peak (near 480 nm). Regarding ERG, both *H. dromedarii* and *A. variegatum* had a maximum peak at 470 nm and *H. dromedarii* expressed another band in the UV range.

H. dromedarii also has higher spatial acuity due to having more photoreceptors (both in their eyes, and extraretinal photoreceptors), a smaller field of vision, and better perception formation than *A. variegatum* (Kaltenrieder et al., 1989). The researchers suggested that *Hyalomma* uses its visual system to seek its host, but *Amblyomma* does not; which leads me to think that maybe *Amblyomma* is not able to distinguish shapes as well as *Hyalomma*. The visual system morphology within ixodid ticks can differ substantially among taxa. For example, *Ixodes ricinus* does not possess corneated eyes, but has two rows of 20 to 21 photoreceptors that allow the tick to sense changes in light intensity located on its dorsolateral side (Perret et al., 2003). *A. americanum*, on the other hand, have eyes located on the scutum (Phillis & Cromroy, 1977).

Phillis & Cromroy (1977) also suggested that the eye of *A. americanum* retains its structural components as the tick passes through its life cycle with only the size increasing through development. At larval stages, the lone star tick's eye is composed of twenty-five to thirty photoreceptor neurons. At adult stages the eye contains around thirty to forty photoreceptor neurons. The photoreceptor neurons have the same structure, throughout the tick's life cycle. This means that the photoreceptors do not change despite the sex, age, or life stage of the tick (Phillis & Cromroy, 1977).

Despite the different ways ticks may locate hosts using the Haller's organ (e.g. via chemosensation, olfaction, and the detection of mechanical stimuli), we do not know if, or how, ticks use their visual system for host detection. There is conflicting information regarding how ticks perceive light. Some sources, such as Phillis & Cromroy, state that *A. americanum* lacks pigment, yet Kaltenrieder et al. (1989), proposed that *A. variegatum* is sensitive to blue light in the electromagnetic spectrum. Furthermore, the iconic yellow spot on adult female *A. americanum* suggests a potential visual signal, perhaps seen by males through color, contrast, edge-detection, or combination of the above. It is also not known if the yellow spot on adult female *A. americanum* serves other functions, such as allowing the tick to blend in with certain backgrounds, or if they signal distastefulness to potential predators. Therefore, more research needs to be done to characterize morphological, behavioral, and molecular aspects of the visual system of ixodid ticks.

Phototransduction

Phototransduction is the act of changing light energy into electrical/neuronal energy. The human eye has photoreceptors which are sensory receptor cells that are specialized to be activated when they receive a light stimulus. Photoreceptors, divided into rods and cones because of their shapes, initiate light response in the retina. The other structures in the retina (such as the retinal ganglion cells) have other roles in the transmission of neuronal information to the central nervous system. Photoreceptors change photons (particles of light) into electrical signals for the nervous system to interpret. This change from light energy to neural energy can occur due to the molecular proteins that are housed in the photoreceptors' membrane. Opsins are the protein components of the visual pigments; they change the light energy to electrical/neuronal energy (Randall et al., 2001). In the retina, opsins attach to retinal (a molecule that absorbs light when it is in its 11-cis conformation) to form visual pigments termed rhodopsins (Randall et al., 2001). Rhodopsin is mostly involved in dim light perception, and does not detect light wavelengths corresponding to colors. Phototransduction occurs in the disks of the outer segment of the rods (Randall et al., 2001). Rods and cones, in vertebrates, have a cilium that allows each type of photoreceptor cell to connect the outer segments that have the photoreceptive membranes of the respective photoreceptor cell, with the inner segments (which houses the nucleus and other structures) (Randall et al., 2001). Invertebrates, however, do not contain this cilium, but they have microvilli that connect the inner and outer segments of rods and cones (Randall et al., 2001). The visual pigment in invertebrates is in the microvilli, therefore, the microvilli that have visual pigments are organized into rhabdomeres (Randall et al., 2001).

Pigments are molecules that absorb a specific wavelength of light and reflect others (Randall et al., 2001). Green chlorophylls and the carotenoids are the most abundant pigments in

nature (Wolken, 1971). The photoreceptors in invertebrates can take the form of ocelli, compound eyes, eyespots, among others (Wolken, 1971). They also possess a pineal gland that functions similarly to the vertebrate retina (Wolken, 1971). Other invertebrates can detect light through their skin, because of photosensory cells that are beneath the animal's skin (i.e. dermal light sense) (Wolken, 1971).

As previously stated, rhodopsin is located in the outer segments of the rods of vertebrates, and in the photoreceptors of invertebrates. Rhodopsin is composed of an opsin protein, and retinal, the light-absorbing molecule. In the retina, retinal has two conformations (Randall et al., 2001). When light is absent, retinal's conformation is 11-cis, because opsin and retinal are bound by a nitrogenous base in a covalent link (Randall et al., 2001). When light hits 11-cis retinal, its shape changes, which causes opsin's shape to change too (Randall et al., 2001). The conformational change from cis to trans, causes the activation of G-transducin by rhodopsin (Randall et al., 2001). Afterwards, when light hits rhodopsin, rhodopsin is active, activates G-transducin (has an important role in light transduction) (Randall et al., 2001). G-transducin activates subunits of phosphodiesterase (PDE, an enzyme). PDE hydrolyzes cGMP (a secondary messenger) into 5'-GMP, rendering it unable to perform its original signaling functions (Randall et al., 2001). When cGMP is low, sodium channels are closed/dark current stops, and potassium ions cause cell hyperpolarization (which occurs in vertebrate photoreceptors, when light stimulus increases) (Randall et al., 2001). cGMP regenerates with the help of guanylyl cyclase (GC, an enzyme) after the light stimulus is over, and the dark current resumes (Randall et al., 2001).

Study objectives

The objective of this study is to characterize the genes involved in the visual system of hard-bodied ticks by analyzing transcriptomes and whole-genome sequences (WGS) of ixodid ticks . Gene characterization is achieved by importing the tick transcriptome sequences and WGS into a workflow that annotates the absence or presence of phototransduction genes. The absence or presence of these genes could help in understanding how the visual system of hard-bodied ticks function, in developing alternative tick-bite mitigation strategies, and in determining the visual capabilities of ticks, such as color perception. Therefore, with my results I expect to determine which components of the phototransduction pathway (such as opsin, transducin, recoverin, arrestin, among others) are present in hard-bodied ticks.

METHODOLOGY

To answer our research objective, we obtained 15 tick transcriptome sequences from the NCBI Transcriptome Shotgun Assembly (TSA) Database (Sequence Set Browser :: NCBI (nih.gov)); eight from *Amblyomma americanum*, and seven from other ixodid ticks. Seven tick WGS from recently published ixodid tick genomes from the NCBI Genomes Database (Genome List - Genome - NCBI (nih.gov)) (see Table 1) were also included. After gathering the transcriptomes and WGS, the datasets were imported to the Phylogenetically Informed Analysis (PIA) workflow on the Galaxy web server (Speiser et. al, 2014). A general summary of the workflow is shown in Figure 1. PIA is a workflow that houses 109 Light Interacting (LIT) genes from non-model organisms (Speiser et al., 2014). LIT genes are genes that various organisms utilize to build their eyes and other structures regarding light-interaction, such as genes involved in light-detection, light absorption, light refraction, and other genes involved in eye development (Speiser et al., 2014).

The RNA sequences were translated into protein sequences as the necessary input into PIA. The WGS did not have to be transcribed to proteins as the complete proteome sequences for each species were imported as is. After importing the data, the transcriptomes and WGS were analyzed to identify those proteins matching the 109 LIT genes from well-described model organisms present within PIA. The PIA workflow matched the 109 LIT genes from these organisms to the genes available in the ixodid ticks' transcriptomes and WGS that are involved in the visual system. PIA also predicted the genes for each functional gene set, i.e., groups of genes involved in the same biological processes that serve in the visual system. When PIA finished analyzing the datasets, they were summarized in a tabular file that contained the

sequence ID, sequence, and the specific LIT gene annotated to each matching sequence. Using MS Excel, I downloaded the tabular files and used the UNIQUE and COUNTIF functions, to organize the gene names in a single column, and counted the numbers of matches for each LIT gene. The final counts across all transcriptomes and WGS were saved in a separate worksheet.

For the heatmaps, I made an excel spreadsheet with columns containing the abbreviation of the gene names, the full gene names, the transcriptome sequence names. For the WGS, I made a separate file with the same information, but I substituted the names of the ticks whose whole-genome sequences were analyzed. Then, I uploaded those files to Heatmapper (www.heatmapper.ca/expression/), to generate a heatmap of LIT gene counts in each sample. I used the white color to denote a missing number of genes (0 LIT genes found within the dataset), a dark blue color for the low number of gene matches between the datasets and PIA LIT genes, a light blue color for genes that had a moderate match, and a yellow color for genes that yielded a high number of matches.

For both files, I unclustered the rows of the heatmaps to allow for an easier view of each gene. Since the excel tables had a header tab, I had to subtract 1 from the gene number in the excel table to match the heatmap results. For example, gene #1 in the heatmaps corresponds to ‘Alas’, but in the excel table it was in row #2, gene #2 corresponds to ‘Aldh’, but in the excel table it was in row #3, and so forth (for more information regarding gene names, see Appendix A).

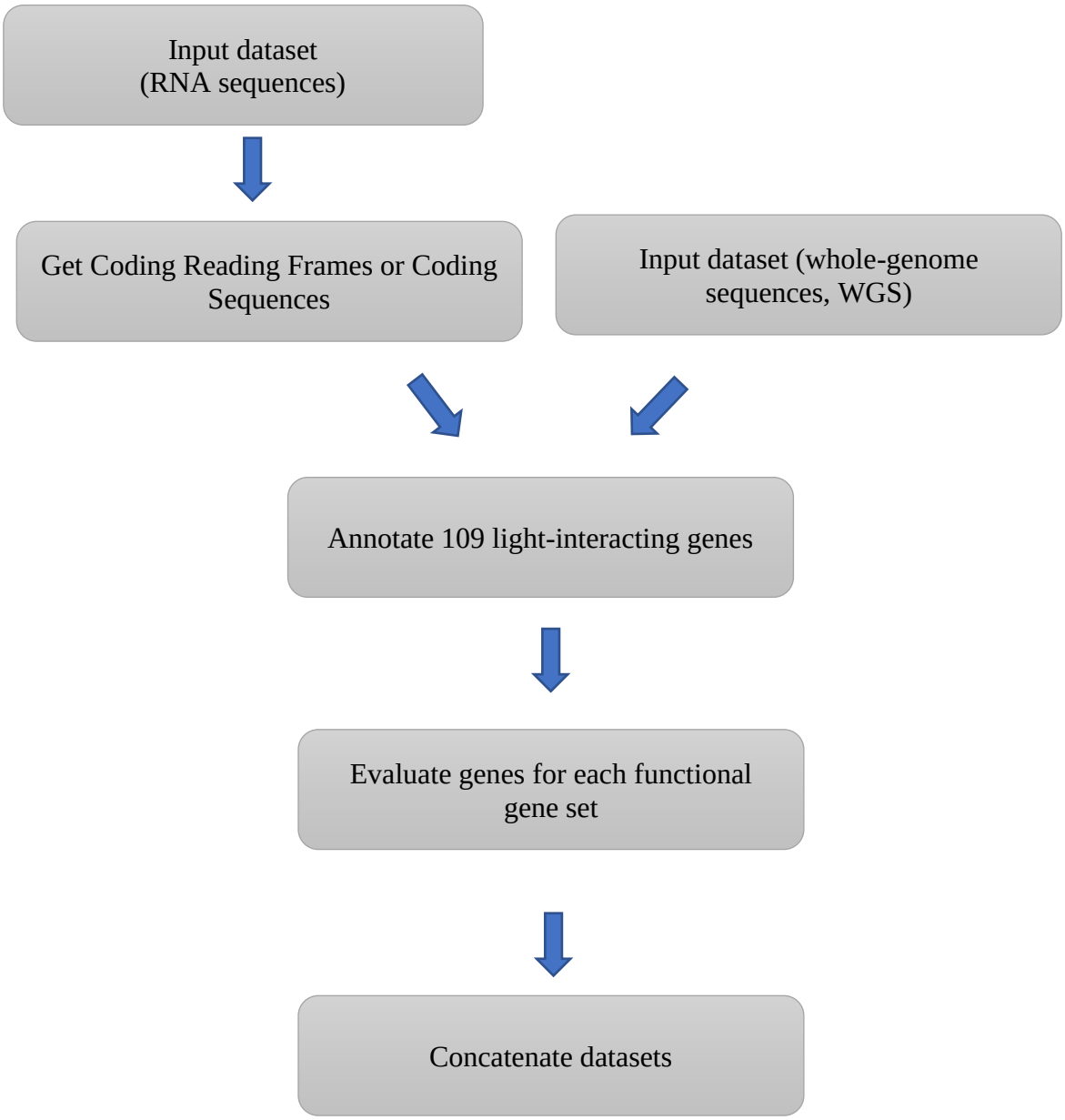

Figure 1: A summary of the Phylogenetically Informed Annotation (PIA) workflow used to analyze light-interacting genes in tick transcriptomes, and whole genome sequences (Speiser et. al, 2014).

RESULTS

Transcriptome sequences

Overall, *A. americanum* and *A. tuberculatum* yielded the fewest number of matches for LIT genes. *A. parvum*, *A. cajennense*, *A. aureolatum*, and *A. sculptum* yielded the highest results for LIT gene matches, suggesting that these organisms exhibit light-detection properties, with the genes that had highest matches in these sequences being Gq alpha (#25 on heatmap), lark (#40 on heatmap), and ovo (#72 on heatmap). For Gq alpha, *A. sculptum* yielded 22 matches, while *A. cajennense* and *A. aureolatum* yielded 18 matches. Lark showed moderate matches for the genes of *A. cajennense* which yielded 17 matches, *A. triste* and *A. sculptum* yielded 16 matches, *A. parvum* and *A. aureolatum* yielded 14 matches. Sequences from *A. americanum* yielded 9, 2, and 0 matches for lark, as well. Ovo was highly encountered in *A. sculptum* with 17 matches, and *A. aureloatum* with 16 matches. D_Cry was only found in *A. sculptum*, with 2 matches for this gene. From the transcriptomes heatmap, it can be observed that the genes that were found in moderate amounts in some transcriptomes were the following: glass, GPRK1, and GPRK2, (#30, #31, #32 on heatmap); inaC (#36 on heatmap); Notch, and Rhodopsin kinase (#42, #43 on heatmap); Retinal specific ATP-binding cassette transporter (#50 on heatmap); 11-cis retinol dehydrogenase, retinol dehydrogenase 8, and Retinaldehyde-binding protein 1 (#54, #55, #56 on heatmap); Retinal binding protein (RALBP, #58 on heatmap); ovo (#72 on heatmap); GTP cyclohydrolase 1, and Eye specific diacylglycerol kinase (#86, #87 on heatmap), among others.

WGS

As for the whole-genome sequences heatmap, Gq alpha, ninaE, c_opsin, and some opsin genes yielded low moderate results. In contrast, ovo yielded moderate results, and in some sequences, opsin yielded high numbers of matches. *I. scapularis* yielded the highest results for Gq alpha, with 24 matches. *D. silvarum*, *H. asiaticum*, and *R. microplus* yielded 22 matches, *I. persulcatus* yielded 20 matches, while *H. longicornis* and *R. sanguineus* yielded 18 matches for the Gq alpha gene. For ninaE, *D. silvarum* yielded 21 matches, *I. scapularis* and *R. sanguineus* yielded 20 matches, *H. longicornis*, *H. asiaticum* and *R. microplus* yielded 19 matches, *and I. persulcatus* yielded 18 matches (the lowest one for ninaE gene). C_opsin was also moderate in numbers of matches, since *D. silvarum* yielded 21 matches, *I. scapularis* and *R. sanguineus* yielded 20 matches, *H. longicornis*, *H. asiaticum*, and *R. microplus* yielded 19 matches, and *I. persulcatus* yielded 18 matches. The WGS in which opsin was moderately encountered were *D. silvarum*, with 21 matches, and *R. sanguineus* with 20 matches. The other sequences had opsin in numerous amounts. *I. scapularis* yielded 40 matches (the highest number of matches for opsin), *H. longicornis*, *H. asiaticum*, and *R. microplus* yielded 38 matches, and *I. persulcatus* yielded 36 matches for the opsin gene.

R. sanguineus yielded 29 matches for ovo, *R. microplus* yielded 27 matches, and *D. silvarum* yielded 25 matches. The other WGS yielded 0 results for ovo (#72 on heatmap). For GTP cyclohydrolase 1, *R. microplus* yielded 12 matches, *H. longicornis*, *I. persulcatus*, *D. silvarum*, *H. asiaticum*, and *R. sanguineus* yielded 11 matches. *I. scapularis* yielded 10 matches for GTP cyclohydrolase 1 (gene #86 on heatmap). There were also other genes shown in the heatmap that had a low number of matches in the WGS, such as: glass, Gprk1, Gprk2, inaC, Rhodopsin kinase, Retinal-specific ATP-binding cassette transporter, 11-cis retinol dehydrogenase, retinol

dehydrogenase 8, Retinaldehyde-binding protein 1, Retinal binding protein (RALBP), and Eye-specific diacylglycerol kinase (genes #30, #31, #32, #36, #43, #50, #54, #55, #56, and #87 on heatmap) yielded 10 matches for all the WGS. Notch (#42 on heatmap) showed 0 to 12 matches across the WGS.

Species	Sample size	Type	Sequences Examined	Number of LIT genes
Amblyomma sculptum	2	Transcriptome	GEEX01, GFAA01	80, 63
Amblyomma triste	1	Transcriptome	GBBM01	61
Amblyomma parvum	1	Transcriptome	GBBL01	49
Amblyomma aureolatum	1	Transcriptome	GFAC01	71
Amblyomma tuberculatum	1	Transcriptome	GIDH01	32
Amblyomma cajennense	1	Transcriptome	GBBK01	60
Amblyomma americanum	8	Transcriptome	GAGD01, GAYW01, GBAI01 - GBAL01, GBZX01, GFBJ01	46, 0, 3, 1, 2, 2, 42, 1
Dermacentor silvarum	1	Genome	GWHAMML	86
Hyalomma asiaticum	1	Genome	GWHAMMK	78
Haemaphysalis longicornis	1	Genome	GWHAMMI	80
Ixodes persulcatus	1	Genome	GWHAMMH	80
Ixodes scapularis	1	Genome	GCF	83
Rhipicephalus sanguineus	1	Genome	GWHAMMM	85
Rhipicephalus microplus	1	Genome	GWHAMMG	81

Table 1: Table describing the ixodid tick species whose transcriptomes and WGS were analyzed in PIA, the sequences that were examined, and the number of LIT genes found in the sequences.

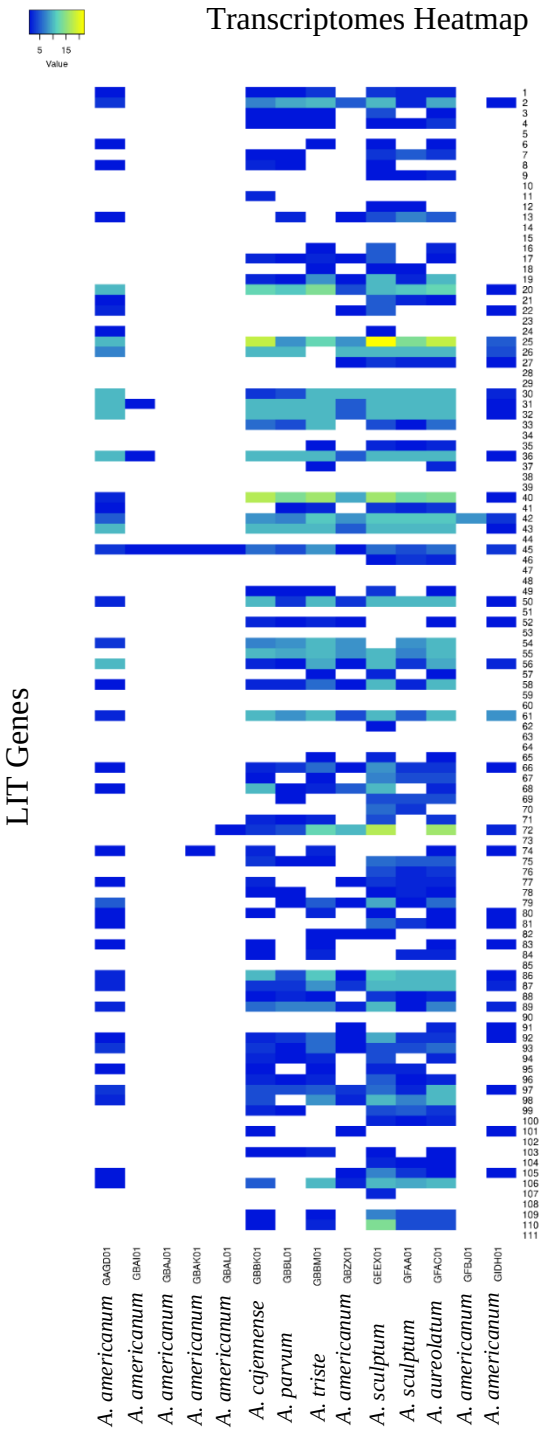

Figure 2: Heatmap showing the number of matches between the transcriptomes and the 109 LIT genes, within the PIA workflow. As stated previously, the white space means that there were no matches for the specified gene in the sequence(s); the dark blue color denotes a low amount of matches; light blue denotes moderate amount of matches, and yellow denotes a high number of LIT genes matches found within the transcriptomes datasets

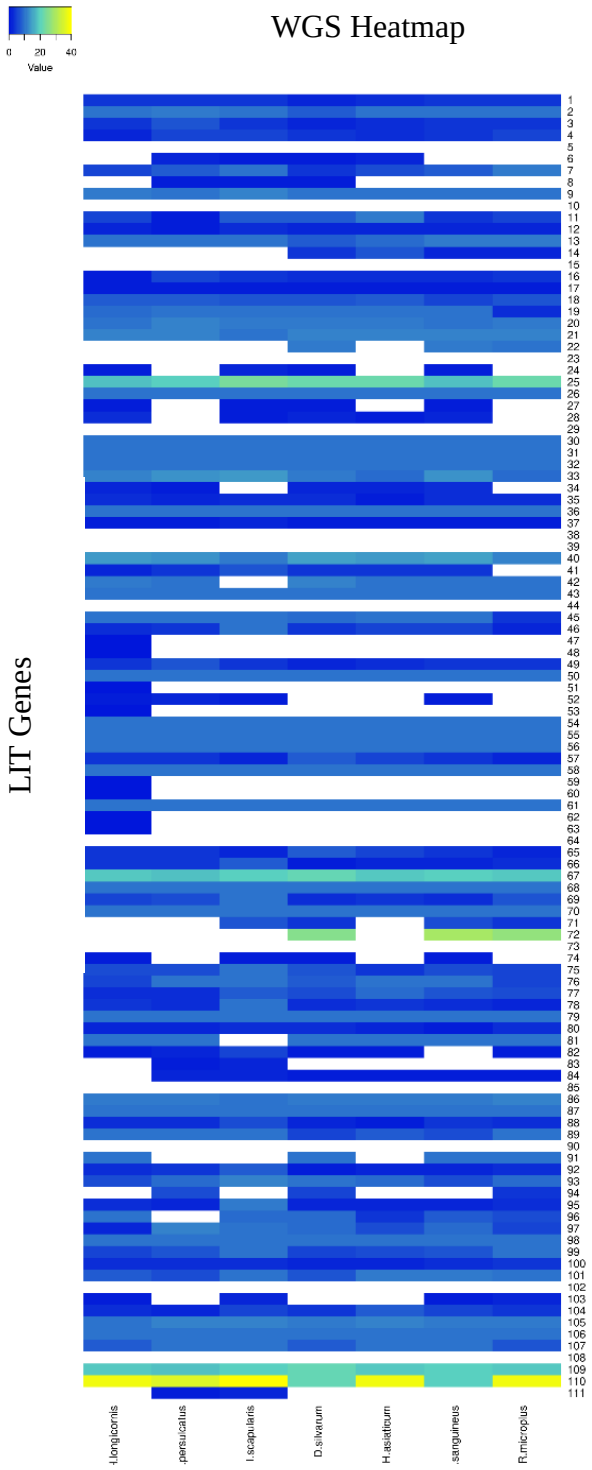

Figure 3: Heatmap showing the number of matches between the WGS and the 109 LIT genes, within the PIA workflow. As stated previously, the white space means that there were no matches for the specified gene in the sequence(s); the dark blue color denotes low number of matches; light blue denotes moderate number of matches, and yellow denotes a high number of LIT genes matches found within the WGS dataset.

DISCUSSION

Gq alpha is essential in activating the photoreceptors for phototransduction (Scott et al., 1995). Gq subunits activate phospholipase C, which hydrolyze phosphatidylinositol 4,5-biphosphate (PIP2) to inositol triphosphate (IP3), and diacylglycerol (DAG) (Mizuno & Itoh, 2009; Scott et al., 1995). IP3 activates calcium release, and DAG activates protein kinase C (PKC) (Mizuno & Itoh, 2009). IP3, and DAG, function in the propagation and amplification of the signal from the Gq subunit (Mizuno & Itoh, 2009; Scott et al., 1995). G-proteins allow the photoreceptor to be sensitive to light (Scott et al., 1995). Therefore, I can surmise that the ticks that possess high numbers of G-proteins, will be more sensitive to light (Scott et al., 1995 found this in *Drosophila*). As such, from the results I can conclude that *A. sculptum* and *I. scapularis* are highly sensitive to light, whereas the ticks that showed a fewer number of matches for Gq alpha may not be as sensitive as *A. sculptum*, and *I. scapularis*. That *A. sculptum* and *I. scapularis* show high sensitivity to light correlates with morphological structures of ticks within their respective genera. For example: *A. americanum* has around 40 photoreceptor neurons in its eyes; *I. ricinus* has almost 20 photosensitive cells that allow it to perceive changes in light. Even though I am not aware of the number of photoreceptors *A. sculptum* and *I. ricinus* may have, based on the number of photoreceptors that their congeners possess, I would infer that *A. sculptum* and *I. scapularis* may have a similar number of photoreceptors that allow them to detect light changes. *I. scapularis* also showed the highest number of matches for opsin, an essential protein that allows the phototransduction pathway to take place, changing light energy into electrical energy.

Lark has been found to interact in the circadian rhythm of *Drosophila,* and in monitoring the ecdysis of the adults (Huang et al., 2007). Thus, it is reasonable that this gene has been found in the transcriptomes and in the WGS. The counts of Lark ranged from 0 – 17 and 11 – 15 in the transcriptomes and WGS, respectively. In the transcriptomes, it might have been found in higher numbers than in the WGS because the ticks whose tissue was sampled were in the process of molting, so they showed higher numbers of lark to enable them to break out of their shells to go into their next life stage. Two of the sampled ticks were adults (*A. americanum*, *A. tuberculatum*), and another two were sampled as whole ticks (both were *A. americanum* ticks). Perhaps the other ticks whose transcriptomes were analyzed were in the process of becoming adults, and that is why they showed higher numbers for lark. The WGS exhibited a lower number of lark protein than the transcriptomes. This may be in part because the WGS must have been taken from adult ticks. Therefore, those ticks already went through their whole life stage, and they would not need as much lark protein as they might have had when they were in the process of advancing through their life cycle.

11-cis retinol dehydrogenase oxidizes 11-cis retinol into 11-cis retinal as part of the visual cycle, in the retinal pigment epithelium. As discussed earlier, when light hits 11-cis retinal, it changes to all-trans retinal (Cideciyan et al., 2000). The all-trans retinal must be disposed of, so that opsin is able to bind to 11-cis retinal which allows the visual cycle to continue (Cideciyan et al., 2000). Some transcriptomes showed low to moderate numbers of 11-cis dehydrogenase, and since this gene is involved in allowing the regeneration of the visual cycle, this may mean that the ticks that possess this gene may have the ability to perceive light in some way. The ticks that did not show matches for 11-cis retinol dehydrogenase may be able to

use another component, or pathway, to oxidize retinol to retinal. Something similar was studied by Cideciyan et al. (2000) in an investigation of the role of 11-cis retinal in a human individual who had a mutation in the RDH5 gene. However, the researchers found that in the individual with the mutated RDH5, the 11-cis retinal dehydrogenase pathway in the retinal pigment epithelium was not used, and they suggested that another oxidation pathway may have been used instead (Cideciyan et al, 2000).

D_Cry/CRY2, a blue light receptor gene, is also another gene that may be involved in the regulation of the circadian rhythm in *Drosophila melanogaster*, plants and humans (Egan et al., 1999). In *Drosophila*, extraretinal photoreceptors sensitive to blue light allow for the resetting of the biological clock (Egan et al., 1999). Researchers noted that eyeless and mutant *Drosophila* have the ability to adjust their clock with the variation of light in the environment (Egan et al., 1999). Other researchers noticed that *Drosophila* mutants degrade the timeless (light sensor) gene as part of their visual transduction process (Egan et al., 1999). CRY2 allows plants to flower (Egan et al., 1999). It is also shown that *Drosophila* are responsive to blue/green wavelengths in the regulation of their circadian clock (Egan et al., 1999). In the tick transcriptomes there were only two matches for D_Cry/CRY2 gene, and in the WGS there were matches across all the sequences, in low numbers. This suggests that ixodid ticks may, or may not, use D_Cry/CRY2 in the regulation of their circadian rhythm (Egan et al., 1999).

To conclude, we found that ixodid ticks' transcriptomes and WGS showed matches to some of the 109 LIT genes, regarding different components that may be involved in the phototransduction pathway. These components included Gq alpha, lark, 11-cis retinol dehydrogenase, and D_Cry/CRY2 gene (discussed above). Other genes that were found in the

transcriptome and WGS of ixodid ticks were recoverin, arrestin, phospholipase C, ovo (though it appears it is mostly responsible for spermatogenesis in mice; Dai et al., 1998), inaC, opsin, r_opsin, c_opsin, among other genes. These genes may suggest that ticks are sensitive to light, some by using potential extraretinal photoreceptors (*I. ricinus, H. dromedarii*); others by using their eyes (*A. americanum*), and others by using the Haller's organ to detect infrared light (*D. variabilis*). However, it is not known which pathways ticks utilize to change retinol to retinal and allow the visual cycle to resume when light hits retinal, which may be an interesting area to investigate in the future. Another area for future research may be to investigate if our findings could be further supported by performing phototaxis behavioral experiments in the ticks that we found to be highly light-sensitive.

APPENDIX: Gene names

Gene	Full gene name
1. Alas	aminolevulinate synthase
2. Aldh	Omega-crystallin
3. Arr1	Arrestin-1
4. black	cysteine sulfinic acid decarboxylase
5. CG1885	Uroporphyrinogen-III synthase
6. cinnabar (cn)	Kynurenine 3-monooxygenase
7. Clk	Clock
8. Clot	Glutaredoxin class of the Thioredoxin-like enzyme superfamily
9. Cng	cGMP-gated cation channel alpha-1
10. Coprox	Coproporphyrinogen-III oxidase
11. Cry	drosocrystallin (D_crystallin)
12. cwo	clockwork orange
13. cyc	Cycle
14. dac	dachsund
15. Dat	Dopamine N-acetyltransferase
16. Ddc	DOPA decarboxylase
17. Dhpr	dihydropteridine reductase
18. dpp	decapentaplegic
19. ebony/tan	NBAD synthase/ hydrolase (NBAD)
20. egfr	epidermal growth factor receptor
21. en	engrailed
22. ey	eyeless
23. Eya	eyes absent
24. Fech	Ferrochelatase
25. Galpha49B	Gq_alpha
26. Gbeta76C	Gq_beta
27. Ggamma30A	Gq_gamma
28. Ggamma30A	Gngt1
29. G-ialpha65A	guanine nucleotide-binding protein G(t) subunit alpha-1
30. Gl	glass
31. Gprk1	G protein-coupled receptor kinase 1
32. Gprk2	G protein-coupled receptor kinase 2
33. GstS1	S-crystallin
34. hh	hedgehog
35. hoepel1	Melanocyte-specific transporter protein
36. inaC	Protein kinase C

37. Kfase	Kynurenine formamidase (KF)
38. l(3)02640	Porphobilinogen deaminase (Pbgd)
39. laccase2	Laccase2
40. lark	RNA-binding protein lark
41. Mitf	Microphthalmia-associated transcription factor
42. N	notch
43. N/A	Rhodopsin kinase
44. N/A	Retinal guanylyl cyclase 2
45. Rcvrn	Recoverin
46. N/A	Regulator of G-protein signaling 9
47. N/A	Regulator of G-protein signaling 9-binding protein
48. N/A	Rhodopsin
49. N/A	S-arrestin
50. N/A	Retinal-specific ATP-binding cassette transporter
51. N/A	Lecithin retinol acyltransferase
52. N/A	Retinol-binding protein 1
53. N/A	Retinol-binding protein 3
54. N/A	11-cis retinol dehydrogenase
55. N/A	retinol dehydrogenase 8
56. N/A	Retinaldehyde-binding protein 1
57. N/A	Retinoid isomerohydrolase
58. N/A	Retinal-binding protein (RALBP)
59. N/A	tyrosinase
60. N/A	reflectin 1a
61. N/A	Alpha-crystallin A chain (A_crystallin)
62. N/A	beta-crystallin A1 (B_crystallin)
63. N/A	J_crystallin
64. N/A	cryptochrome 1/2
65. ninaB	neither inactivation nor afterpotential B
66. ninaD	neither inactivation nor afterpotential D
67. ninaE	neither inactivation nor afterpotential D
68. ninaG	neither inactivation nor afterpotential G
69. norpA	Phosphoinositide phospholipase C
70. oc	ocelliless
71. Optix	Optix
72. ovo	ovo
73. Pbgs	Delta-aminolevulinic acid dehydratase
74. Pcd	Pterin-4-alpha-carbinolamine dehydratase
75. Pde6	Rod cGMP-specific 3',5'-cyclic phosphodiesterase subunits a/b/c
76. Pdfr	Pigment-dispersing factor receptor, isoform A

77.	Pdp1	PAR-domain protein 1 (par)
78.	per	period
79.	pinta	prolonged depolarization afterpotential is not apparent
80.	ple	tyrosine hydroxylase
81.	Pph13	PvuII-PstI homology 13
82.	Ppox	Protoporphyrinogen oxidase
83.	pr	6-pyruvoyl tetrahydrobiopterin synthase
84.	PrBP	Retinal rod rhodopsin-sensitive cGMP 3',5'-cyclic phosphodiesterase subunit delta
85.	proPO	Prophenoloxidase subunit 1
86.	Pu	GTP cyclohydrolase 1
87.	rdgA	Eye-specific diacylglycerol kinase
88.	rdgB	retinal degeneration B
89.	rdgC	retinal degeneration C
90.	rosy (ry)	Xanthine dehydrogenase
91.	Rx	retinal homeobox
92.	santamaria	scavenger receptor acting in neural tissue and majority of rhodopsin is absent
93.	sepia (se)	glutathione S-transferase omega 1
94.	Six4	Six4
95.	slo	slowpoke
96.	so	sine oculis
97.	Sptr	sepiapterin reductase
98.	st/bw	scarlet/brown
99.	tgo	tango
100.	timeout	timeless
101.	trp	Transient receptor potential protein
102.	Updo	Uroporphyrinogen decarboxylase
103.	vermilion	Tryptophan 2,3-dioxygenase
104.	vri	vrille
105.	Vsx1/2	visual system homeobox
106.	W	white
107.	Wg	wingless
108.	yellow	dopachrome conversion enzyme (DCE)
109.	c_opsin	c_opsin
110.	Opsin	opsin
111.	Cpox	Cpox

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