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REVIEW

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All the better to see you with: a review of odonate color vision with transcriptomic insight into the odonate eye

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Abstract Although dragonflies and damselflies (Insecta: Odonata) represent some of the most advanced visual systems among insects, odonate visual systems are not as well understood as those of model or more economically important insects. Yet, with their large and complex eyes, aquatic and terrestrial life stages, entirely carnivorous lifestyle, exceptional mating behaviors, diversity in coloration, occupancy of diverse light environments, and adult success that is completely dependent on vision, it would seem studying the visual system of Odonata at the molecular level would yield highly rewarding scientific findings related to predator/prey interactions, the physiological and molecular shifts associated with ecological shifts in light environments, and the role of vision on behavioral ecology. Here, we provide a review of odonate color vision. The first odonate opsin sequences are published using a degenerate PCR approach for both dragonfly and damselfly lineages as well as a transcriptome approach for a single species of damselfly. These genetic data are combined with electrophysiology data from odonates to examine genotype/phenotype relationships in this visual system. Using these data, we present the first insights into the evolution and distribution of the visual pigments (opsins) among odonates. The integration of molecular and behavioral studies of odonate vision will help answer long-standing questions about how sensory systems and coloration may coevolve.

Keywords Mike May Festschrift · Odonata · Vision · Opsins · Color

Seth M. Bybee and Kelsy K. Johnson contributed equally to this work.

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Introduction

Insects are the most diverse animal group on the planet and their diversification has been accompanied by an astonishing array of behavioral and physiological innovations. The visual system is no exception to this pattern. Insects display an astonishing array of morphologically, ecologically, and functionally different visual systems (Nilsson 1989; Nilsson and Kelber 2007). Further, major differences in visual ability and visual communication are observable both within and across insect groups (e.g., Roberts et al. 2011; Maksimovic et al. 2011; Bybee et al. 2012; Briscoe et al. 2010; Yuan et al. 2010; Maksimovic et al. 2009; Osorio and Vorobyev 2008; Wakakuwa et al. 2007; Briscoe and Chittka 2001). While the quality of insect vision is generally poorer than that of vertebrates, most insects have the ability to see light in a wider range of wavelengths. Thus, many insects have the potential to both forage and communicate in a spectral realm that is unobservable to the unaided human eye.

Much of our understanding of insect vision results from anatomical studies of insect sensory systems. The "simple" eyes of insects consist of a single structure called an ommatidium. Simple eyes range in visual ability from only light sensitive to image forming. They are found amongst most insects in the form of ocelli on the dorsal surface of the adult head, or as the functional eyes of holometabolous larvae (Land and Nilsson 2002). Compound eyes contain many ommatidia and are the most prevalent eye type among adult insects and immature hemimetablous insects. The type and number of ommatidia within a single compound eye varies across insects. For example, some primitive, cave and leaf litter dwelling insects have only a few ommatidia or are blind and have lost their eyes all together (e.g., *Glacicavicola bathyscoides*), while dragonflies have up to ~30,000 ommatidia (Land and Nilsson 2002). Compound eyes are capable of forming a clearer image and can more efficiently track movement than is possible with the simple eye.

The ommatidia of the insect eye are composed of rhabdomeric photoreceptors and pigment cells (Fig. 1). The rhabdom, a transparent rod found in the center of each ommatidium guides light into the ommatidium, and an image is formed by the average of all the light that has entered each of the rhabdoms (Wehner 1981). The quality of the image that is viewed is determined by the angle that is formed between the ommatidia (Nilsson 1989; Land and Nilsson 2002). Rhabdoms can have open, fused, or tiered structures, which help to determine a photoreceptor cell's spectral sensitivity to light (Snyder et al. 1973). The photoreceptor cells are essentially special sensory neurons that receive light (Kirschfeld 1976). An insect's spectral sensitivity is also determined by the number and diversity of opsins (the genes responsible for color vision) (Chang et al. 1995) and in some cases the screening pigments associated with the ommatidia (Stavenga 2002). Different parts of the eye are capable of capturing different wavelengths of light (Chittka 1997; Endler 1993; Lythgoe 1972; Cronin et al. 2000; Hardie 1986; Peitsch et al. 1992; Warrant and Nilsson 1995), which results from differences in the relative proportions of alternative opsins among regions of the compound eye (Laughlin 1976).

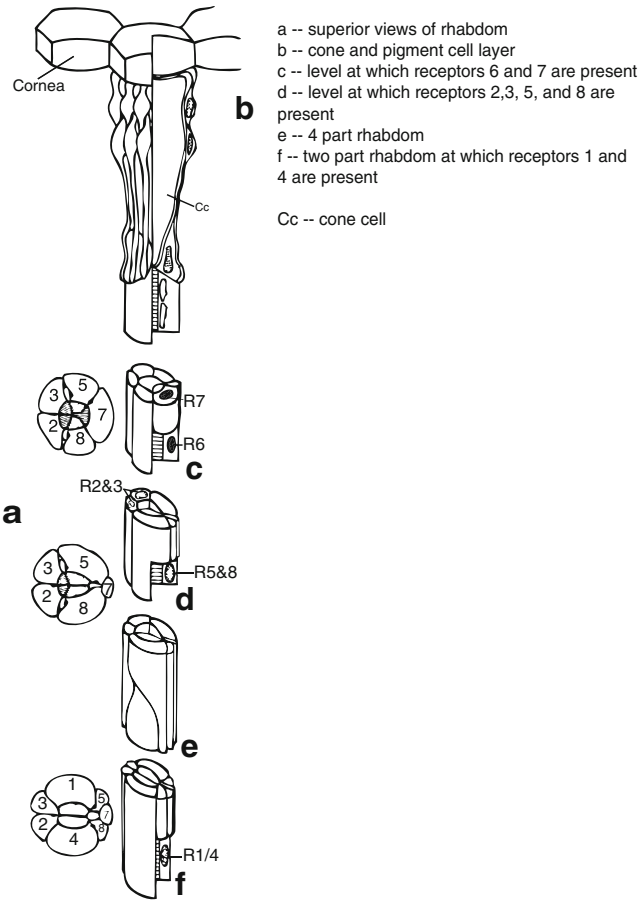

Fig. 1 Diagram of the ventral ommatidium of *Sympetrum* (redrawn from Armett-Kibel and Menertzhagen 1983)

Within some insect groups, the ecological constraints imposed on vision are comparatively well understood. For example, molecular, behavioral, and neuroanatomical data have recently been integrated to determine the perceptibility of visual displays to conspecifics, mutualists, and enemies in some arthropods (e.g., Morehouse and Rutowski 2010; Defrize et al. 2010; Bybee et al. 2012). By contrast, equally complex visual models are presently lacking for odonates (but see Olberg et al. 2000, 2005, 2007), despite their exceptional diversity in visual systems and ecotypes. Filling this gap has obvious potential to illuminate the limits and possibilities of visual system evolution. Below, we provide a review of current knowledge for the odonate visual sensory system. We also present a catalogue of spectral variation observable among odonates and assessments of whether such variation might be coevolving with the odonate eye. Finally, in an effort to characterize the color vision system of an odonate at the molecular level, we produce the first visual transcriptome for Odonata (Zygoptera: *Telebasis salva*). We discuss our findings within the broader context of electrophysiological studies from the literature and some preliminary efforts using degenerate primers and PCR to screen for LW opsins using genomic DNA (gDNA).

Current knowledge of odonate vision

Anatomy and development of odonate compound eyes

The odonate eye has long been recognized to represent the pinnacle of insect visual capacity. For example, the eyes of dragonflies in the family Aeshnidae contain more ommatidia than any other known insect (Land and Nilsson 2002). The odonate eye is the largest of all insect eyes, having an

average diameter of 8 mm (Land and Nilsson 2002) and the ability to perceive light in all directions with the exception of the area directly behind the head (Corbet 1999). Odonate naiads (immature odonates) also possess very large eyes, and during metamorphosis, the ommatidia multiply and the eye expands greatly. Immature odonates have ~170 ommatidia and by maturity adults have ~8000 ommatidia in each eye (Corbet 1999; Sherk 1978a, b), with some species achieving much higher numbers of ommatidia (e.g., *Anax junius* with 28,672 ommatidia present in each eye at maturity; Land and Nilsson 2002).

Ommatidia in naiad eyes are slightly different from those of the adults. Odonate naiad eyes have light and dark bands that develop and lose color with age (Corbet 1999). However, like adults, the naiad ommatidia appear to have receptors for a wide range of wavelengths, including ultraviolet, violet, green, and orange (Seki et al. 1989), though this has not been confirmed at the molecular level. Because the ommatidia develop from the anterior, ommatidia of the naiad will become dorsal ommatidia upon metamorphosis into the adult life stage (Sherk 1978a; Schaller 1960). It seems likely that the developmental changes of the eye across life stages may represent adaptations to differences in the light environments inhabited by immatures (usually aquatic) and adults (terrestrial).

Odonate color vision

Understanding the sensory systems on which selection can act provides greater insight into not only visual system but signal evolution as well (e.g., Cummings 2004). We are curious about what odonate color vision encompasses at the molecular level (opsin diversity), specifically in comparison to other insects groups (e.g., butterflies). Both butterflies and the Odonata have several commonalities. Odonates are comparable in body and wing color, structural pigments, behavior (particularly mating behavior), and habitat types. Additionally, both groups appear to use visual cues for mate recognition. Yet, Odonata differ from butterflies by being generalist predators (rather than feeding on plants, detritus, pollen and/or dung), and lack stringent requirements for host plant identification. Thus, whereas butterfly color vision is subject to selection for a diverse array of specialized tasks (e.g., Briscoe et al. 2010), odonate vision might evolve under less numerous selection regimes.

The odonate eye can detect color from the ultraviolet (UV) (~300 nm) to the long wavelength (LW) (~700 nm) portion of the visible spectrum and is capable of discriminating polarized light (Roberts et al. 2011; Mayer and Labhart 1993; Yang and Osorio 1991; Armett-Kibel and Menertzhagen 1983; Meinertzhagen et al. 1983). Past studies of electrophysiology have demonstrated that dragonflies have from three to five opsin copies for detecting color in different parts of the light spectrum (e.g., *Hemicordulia tau*, UV (330 nm), violet (410 nm), blue (460 nm), green (525 nm) and red (630 nm); Yang and Osorio 1991). A single electrophysiology study of the damselfly eye (Lavoie-Dornik et al. 1988) reanalyzed by Schultz et al. (2008) suggested that the damselfly *Enallagma cyanthigerum* has at least three opsins in the UV (366 nm), blue (B) (410 nm) and green (525 nm) portions of the light spectrum. The findings from these studies have never been verified by an independent molecular screening of the opsins.

Odonate color vision demonstrates retinal specialization. Ommatidia located on the dorsal surface of the eye are more sensitive to UV (~300-400 nm) and B (~401-500 nm) light. Thus, allowing these specialized retinas to more fully view objects against the bright background of the sky (Hisada et al. 1965). Acute zones of the eye contain larger ommatidia that allow more photons to come through per unit. The reduction of diffraction among these larger lenses results in the formation of a clearer image (Land 1981, 1989; Corbet 1999). The acute zones of the dragonfly eye vary by species and are largely dependent on lifestyle and habitat (Corbet 1999). This is especially helpful while hunting in the mornings or evenings and provides an extremely vivid background image that allows small moving objects to be easily spotted (Pritchard 1966; Sherk 1978b, c). Odonates are generalist predators feeding primarily on small insects (e.g., Diptera), but some dragonflies do take larger prey (e.g., bees, wasps and butterflies). Most adult feeding is done in and around terrestrial aquatic environments, where prey is detected against vegetation, the sky, or both as a backdrop.

Functions of body and wing color variation

Although Odonata is a relatively small insect order (~6,000 spp), it encompasses an extraordinary diversity of wing and body coloration and patterns (Fig. 2). To the human eye, many odonates are highly visually conspicuous, which raises the questions of what function coloration serves and how it evolves (e.g., Fuller et al. 2010). The prevalence of sexual dimorphism in color throughout this group and its starring role in many conspecific displays suggests the possibility that color may commonly evolve to serve communication purposes. Visual communication is likely paramount for odonates in recognizing conspecific mating partners and sexual rivals, as there appears to be little evidence for auditory, touch, or chemical communication among adults throughout the order (but see Rebora and Piersanti 2010).

Species recognition

Gorb (1998) conducted studies of coenagrionid damselflies in which he manipulated morphology and coloration of

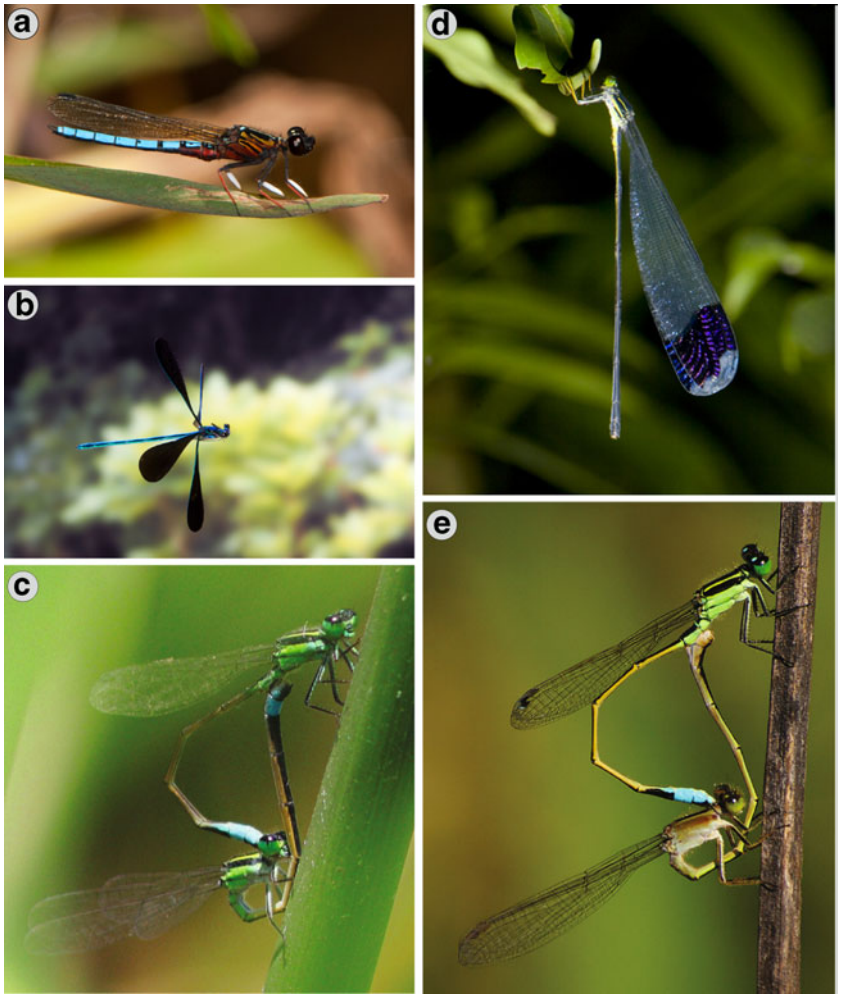

Fig. 2 Image representing the body and wing coloration of damselflies (**a-e**). (**a**) *Platycyphya caligata* courtesy of J. Abbott. (**b**) *Calopteryx maculata* courtesy of J. Abbott. (**c**) An andromrophic mating wheel of *Ischnura ramburii* with male on top and andromorph female on the bottom. Courtesy of S. Coleman. (**d**) *Megaloprepus coerulatus* courtesy of T. Davenport. (**e**) An gynomrophic mating wheel of *Ischnura ramburii* with male on top and gynomorph female on bottom. Courtesy of S. Coleman

female models. This work shows that color is an essential component of mate recognition; males were responsive to a subset of models only when a species typical coloration was provided. Further evidence for the role of color in species recognition comes from parapatric damselflies in the family Calopterygidae. In an unusual form of character displacement, species differences in male traits (wing spotting) are exaggerated in sympatry, apparently due to selection to prevent (presumably unprofitable) interspecific territorial aggression (e.g., Tynkkynen et al. 2004; Anderson and Grether 2009). These same traits are also used by females in this group to identify conspecific mates (Svensson et al. 2007). Schultz et al. (2008) demonstrated that male coloration was more conspicuous during the time of day they were actively searching for females, suggesting that males of different species are more noticeable to the damselfly visual system due to light environments created by changes in sunlight position. We suspect that further studies of closely-related odonate taxa with different body and/or wing colors will reveal further support for color's role in intra- and interspecies recognition (Shaw and Mullen 2011).

Sex-limited color polymorphism

While male-limited color polymorphism has only been described in a small number of odonates, female-limited polymorphism occurs in >100 species (Fincke et al. 2005). The prevalence of female-polymorphism is commonly thought to reflect adaptation to sexual conflict (reviewed by Van Gossum et al. 2008). Polymorphic odonates are usually comprised of a female morph with male-like coloration (the "andromorph"; Fig. 2c), which co-occurs alongside counterparts that have distinctive patterns and/or colors ("gynomorphs", or "heteromorphs"; Fig 2e). Alternative female morphs are differentiated by allelic variation in all studied cases (see Van Gossum et al. 2008), suggesting the operation of balancing selection in polymorphism maintenance.

One hypothesis holds that andromorph females mimic conspecific males in order to avoid excessive male–female interactions (Robertson 1985). Alternatively, males may learn to recognize the prevailing morph within a population, exerting frequency-dependent harassment on either morph

(e.g., Fincke 2004). Whereas a single copulation provides ample viable sperm for a female's reproductive life (e.g. Fincke 1987; Cordero 1991), males can mechanically remove stored sperm with their genitalia (Waage 1979). The removal of another males stored sperm from females through repeated mating bouts may decrease female fitness in the process by inhibiting foraging (Sirot et al. 2003), elevating predation risk (Forbes 1994), and/or imposing physical trauma. Although the precise mechanism by which male–female interactions maintain polymorphism remains unclear (e.g., Sirot et al. 2003; Cooper 2010) and may vary according to species, sexual conflict's role in maintaining color polymorphism has been extensively supported by field and lab studies (e.g., Svensson et al. 2005; Takahashi et al. 2010). The widespread incidence of female-limited polymorphism thus showcases the vast and untapped potential of odonates for investigating the coevolution of color and color vision.

By way of example, within the female-polymorphic genus *Ischnura,* even closely related taxa often differ in the presence and absence of female polymorphism and/or in the spectral properties of developmental and genetic morphs (e.g., Van Gossum et al. 2010). These differences will result in varying challenges for, and selection on, the visual systems of mate-searching males. Yet, while proposed mechanisms of color polymorphism maintenance emphasize morph differences in detectability to males and/or predators, studies that manipulate color are presently lacking (but see Iserbyt and Van Gossum 2011), as are details of the visual systems involved (but see Schultz et al. 2008). Hence, the sensory ecology of sex-limited polymorphism remains relatively unexplored.

Other examples of color variation

Throughout the lifespan of Odonata, many undergo various color changes, particularly those of aeshnids (Paulson 1966), corduliids (Williams 1976), petalurids (Clement and Meyer 1980) and coenagrionids. Many color changes in Odonates are associated with increasing age and sexual maturation (Ueda 1989). For example, the wings of *Aeshna cyanea* change color as they become more sexually mature (Kaiser 1985). Their wings may become more pigmented and lose their clear appearance (Corbet 1999). It is usual for the wings of some Anisoptera such as *Nannophya pygmaea* to attain a white wing margin after sexual maturation (Yamamoto 1968).

Although many of the examples cited above show how sexual selection can act on odonate phenotypes, there is also evidence to suggest that natural selection imposed by physical and/or ecological environments can diversify odonate color. For example, in the Hawaiian endemic damselfly *Megalagrion calliphya,* clinal variation in male and female color may have evolved in response to spatial variation in the need for antioxidant protection from ionizing solar radiation (Cooper 2010). In a handful of taxa in the families Polythoridae and Pseudostigmatidae, Batesian mimicry is thought to have evolved to deter predators. Mimetic taxa have been proposed to resemble sympatric, toxic butterflies (Corbet 1999, Pers. Comm. R. Reed) and even aposematic black and yellow to mimic bees and wasps (e.g., *Nannothemis bella*). The role of predation in odonate color evolution is further suggested by occurrences of pronounced sexual dimorphism in most dragonfly and damselfly species. In dimorphic taxa, females typically exhibit more drab colors than males, a likely consequence of selection for crypsis by visually orienting predators (e.g., birds and even other odonates) and by sexual asymmetry in sexual selection.

Materials and methods

Taxon sampling

Our targeted taxa for these PCRs were *Phenes raptor*, *Petalura gigantea*, *Rionaeschna multicolor*, *Lestes congener* and *Libellago semiopaca*. Transcriptome sequencing of the visual tissues was conducted on a single taxon, *Telebasis salva*. We chose to target a damselfly for transcriptomics because physiological estimates of opsin diversity for Odonata are focused almost entirely within the dragonflies.

DNA extraction

Genomic DNA was extracted using the QIAGEN DNeasy extraction kit. Each PCR reaction was amplified using degenerate LW opsin primers (IVRH-F5: GCGAATTCCGS GANCARGCMA; IVRH-R8: YGTNTAYKSYRTHGGAT CCCG; Hariyama et al. 1993) on ABI 9700 thermal-cycling machines using the following protocol and Elongase Taq (92°C (2 min) [92°C (1 min) 50°C (1 min) 68°C (1 min 30 s)×40 cycles] 68°C (7 min)). The resulting DNA fragment from this primer set and protocol yielded a DNA fragment of ~550 bp that included an intron of ~300 bp. We tried several combinations of primers from the literature and those designed in the lab but were unable to isolate either UV or B sensitive opsins form gDNA from our chosen taxa. Electrophoresis was performed on a 1 % agarose gel to identify successful reactions and monitor for contamination. Products were cleaned using a PrepEase Purification (USB Corporation) 96-well plate, followed by a reaction to add a 3' A-overhang for cloning. The products were then cloned using the Invitrogen TOPO TA Cloning kit (Invitrogen Corporation). We cultured the clones on Luria Broth plates containing ampicillin (50 mg/ml) and X-gal (40 mg/ml) and allowed them to incubate for 12–16 hours. After incubation

(37°C for 16–24 hours), 50 colonies were picked from the successful clones and added to PCR tubes containing 50 μl of a 1xTE Buffer solution. We PCR amplified the colonies with the M13 primers. The products were size selected and then sequenced using BigDye (Applied Biosystems Inc., version 3.1) chain terminating chemistry, cleaned using sephadex (GE Healthcare) and fractionated on the ABI 3730 XL DNA Analyzer (BYU DNA Sequencing Center). We cleaned the sequences using sephadex (GE Healthcare) and then submitted them to the BYU DNA Sequencing Center (DNASC). The sequences were assembled and edited using Sequencher 4.9 (GeneCodes Corporation). We performed a nucleotide BLAST search on each sequence using the NCBI BLAST function to identify the spectral class of each opsin sequence through functional homology.

Transcriptome

RNA was extracted from the head of a 7–8th instar naiad (estimated from size, ~1.5 cm, and wing buds not being longer than the width of the head) using a Nucleospin® RNA II column. Approximately 1 μg of RNA was used to construct a cDNA library using Clontech's SMARTer cDNA kit and standard protocol. Following first strand synthesis and cDNA amplification the cDNA was purified using a Qiagen Qiaquick PCR Purification kit. Purified cDNA was fragmented to ~600 bp via nebulization and further purified using two MinElute PCR Purification kit columns. Ends of the purified, nebulized cDNA are polished and ligated with GS FLX Titanium primers and small fragment cDNAs are removed via AMPure beads. The ligation is tested via PCR to ensure that the proper adapters were ligated. Following adapter verification another PCR is carried out to optimize for the number of cycles to get a consistent visible smear between 300–2000 bp on a gel. Once the optimal cycles is determined another round of PCR is carried out to amplify the cDNA followed by gel purification using a 1 % MetaPhor Agarose gel with 1× TAE. From this gel the 500–700 bp swath is removed and gel purified using the QiaQuick Gel Extraction kit. The cDNA was then included in an emulsion PCR and prepared for 454 sequencing using the GS FLX Titanium General Library Preparation Method Manual. Transcriptome sequencing was performed on the Roche 454 platform through the BYU DNA Sequencing Center.

Results

From the degenerate PCR, we were able to isolate only the LW opsin copies. Other attempts to design degenerate primers to isolate and amplify the UV and B opsins from gDNA were not successful. From our small taxon sampling, we found a single LW copy for all odonates sampled. The transcriptome approach was much more successful in isolating multiple opsin classes. We recovered one UV, B and LW from *T. salva.* These data combined with what is known from other degenerate PCR efforts (Jananda 2011) and physiological estimates are summarized in Fig. 3.

Discussion

Odonate opsin evolution

Taking all data together (electrophysiology, degenerate PCR and transcriptome) and mapping these data on an established phylogenetic estimate for the group (Bybee et al. 2008), the first glimpse for the evolution of the odonate color vision systems is possible. There is evidence that all three opsin copies are found throughout the order, with two exceptions where physiological data did not provide evidence for UV and B in *Libellula needhami* and *Aeshna tuberculifera*, respectively (but this is a negative result and still must be corroborated by an independent molecular investigation of the opsins). Both B and LW appear to have duplicated during odonate evolution. Both LW and B classes appear to have been duplicated in the most derived lineage of dragonflies (Libelluloidea). One libelluloid (*Libellula needhami*) appears to have a simplified visual system compared to its close relatives (Horridge 1969). However, we make this point with caution as this is only one species from a superfamily of ~1000 species.

It is likely that the ancestral condition for color vision involved only a single copy of each opsin class. The more primitive lineage of dragonflies and all damselfly lineages represented in this study support this hypothesis (Fig. 3). Damselflies appear to have a simple color vision system consisting of a single copy from each opsin class. However, the taxon sampling is skewed toward pond damselflies (Coenagrionidae) and it is likely that as the sampling expands to include groups with more color and behavioral complexity (e.g., more calopterygoids or even the polymorphic coenagrionids), we will encounter more complex visual systems.

Color and color vision

It is interesting to note that the most derived lineages from dragonflies (libelluloids) and damselflies (pond damselflies), which are also the most diverse lineages in their respective suborders, appear to have visual systems evolving in two completely different fashions (Fig. 3). The majority of wing color diversity among dragonflies is contained among the libelluloids and this is perhaps one explanation for the large diversification of opsins within this

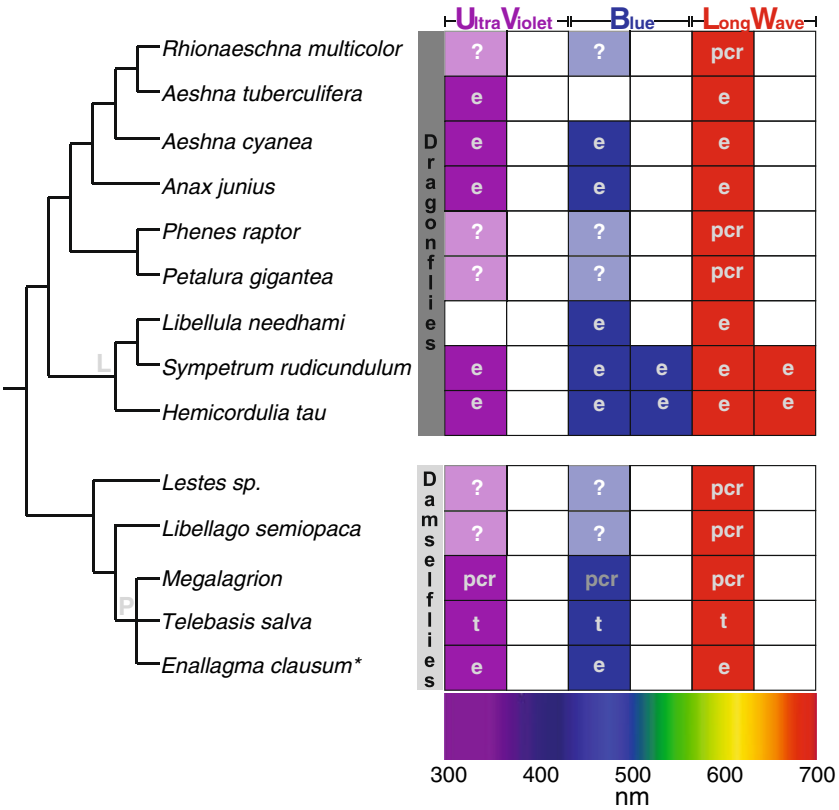

Fig. 3 Synthesis of data derived from research focused on odonate color vision systems using techniques in electrophysiology (e), polymerase chain reaction (pcr) and transcriptomics (t). Where data are uncertain a "?" is placed. Lighter colored boxes represent predictions of what we anticipate will be found with continued efforts to discover the molecular composition of the odonate color vision. Phylogeny simplified from Bybee et al. (2008). The clade containing Libelluloidea is represented by an "L" and a "P" designates the pond damselfly clade

lineage. The other extremely speciose lineage of odonates is Coenagrionidae or pond damselflies. Compared to more spectacularly ornamented groups, pond damsels are not generally characterized as being exceptionally colorful, although some lineages are certainly colorful and others have color-centered behaviors (e.g., female polychromatism). Based on our results for *T. salva,* they appear to have retained the ancestral color vision system. We note, however, that this diverse group merits further study. For example, given the visual challenges female polychromatism presents for mate-searching males, it will be interesting to see what visual systems are apparent within color-polymorphic species.

The visual systems of the more spectacularly ornamented odonates present exciting research opportunities. *Lestes disjuncta* (Lestidae) and Coenagrionidae represent the most primitive and derived damselfly lineages respectively and both groups having only a single LW copy, which is not entirely surprising as neither group is known for extensive color or color centered behaviors. That *Libellago semiopaca* did not have any detectable LW opsin duplicates is surprising. Perhaps, due to degenerate primers that are not optimized to pick-up opsin duplicates among odonates. *L. semiopaca* is a member of the Chlorocyphidae, one of the most colorful of all odonate groups, and a group that is well known for its charismatic courtship behaviors that have color as a central focus (Fig. 2a). Despite these findings, it is very likely that a more extensive and intensive (e.g., transcriptomic) investigation of the visual pigment genes among species from this family as well as others from its superfamily (Calopterygoidea) is likely to reveal a complex and unique evolutionary history of color vision.

Our limited taxon sampling combined with a degenerate primer/PCR approach that resulted in a 219 bp fragment (once the intron was removed) of the LW opsin gene does not makes it easy to draw any broad conclusions concerning odonate opsins generally. This compounded with the scarcity of opsins from non-holometablous insects for close comparison makes it difficult to identify any major differences between odonate opsins and those of other insects. When comparing the UV opsin genes from several Hawaiian *Megalagrion* species (Jananananda 2011) it appears that the UV opsin among pond damselflies is highly conserved at the amino acid level, but extremely divergent (as expected) from Orthoptera UV opsins (Supplemental Figure 1). For the LW opsin our data set has the most overlap among sequences beginning after helix V and terminating just before the end of Helix IIV. Again, the amino acids among the Odonata LW opsins are conserved. Comparing the full length LW from *T. salva* with orthopteran opsins shows that ~17 % of amino acid changes are odonate specific (Supplemental Figure 2). An alignment of *T. salva's* B opsin with an orthopteran B opsin can be found in Supplemental Figure 3.

How complete are the data for estimates of odonate color vision genes?

Even though our current view of odonate visual evolution is still very much incomplete, we present the most comprehensive view to date and the first ever estimates of opsin gene expression from odonate visual tissue. There are three main limitations of this current study which include (1) incompleteness of taxon sampling, (2) reliance on elector-physiological data, and (3) negative results obtained via PCR due to primer issues. For example, we know from physiological data alone that the odonate eye, especially for dragonflies, appears to be much more complex than what was recovered from the degenerate PCR. Comparable limitations are also present in physiological estimates of opsins, where there are many complex measurements that must be taken to produce an excellent estimate. The advantage of the transcriptome approach is that it does not rely on primer directed PCR, is very sensitive and usually yields full-length genes. However, transcriptomics, as with physiological data, is limited by what genes are expressed in the tissue (i.e., ommatidia) examined. For example, our transcriptome is from an immature *T. salva*. It could be that the copies we recovered using a transcriptome approach were those expressed during the immature stage and that additional opsin copies will be expressed during the adult life stage.

Moving forward

This research represents only a first pass toward a more in-depth examination of the evolution of odonate visual systems at the molecular level that will involve transcriptomics, directed sequencing, and degenerate PCR. We find compelling molecular evidence that odonate color vision is as complex as physiological data suggest and very likely as diverse as what has been observed in other insect groups with similar behavioral adaptations and ecological niches. We anticipate that further research on odonates, the pinnacle of insect visual acuity, will provide a rich and diverse view of the evolution of color vision.

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References

Anderson, C. N., & Grether, G. F. (2009). Interspecific aggression and character displacement of competitor recognition in *Hetaerina* damselflies. *Proceedings of the Royal Society B, 277*, 549–555.

Armett-Kibel, C., & Menertzhagen, I. A. (1983). Structural organization of the ommatidium in the ventral compound eye of the dragonfly *Sympetrum*. *Journal of Comparative Physiology A, 151*, 285–294.

Briscoe, A. D., & Chittka, L. (2001). The evolution of color vision in insects. *Annual Review of Entomology, 46*, 471–510.

Briscoe, A. D., Bybee, S. M., Bernard, G. D., Yuan, F., Sison-Mangus, M. P., Reed, R. D., et al. (2010). Positive selection of a duplicated ultraviolet-sensitive visual pigment coincides with wing pigment evolution in *Heliconius* butterflies. *Proceedings of the National Academy of Sciences, U.S.A., 107*, 3628–3633.

Bybee, S. M., Ogden, T. H., Branham, M. A., & Whiting, M. F. (2008). Molecules, morphology and fossils: A comprehensive approach to odonate phylogeny and the evolution of the odonate wing. *Cladistics, 24*, 477–514.

Bybee, S. M., Yuan, F., Ramstetter, M. D., Llorente-Bousquets, J., Reed, R. D., Osorio, D., et al. (2012). UV photoreceptors and UV-yellow wing pigments in *Heliconius* butterflies allow a color signal to serve both mimicry and intraspecific communication. *The American Naturalist, 179*, 38–51.

Chang, B. S. W., Crandall, K. A., Carulli, J. P., & Hartl, D. L. (1995). Opsin phylogeny and evolution: A model for blue shifts in wavelength regulation. *Molecular Phylogenetics and Evolution, 4*, 31–43.

Chittka, L. (1997). Bee color vision is optimal for coding flower colors, but flower colors are not optimal for being coded—why? *Israeli Journal of Plant Science, 45*, 115–127.

Clement, S. L., & Meyer, R. P. (1980). Adult biology and behavior of the dragonfly *Tanypteryx hageni* (Odonata: Petaluridae). *Journal of the Kansas Entomological Society, 53*, 711–719.

Cooper, I. A. (2010). Ecology of sexual dimorphism and clinal variation of coloration in a damselfly. *The American Naturalist, 176* (5), 566–572.

Corbet, P. (1999). *Dragonflies: Behavior and ecology of Odonata*. Ithaca: Cornell University Press.

Cordero, A. (1991). Fecundity of *Ischnura graellsii* (Rambur) in the laboratory (Odonata: Coenagrionidae). *Odonatologica, 20*(1), 37–44.

Cronin, T. W., Jarvilehto, M., Weckstrom, M., & Lall, A. B. (2000). Tuning of photoreceptor spectral sensitivity in fireflies (Coleoptera: Lampyridae). *Journal of Comparative Physiology A, 186*, 1–12.

Cummings, M. E. (2004). Modelling divergence in luminance and chromatic detection performance across measured divergence in surfperch (Embiotocidae) habitats. *Vision Research, 44*(11), 1127–1145.

Defrize, J., Théry, M., & Casas, J. (2010). Background colour matching by a crab spider in the field: A community sensory ecology perspective. *The Journal of Experimental Biology, 213*, 1425–1435.

Endler, J. A. (1993). The color of light in forests and its implications. *Ecological. Monographs, 63*, 1–27.

Fincke, O. M. (1987). Female monogamy in the damselfly *Ischnura verticalis* Say (Zygoptera: Coenagrionidae). *Odonatologica, 16*, 129–143.

Fincke, O. M. (2004). Polymorphic signals of harassed female odonates and the males that learn them support a novel frequency-dependent model. *Animal Behavior, 67*, 833–845.

Fincke, O. M., Jödicke, R., Paulson, D., & Schultz, D. T. (2005). The evolution and frequency of female color morphs in Holarctic

Odonata: Why are male-like morphs typically the minority? *International Journal of Odonatology, 8*, 183–212.

Forbes, M. (1994). Tests of hypotheses for female-limited polymorphism in the damselfly, Enallagma boreale Selys. *Animal Behaviour, 47*, 742–726.

Fuller, R. C., Noa, L. A., & Strellner, R. S. (2010). Teasing apart the many effects of lighting environment on Opsin expression and foraging preference in bluefin killifish. *American Naturalist, 176* (1), 1–13.

Gorb, S. N. (1998). Visual cues in mate recognition by males of the damselfly, *Coenagrion puella* (L.) (Odonata: Coenagrionidae). *Journal of Insect Behavior, 11*(1), 73–92.

Hardie, R. C. (1986). The photoreceptor array of the dipteran retina. *Trends in Neuroscience, 9*, 419–23.

Hariyama, T., Ozaki, K., Tokunaga, F., & Tsukahara, Y. (1993). Primary structure of crayfish visual pigment deduced from cDNA. *FEBS Letters, 315*(3), 287–292.

Hisada, M., Tamasige, M., & Suzuki, N. (1965). Control of the flight of the dragonfly *Sympetrum darwinianum* Selys I. Dorsophotic response. *Journal of the Faculty of Science Hokkaido University, Series 6, Zoology, 15*, 568–577.

Horridge, G. A. (1969). Unit studies on the retina of dragonflies. *Zeitschrift fur Vergleichende Physiologie, 62*, 1–37.

Iserbyt, A., & Van Gossum, H. (2011). Show your true colour: Cues for male mate preference in an intra-specific mimicry system. *Ecological Entomology, 36*, 544–548.

Janananda, B. G. (2011). Characterization of changes in Megalagrion opsin genes to detect signatures of selection. *Open Access Theses.* Paper 259.

Kaiser, H. (1985). Availability of receptive females at the mating place and mating chances of males in the dragonfly *Aeschna cyanea*. *Behavioral Ecology and Sociobiology, 18*(1), 1–7.

Kirschfeld, K. (1976). The resolution of lens and compound eyes. In F. Zettler & R. Weiler (Eds.), *Neural principles in vision* (pp. 354–370). Springer: Berlin.

Land, M. F. (1981). Optics and vision in invertebrates. In H. Autrum (Ed.), *Handbook of sensory physiology, Vol. VII/6B* (pp. 471–592). Berlin: Springer.

Land, M. F. (1989). Variations in the structure and design of compound eyes. In D. G. Stavenga & R. C. Hardie (Eds.), *Facets of vision* (pp. 90–111). Berlin: Springer-Verlag.

Land, M. F., & Nilsson, D. E. (2002). *Animal eyes*. Oxford: Oxford University Press.

Laughlin, S. B. (1976). The sensitivities of dragonfly photoreceptors and the voltage gain of transduction. *Journal of Comparative Physiology, 111*, 221–247.

Lavoie-Dornik, J., Pilon, J.-G., Gogala, M., & Ali, M. A. (1988). Electrophysiological study of compound eye growth in *Enallagma cyathigerum* Charpentier and *Enallagma clausum* Morse Zygotpera Coenagrionidae. *Odonatologica, 17*(4), 337–356.

Lythgoe, J. N. (1972). The adaptation of visual pigments to the photic environment. In H. Dartnall (Ed.), *Handbook of sensory physiology, vol. 7, part 1: Photochemistry of vision* (pp. 566–603). Berlin: Springer-Verlag.

Maksimovic, S., Cook, T. A., & Buschbeck, E. K. (2009). Spatial distribution of opsin-encoding mRNAs in the tiered larval retinas of the sunburst diving beetle Thermonectus marmoratus (Coleoptera: Dytiscidae). *Journal of Experimental Biology, 212*(23), 3781–3794.

Maksimovic, S., Layne, J. E., & Buschbeck, E. K. (2011). Spectral sensitivity of the principal eyes of sunburst diving beetle, Thermonectus marmoratus (Coleoptera: Dytiscidae), larvae. *Journal of Experimental Biology, 214*(21), 3524–3531.

Mayer, E. P., & Labhart, T. (1993). Morphological specializations of dorsal rim ommatidia in the compound eye of dragonflies and damselflies (Odonata). *Cell & Tissue Research, 272*, 17–22.

Meinertzhagen, I. A., Menzel, R., & Kahle, G. (1983). The identification of spectral receptor types in the retina and lamina of the dragonfly *Sympetrum rebicundulum*. *Journal of Comparative Physiology, 151*, 295–310.

Morehouse, N. I., & Rutowski, R. L. (2010). In the eyes of the beholders: Female choice and avian predation risk associated with an exaggerated male butterfly color. *American Naturalist, 176*, 768–784.

Nilsson, D.-E. (1989). Optics and evolution of the compound eye. In D. G. Stavenga & R. Hardie (Eds.), *Facets of vision*. Berlin: Springer.

Nilsson, D.-E., & Kelber, A. (2007). A functional analysis of compound eye evolution. *Arthropod Structure & Development, 36*, 373–385.

Olberg, R. M., Worthington, A. H., & Venator, K. R. (2000). Prey pursuit and interception in dragonflies. *Journal of Comparative Physiology A, 186*, 155–162.

Olberg, R. M., Worthington, A. H., Fox, J. L., Bessette, C. E., & Loosemore, M. P. (2005). Prey size selection and distance estimation in foraging adult dragonflies. *Journal of Comparative Physiology A, 191*, 791–797.

Olberg, R. M., Seaman, R. C., Coats, M. I., & Henry, A. F. (2007). Eye movements and target fixation during dragonfly prey-interception flights. *Journal of Comparative Physiology A, 193*, 685–693.

Osorio, D., & Vorobyev, M. (2008). A review of the evolution of animal colour vision and visual communication signals. *Vision Research, 48*, 2042–2051.

Paulson, D. R. (1966). *The dragonflies (Odonata: Anisoptera) of southern Florida*. Dissertation, University of Miami, Florida.

Peitsch, D., Feitz, A., Hertel, H., de Souza, J., Ventura, D. F., & Menzel, R. (1992). The spectral input systems of hymenopteran insects and their receptor-based colour vision. *Journal of Comparative Physiology A, 170*, 23–40.

Pritchard, G. (1966). On the morphology of the compound eyes of dragonflies (Odonata; Anisoptera). *Proceedings of the Royal Entomological Society of London A, 43*, 333–336.

Rebora, M., & Piersanti, S. (2010). The antennal sensory function in the oldest pterygote insects: An ultrastructural overview. In D. J. Mendez-Vilas (Ed.), *Microscopy: Science, technology, applications and education*. Badajoz: Formatex Research Center.

Roberts, N. W., Porter, M. L., & Cronin, T. W. (2011). The molecular basis of mechanisms underlying polarization vision. *Philosophical Transactions of the Royal Society B, 366*, 627–637.

Robertson, H. M. (1985). Female dimorphism and mating behaviour in a damselfly, *Ischnura ramburii*: Females mimicking males. *Animal Behavior, 33*, 805–809.

Schaller, F. (1960). Ètude du développement postembryonnaire d'*Aeschna cyanea* Müll. *Annales des Sciences Naturelles-Zoologie et Biologie Animale, 2*, 751–868.

Schultz, T. D., Anderson, C. N., & Symes, L. B. (2008). The conspicuousness of colour cues in male pond damselflies depends on ambient light and visual system. *Animal Behaviour, 76*, 1357–1364.

Seki, T., Fujishita, S., & Obana, S. (1989). Composition and distribution of retinal and 3-hydroxyretinal in the compound eye of the dragonfly. *Experimental Biology, 48*, 65–75.

Shaw, K. L., & Mullen, S. P. (2011). Genes versus phenotypes in the study of speciation. *Genetica, 139*(5), 649–661.

Sherk, T. E. (1978a). Development of the compound eyes of dragonflies (Odonata) II. Development of the larval compound eyes. *Journal of Experimental Zoology, 203*, 183–200.

Sherk, T. E. (1978b). Development of the compound eyes of dragonflies (Odonata) III. Adult compound eyes. *Journal of Experimental Zoology, 203*, 61–80.

Sherk, T. E. (1978c). Development of the compound eyes of dragonflies (Odonata) IV. Development of the adult compound eyes. *Journal of Experimental Zoology, 203*, 183–200.

Sirot, L. K., Brockmann, H. J., Marinis, C., & Muschett, G. (2003). Maintenance of a female-limited polymorphism in Ischnura ramburi (Zygoptera:Coenagrionidae). *Animal Behavior, 66*, 763–775.

Snyder, A. W., Menzel, R., & Laughlin, S. B. (1973). Structure and function of the fused rhabdom. *Journal of Comparative Physiology., 87*, 99–135.

Stavenga, D. G. (2002). Colour in the eyes of insects. *Journal of Comparative Physiology A- Neuroethology Sensory Neural and Behavioral Physiology, 188*(5), 337–348.

Svensson, E. I., Abbott, J., & Härdling, R. (2005). Female polymorphism, frequency dependence, and rapid evolutionary dynamics in natural populations. *American Naturalist, 165*, 567–576.

Svensson, E. I., Karlsson, K., Friberg, M., & Eroukhmanoff, F. (2007). Gender differences in species recognition and the evolution of asymmetric sexual isolation. *Current Biology, 17*, 1943–1947. doi:10.1016/j.cub.2007.09.038.

Takahashi, Y., Yoshimura, J., Morita, S., & Watanabe, M. (2010). Negative frequency-dependent selection in female color polymorphism of a damselfly. *Evolution, 64*, 3620–3628. doi:10.1111/j.1558-5646.2010.01083.

Tynkkynen, K., Rantala, M. J., & Suhonen, J. (2004). Interspecific aggression and character displacement in the damselfly *Calopteryx splendens*. *Journal of Experimental Biology, 17*, 759–767. doi:10.1111/j.1420-9101.2004.00733.x.

Ueda, T. (1989). Sexual maturation, body colour changes and increase of body weight in a summer diapause population of the damselfly *Lestes sponsa* (Hansemann) (Zygoptera: Lestidae). *Odonatologica, 18*, 75–87.

Van Gossum, H., Sherratt, T. N., & Cordero Rivera, A. (2008). The evolution of sex-limited colour polymorphism. In A. Córdoba-Aguilar (Ed.), *Dragonflies: Model organisms for ecological and evolutionary research* (pp. 219–229). Oxford: Oxford University Press.

Van Gossum, H., Bots, J., Van Heusden, J., Hammers, M., Huyghe, K., & Morehouse, N. I. (2010). Reflectance spectra and mating patterns support intraspecific mimicry in the colour polymorphic damselfly *Ischnura elegans*. *Evolutionary Ecology, 25*, 139–154.

Waage, J. K. (1979). Dual function of the damselfly penis: Sperm removal and transfer. *Science, 203*, 916–918.

Wakakuwa, M., Stavenga, D. G., & Arikawa, K. (2007). Spectral organization of ommatidia in flower-visiting insects. *Phytochemical and Photobiology, 83*, 27–34.

Warrant, E. J., & Nilsson, D.-E. (1995). The absorption of white light by photoreceptors. *Vision Research, 38*, 195–207.

Wehner, R. (1981). Spatial vision in arthropods. In H. Autrum (Ed.), *Handbook of sensory physiology, Vol. VII/6C* (pp. 287–616). Berlin: Springer.

Williams, C. E. (1976). *Neurocordulia (Platycordulia) xanthosoma* (Williamson) in Texas (Odonata: Libellulidae: Corduliinae). *Great Lakes Entomologist, 9*, 63–73.

Yamamoto, Y. (1968). A note on a gynandromorphic specimen of the dragonfly, *Lyriothemis pachygastra* Selys. *New Entomologist, 17* (2), 17–21.

Yang, E.-C., & Osorio, D. (1991). Spectral sensitivities of photoreceptors and lamina monopolar cells in the dragonfly, *Hemicordulia tau*. *Journal of Comparative Physiology A, 169*, 663–669.

Yuan, F., Bernard, G. D., Le, J., & Briscoe, A. D. (2010). Contrasting modes of evolution of the visual pigments in *Heliconius* butterflies. *Molecular Biology and Evolution, 27*, 2392–2405.