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Opsin Evolution in Flower-Visiting Beetles

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Flowers have evolved signals that exploit the sensory systems of insect visitors. In the case of visual cues, color signals are thought to have been shaped in large part by the spectral sensitivity of key pollinators, such as hymenopterans. Beetles were some of the first plant pollinators, pre-dating the angiosperm radiation but with the exception of a few well-studied species, the evolution of flower-visiting beetle visual systems is poorly understood. Thus, the ability of beetles to detect and distinguish flower color signals and perhaps their potential role in shaping flower coloration is not well understood. Traditional models of pollinator visual systems often assume a putative tri- or tetrachromatic flower-visitor, as is found in bees, flies and butterflies. Beetles are unique among modern pollinators as ancestrally they did not possess the machinery for trichromatic vision, lacking the blue-sensitive photoreceptor class. Research on the evolution of visual genes responsible for wavelength sensitivity (opsins) has revealed that beetles with putative tri- and tetrachromatic visual systems have evolved independently, along multiple lineages. We explore the evolution of beetle visual genes using newly generated and publicly available RNA-seq data from 25 species with flower associations, including previously unexplored key flower-visitor groups and 20 non-flower visiting relatives. Our findings serve as a resource to inform and guide future studies on beetle-flower interactions, where insight from both signal and receiver is needed to better understand these poorly explored systems.

Keywords: opsin, insect vision, pollinators, gene duplication, coleoptera (beetles)

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INTRODUCTION

Beetles were some of the earliest pollinators and remain the primary pollinators of ancient plant group, gymnosperms (Toon et al., 2020), while also functioning as common pollinators of more recent angiosperm (flowering plant) radiations. Angiosperm radiations during the Cretaceous co-occurred with accelerated diversification among the holometabolous insects that comprise the dominant insect pollinators: hymenopterans, lepidopterans, dipterans and coleopterans (Doyle, 2012; Misof et al., 2014). This co-radiation is thought to be in part due to the establishment of close associations or mutualisms between pollinator and plant, which likely contributed to the diversification of floral pollinator signals seen in modern flowers (Bronstein et al., 2006; Cardinal and Danforth, 2013). It is estimated that there are over 77,000 extant beetle species with flower associations (Wardhaugh, 2015) and flower visitation in these species has arisen *via* various evolutionary routes. While some species have retained existing ancestral gymnosperm associations (e.g., Boganiidae) (Cai et al., 2018), pollination behavior likely transitioned in some beetle lineages

from gymnosperms to angiosperms (e.g., Oedemeridae and Kateretidae) (Peris et al., 2017, 2020). In other beetle lineages, flower associations evolved without preexisting pollination behavior (e.g., Glaphyridae) (Sabatinelli et al., 2020), as in Anthophila (bees) (Cardinal and Danforth, 2013; Peters et al., 2017).

Traditionally, flowers visited by beetles have been described as dull in coloration and highly scented, suggesting that beetles do not use or are not reliant on spectral cues to detect flowers (Faegri and Van der Pijl, 1979). Perhaps unsurprisingly, considering the diversity of beetles, as more beetle-flower interactions have been described it has become clear that many beetle species do use spectral information for flower detection. Pollinators in the families Meloidae (blister beetles), Glaphyridae (bumble-bee scarabs), and Scarabaeidae (monkey beetles), use color alone as a cue for flower detection (Dafni et al., 1990; Steiner, 1998; Van Kleunen et al., 2007; Paudel et al., 2017). In carabids and fireflies, heavier investment in vision (larger eyes) rather than olfaction (reduced antennae) has been shown to be driven by visually mediated tasks (Bauer and Kredler, 1993; Stanger-Hall et al., 2018). It is not yet known whether there are similar trade-offs between flower visiting beetles that utilize predominantly visual or olfactory cues. Angiosperms that are pollinated primarily by beetles span at least 34 families (**Figure 1**; Bernhardt, 2000) thus there are likely many beetle-flower associations still to be described and the role of visual cues to be determined.

The role of insect pollinator visual systems in shaping floral color cues has been well studied in the context of hymenopteran pollination systems (e.g., Chittka, 1996; Dyer et al., 2012). The spectral sensitivities of hymenopteran UV-, blue- and green-sensitive photoreceptors are positioned for optimum wavelength discrimination of floral spectral cues (Chittka and Menzel, 1992). Rather than tuning of insect spectral sensitivity, this relationship is thought to have arisen from spectral tuning of floral reflectance, to existing photoreceptor sensitivities (Chittka, 1996; Dyer et al., 2012). The underlying sensitivity of a photoreceptor is determined by the structure of a GPCR protein (opsin), which is coupled to a light-absorbing chromophore pigment. Unlike the vast majority of insects studied, including bees and butterflies, beetles lack a key opsin (SW) that typically confers sensitivity to blue wavelengths of light (Jackowska et al., 2007), lost prior to the radiation of beetles (Sharkey et al., 2017). Based on spectral sensitivity measurements of beetles with this ancestral condition, it is assumed that the ancestor of all beetles had a dichromatic UV-green color visual system (Gribakin, 1981; Warrant and McIntyre, 1990).

The lack of a dedicated blue-sensitive photoreceptor channel impacts the discriminatory capabilities of certain wavelengths, particularly within the violet-blue region of the light spectrum where photon catch is low. The ancestral beetle visual system is less complex than bees, for example, which have retained all three insect opsin classes UV, SW and LW (long wavelength), resulting in UV- blue- and green-sensitivity. Opsin duplication events are the major route for acquiring additional spectral channels. Duplications alone do not lead to novel spectral sensitivities; selection for changes in function is required via mutations that lead to changes in protein (subfunctionalization). In a number of coleopteran lineages, duplications of the UV opsin and subsequent subfunctionalization has led to the ability of these taxa to perceive blue wavelengths (Sharkey et al., 2017) by essentially "re-evolving" a dedicated blue-sensitive photoreceptor, for example in coccinellids (Lin, 1993) and chrysomelids (Döring and Skorupski, 2007). There is also evidence that flower-visiting lineages of beetles in the families Scarabaeidae, Nitidulidae, and Curculionidae have expanded their opsin repertoire, likely expanding their wavelength sensitivities (Sharkey et al., 2017). It is not known if this is true across the diversity of beetle pollinators.

We explore opsin expansions across the diversity of beetle pollinators using publicly available RNA-seq data, opsin sequences and an additional nine transcriptomes generated in this study. We aimed to investigate the evolution of the visual genes (opsins) that underpin sensitivity to spectral (color) information. Transcriptome data allow us to examine the diversity of opsin genes and hence putative wavelength sensitivities, from these flower-visiting species and their close relatives. In addition, we examine relative eye size in these species as an indicator of visual system investment.

MATERIALS AND METHODS

RNA Extraction and Assembly

Seven flower-visiting beetle species (*Trirhabda eriodictyonis*, *Sibinia setosa*, *Tychius meliloti*, *Anthrenus lepidus*, *Mordella albosuturalis*, *Nitops pallipennis,* and *Anaspis rufa*) were collected from flowers in southern Utah into RNAlater and later frozen (–80°C) until processed. Multiple individuals were pooled for RNA extraction with the exception of *T. eriodictyonis* where one male was used. Total RNA was extracted from adult whole bodies using Nucleospin spin columns (Clontech) and reverse transcribed into cDNAs using the Illumina TruSeq RNA v2 kit. Sequencing was done on an Illumina HiSeq 2,500 generating 100 bp paired-end reads (BYU DNA sequencing center). RNA from two additional nitidulid species was extracted using the RNeasy Mini kit (Qiagen) and transcribed with the KAPA Stranded mRNA-Seq kit (Roche). Sequencing was completed on an Illumina HiSeq 2,500 generating 250 bp paired-end reads (BYU DNA sequencing center). Additionally, paired-end RNA-seq data from 36 species were downloaded from the Sequence Read Archive (SRA). Data were trimmed using Trimmomatic (Bolger et al., 2014), removing adapter sequences and poor-quality bases using the parameters: SLIDINGWINDOW:4:5 LEADING:5 TRAILING:5 MINLEN:25. Trinity (v2.11.0) (Grabherr et al., 2011; Haas et al., 2013) was used to assemble the remaining reads. Completeness of assemblies was estimated by searching each for the presence of 1367 insect Benchmarking Universal Single-Copy Orthologs using BUSCO v5 (Simão et al., 2015; Seppey et al., 2019). See **Supplementary Table 1** for all species information. Opsin sequences have been deposited in GenBank with accession numbers MW885977—MW886096 and RNA-seq data have been uploaded to the Sequence Read Archive (PRJNA718629).

FIGURE 1 | Flower-visiting beetle families included in this study. From left to right: Cetoniinae (*Trichaulax philipsii*), Buprestidae (*Acmaeodera* sp.), Lycidae (*Lycus* sp.), Curculionidae and Nitidulidae (*Aethina concolor*). Images by: Chris Moeseneder, GSP, Russ Anderson, Steven Marshall, GSP.

Opsin Search

Coding regions were predicted using TransDecoder v5.5.0,[1] retaining the longest open reading frame (ORF). All predicted ORFs were also BLASTed[2] against a database of known arthropod opsins (orthodb EOG8NKF98), with the addition of Lampyridae and *Thermonectus marmoratus* full-length opsin copies with an *e*-value of 0.001. All remaining ORFs were searched against our insect opsin database using hmmscan in HMMER (v3.3) (Eddy, 2011). Cross-contamination and pseudogenes were removed using phylogenies of DNA and protein opsin sequences and by examining alignments. Opsins with > 99% similarity in protein sequence, likely structurally and functionally identical, were removed (CD-hit v4.8.1; Li and Godzik, 2006; Fu et al., 2012). Duplicates with 100% sequence identity using local alignment (BLASTp) were considered to be one opsin gene (*Anthocomus equestris* and *Pharaxonotha floridana*; see asterisks **Figure 2** and **Supplementary Table 2**).

Final DNA sequences and additional insect opsins were subject to codon alignment (MAFFT v7.453; Katoh et al., 2002; Katoh and Standley, 2013) with automatic alignment strategy detection. Maximum likelihood opsin DNA gene trees were generated using IQ-tree (v1.6.12) (Minh et al., 2013; Nguyen et al., 2015). The substitution model GTR + F + I + G4 was selected automatically using ModelFinder (Kalyaanamoorthy et al., 2017). A species topology was generated in Mesquite 3.2 (Maddison and Maddison, 2018) based on McKenna et al. (2019). For statistical analyses, beetle species were categorized as obligate flower-visitors that require floral resources for food or reproduction (A), facultative flower visitors with known floral associations (e.g., facultative pollinators) but no reliance on floral resources for food or reproduction (B) and non-flower visitors with no known association with flowers (C).

Eye Size Measurements and Statistics

Relative eye size was generated using the measurement tools in Adobe Photoshop v.19.1.6 from high resolution habitus photos (available upon request due to copyright) with an unimpeded dorsal view of the head. Two measurements were taken for each photo: total head width including eyes, and interocular distance (see **Supplementary Figure 1** for example image). These two measurements were used to generate the total width of the eyes in the dorsal view and used to calculate a percentage of lateral head space dedicated to eye tissue as viewed dorsally. We tested for a relationship between UV and LW opsin copy number (Pearson's correlation test) and if flower visitation behavior predicted relative eye-size (one-way ANOVA). Both were included as predictors in the final MANOVA. Because LW and UV were not correlated, we chose to test the effects of relative eye size and flower visitation on both LW and UV opsin copy number (MANOVA). For the purpose of this analysis, species without UV opsin copies were omitted. All data met required assumptions, and no data transformations were performed. All eye size statistical tests were executed in SPSS 27 (IBMCorp.).

RESULTS

Opsin Duplications

A transcriptomics approach was taken to explore the putative wavelength sensitivities of flower visiting and non-flower visiting beetle species. To determine possible wavelength sensitivity expansions, RNA-seq data from 45 species spanning 26 families were mined for opsins and data were analyzed for the presence of duplication events. Nine transcriptomes from flower-visiting species were generated for this study to expand the diversity of sampled lineages. We recovered 120 opsin copies, 92 of which were full length copies with a minimum length of 101 amino acids. We classified 18 beetle species as obligate flower visitors (category A), seven as facultative flower visitors (category B), and 20 with no known floral associations (category C). Additionally, we included 18 previously published beetle opsins from five category A species, 11 opsins from four category B species and 14 opsins from five category C species (Sharkey et al., 2017; **Supplementary Table 1**).

In our dataset both obligate (A) and facultative (B) flower visitors had 2-fold higher proportions of UV duplications present, 39.1 and 45.5%, respectively, than non-flower visitors (C) with only 20%. LW duplications were more common in obligate flower visitors (52.2%) than either facultative (18.2%) or non-flower visitors (12%). The prevalence of either opsin duplication was highest in obligate flower visitors (73.9%), lower in flower-associated species (54.5%) and lowest in non-flower visitors sampled (28%). We report 33 independent opsin duplication events (12 UV and 21 LW), which have occurred across the diversity of Coleoptera (**Figure 2**). Duplication events occurred

[1] http://transdecoder.github.io

[2] https://blast.ncbi.nlm.nih.gov/

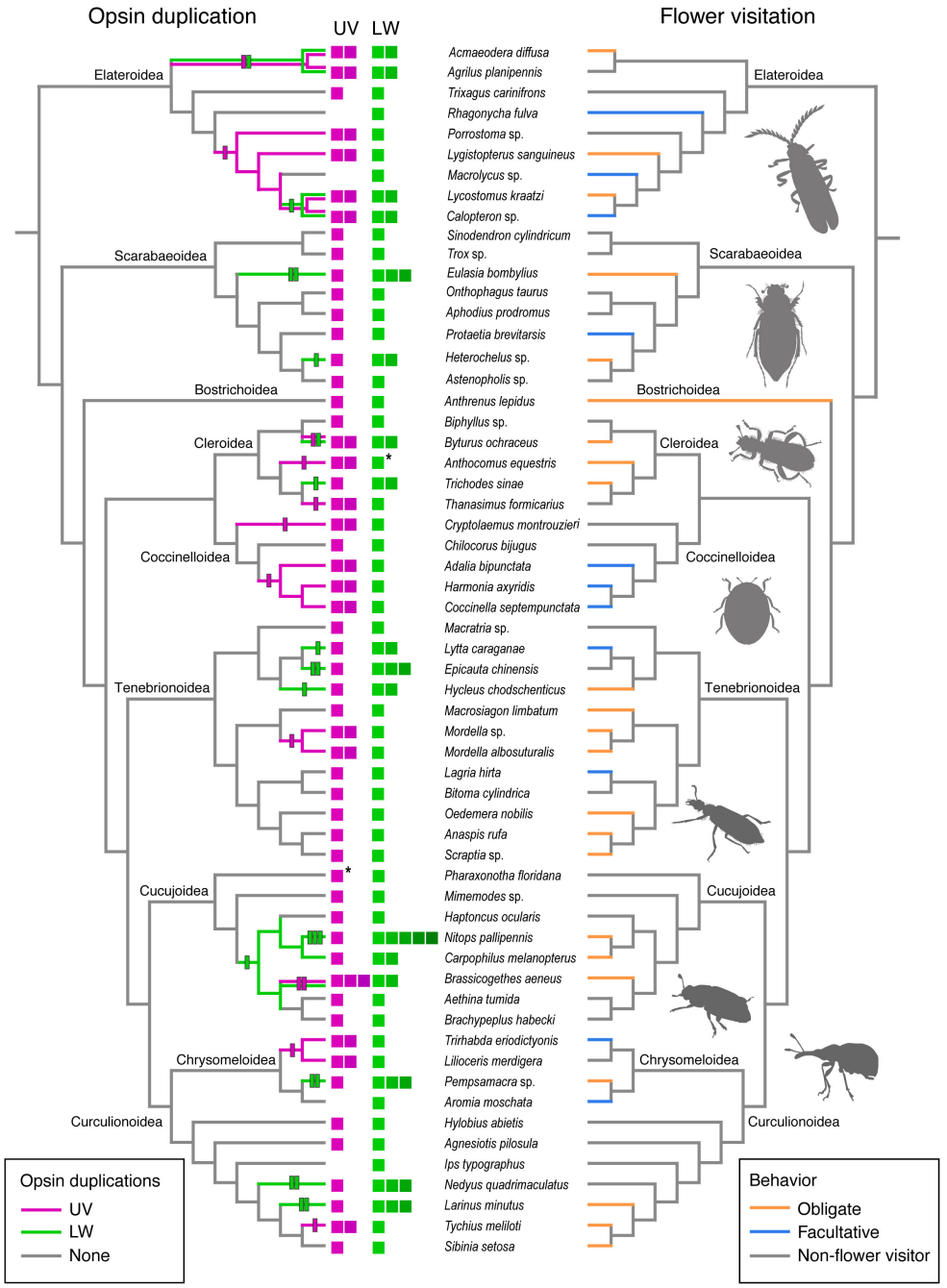

FIGURE 2 | Patterns of opsin duplication and flower-visitation behavior for 59 beetle species. The cladograms represent a simplified version of beetle relationships after McKenna et al. (2019) with the position of superfamilies indicated. The left-hand cladogram shows opsin duplication events with opsin copy number at the terminals and duplication events indicated on the branches. Duplication events were estimated using the opsin tree (see **Supplementary Figure 2**). Asterisks (*) denote cases where additional duplication copies were found but determined not to be functionally distinct (see Opsin Search Methods text). The right-hand cladogram reflects known behavioral categories for the level of flower visitation. No attempt at ancestral reconstruction was made due to the available taxon sampling.

within all but one superfamily, Bostrichoidea, which has only a single flower-visiting lineage (the Dermestidae) represented by *Anthrenus lepidus*. Percent identity (sequence similarity) of aligned duplicate opsins (BLASTp, see text footnote 2) ranged from 64 to 98% sequence identity and a mean of 80% (see **Supplementary Table 2**).

Within Scarabaeoidea only flower visiting scarabs exhibit opsin duplications. These duplications were independent and

solely among LW opsins in *Heterochelus* sp. and *Eulasia bombylius* with one and two duplications, respectively (**Figure 2**). Notably, *Protaetia brevitarsis*, which can commonly be found on flowers (B) but is not considered an obligate flower visitor, did not have opsin duplicates. Among the five Lycidae (Elateroidea) species sampled, the UV opsin duplicates present were orthologous (**Figure 2** and **Supplementary Figure 2**) and therefore represent an early duplication event in this group. Two LW opsin copies were present in two lycid species *Lycostomus kraatzi* and *Calopteron* sp. However, this was not linked to flower visiting behavior with the former likely an obligate (A) and the latter only facultatively associated with flowers (B). Rather, these species are among the most derived members of this group and therefore highlight a potential ancestral LW opsin duplication event that occurred during the lycid radiation. All flower visiting members (A) of Cleroidea have either UV or LW duplicates. Each duplication event was independent, including within the subfamily Clerinae where a UV duplication occurred in *Thanasimus formicarius* and a LW duplication in the flower visitor *Trichodes sinae*.

Coccinellid opsin UV duplicates have been previously examined (Sharkey et al., 2017). We add the species *Chilocorus bijugus* (tribe: Chilocorini), which has no known associations with flowers. No UV duplicate was recovered for this species making this species unique among the coccinellids sampled thus far. Our opsin phylogeny (**Supplementary Figure 2**) suggest that two separate UV duplication events occurred, one prior to the diversification of the tribe Coccinellini and the other along lineage sister to the rest of the coccinellids, *Cryptolaemus montrouzieri* (tribe: Coccidulini). We note only two opsin duplication events within the Tenebrionoidea, within the blister beetles Meloidae (*Hycleus chodschenticus*) (LW duplication) and tumbling flower beetles Mordellidae (*Mordella* sp. and *Mordella albosuturalis*) (UV duplication). Opsin orthologs are present in both *Mordella* species, suggesting that this duplicate may be shared among other members of this genus.

A close relationship between flower visitation and opsin copy number can be clearly seen among the cucujoids (**Figure 2**). Single UV and LW opsin copies are present in all non-flower visiting species but UV and/or LW opsin duplications are present among all flower-visiting nitidulids sampled (**Figure 2**). The LW opsin duplicates form two clades shared among these species suggesting an early LW duplication event in Nitidulidae that has potentially been lost in the three non-flower visiting lineages. Two of the three obligate flower visiting weevils (Curculionidae) species possess additional opsin copies.

While duplications more commonly occurred amongst flower visiting species (categories A and B), than in non-flower visitors (C), 26% of obligate flower visitors have retained the ancestral UV-LW opsin condition. Among the obligate flower visitors, four of the seven tenebrionid species sampled have single opsin copies, including both members of Scraptiidae (*Scraptia* sp. and *Anaspis rufa*), *Oedemera nobilis* (Oedemeridae), and *Macrosiagon limbatum* (Ripiphoridae) (**Figure 2**). Carpet beetle *Anthrenus lepidus* (Dermestidae: Bostrichoidea), *Biphyllus* sp. (Biphyllidae: Cleroidea) and two weevil species, *Tychius meliloti* and *Sibinia setosa* (Curculionidae: Curculionoidea) also did not have opsin duplications. We did not recover UV opsins from four non-obligate flower visiting species, *Rhagonycha fulva*, *Macrolycus* sp., *Aromia moschata* or *Ips typographus* but LW opsins were recovered from all of these species.

Eye Size, Flower Visitation and Opsin Copy Number

Relative eye size was used as an approximate measure of visual investment across the species in this study. We found no significant correlation between UV and LW opsin copy number ($p = 0.893$). Flower visitation category (A, B or C) did significantly predict relative eye size ($F = 8.52$, $df = 2$, $p < 0.001$). *Post hoc* tests revealed obligate flower visiting species (A) have significantly larger eyes than those without any floral association (C) ($p < 0.001$, mean difference = 11.15%). Flower visitation category in conjunction with relative eye size was a significant predictor of LW copy number ($F = 3.895$, $df = 32$, $p = 0.015$), but not UV copy number ($F = 2.06.11$, $df = 32$, $p = 0.122$). While the overall model showed evidence for the combination of both flower visitation and eye size influencing LW copy number, neither was significant individually.

DISCUSSION

A previous study (Sharkey et al., 2017) found opsin duplications in four of five flower-visiting beetle species. While LW opsin duplications were not common generally, in the coleopterans sampled, they were ubiquitous among these flower-visitors. These findings suggested that there may be a selective advantage for duplications, such as an expansion of wavelength sensitivity, among species that may use floral cues. Our opsin analysis across numerous flower-visiting species of coleopterans suggests that associations with flowers has often led to or precedes the expansion of wavelength sensitivity through opsin duplication. We cannot attribute an opsin duplication event strictly to the evolution of flower visitation behavior due to associated factors, such as diurnality (Sondhi et al., 2021) and other visually guided behaviors (e.g., host-plant seeking), for which expanded wavelength sensitivity may be beneficial. However, by including closely related non-flower visiting relatives in this study, we show that duplication events and particularly LW opsin duplications, do commonly occur along lineages associated with flowers, more so than those that have no floral associations. The frequency of duplications varies among other pollinator groups. Hymenopterans exhibit relatively stable opsin copy numbers (Spaethe and Briscoe, 2004; Oeyen et al., 2020) but in contrast the opsin repertoire of lepidopterans is highly variable, particularly within the butterflies, Papilionoidea (Sondhi et al., 2021). Our finding that there have been many independent duplication events in flower-visiting coleopterans may be attributed to the ancestral loss of the SW opsin in this group, or as in butterflies, may suggest increased spectral richness of the visual system.

Opsin duplications do not in all cases lead to new photoreceptor sensitivities, as is the extreme case in odonates, where there is a large excess of opsin copies (Futahashi et al.,

2015; Suvorov et al., 2016) compared to measured photoreceptor sensitivities (Laughlin, 1976; Yang and Osorio, 1991). In contrast, it has been shown that beetles are relatively conservative in opsin copy number (Sharkey et al., 2017). Therefore, opsin duplicates can be used as a guide for photoreceptor sensitivity diversity. For example, all sampled jewel beetles (Buprestidae) and *Carabus* sp. (Carabidae) have 2 UV and 2 LW opsin copies (Lord et al., 2016; Sharkey et al., 2017) that underpin UV-, blue-, green-, and red-sensitive photoreceptors (Hasselmann, 1962; Meglič et al., 2020), demonstrating increased function of photoreceptor sensitivity for these groups as a result of opsin duplication. Further, the presence of blue-sensitive photoreceptors also aligns well with the presence of a UV opsin duplication, in coccinellids (Lin and Wu, 1992) and chrysomelids (Döring and Skorupski, 2007). Additionally, species with ancestral UV-green sensitivity and complementary opsin data only possess a single UV and a single LW opsin copy (e.g., Lampyridae and *Dendroctonus*) (Groberman and Borden, 1982; Lall et al., 2010; Martin et al., 2015; Sander and Hall, 2015).

UV opsins were absent in four species. The UV opsin typically has lower expression levels as often UV-sensitive photoreceptors are less numerous than long wavelength-sensitive receptors in insects. Thus, we cannot be sure whether this finding reflects inadequate sequencing depth in these species or a true opsin loss. The use of head or eye tissue rather than whole body specimens, or deeper sequencing, may be necessary for future studies. Additionally, opsin expressed in photoreceptors with low abundance, such as those used in highly specialized regions (e.g., the polarization-sensitive dorsal rim area) may also have low signal. Further study to determine opsin abundance and expression patterns is required to better understand how well opsin copy number is estimated from whole body specimens, in particular those with reduced eyes.

Flowers commonly frequented by beetles have been traditionally described as dull in coloration (white, green or yellow) but highly scented, thought to exploit existing attraction to certain volatile compounds, such as skatole, attractive to coprophagous beetles (Schiestl and Dötterl, 2012). Despite compelling evidence that many beetles use floral visual cues for flower visitation, the study of associated visual adaptations is lacking. Scarab pollinators provide compelling evidence for vision as the primary cue for flower detection. Bumble-bee scarabs (Glaphyridae) and monkey beetles (Hopliini) use the dark center of a flower as a visual cue, termed "beetle marks" (Dafni et al., 1990; Johnson and Midgley, 2001). Other non-scarab beetle pollinators are also attracted to dark spots on flowers, e.g., nitidulids (Free and Williams, 1978) and mordellids (Westmoreland and Muntan, 1996).

In scarab beetles, anthophagy (flower feeding) has evolved at least seven times (Ahrens et al., 2014). Three of these lineages have been sampled in this study: Glaphyridae, Hopliini and Cetoniinae. We found LW opsin duplications among the two lineages that are known to have preferences for red and orange flowers, Glaphyridae (Sabatinelli et al., 2020) and Hopliini (Johnson and Midgley, 2001). The bumble-bee scarab *Pygopleurus israelitus* (Glaphyridae) has both green- and red-sensitive photoreceptors (λ_{max}: 631 nm) the latter of which increase the conspicuousness of red bowl-shaped flowers they visit (Martínez-Harms et al., 2012). This suggests a role for LW opsin duplication and subfunctionalization to expand long-wavelength sensitivity for floral detection. Electrophysiological measurements of *Protaetia brevitarsis* (Cetoniinae), a facultative flower visitor, are in agreement with our finding that this species only has UV and green sensitivity (Lin and Wu, 1992) but curiously this species exhibits attraction to red over green stimuli (Cai et al., 2021). We have yet to find any opsin duplications in the eight additional scarabs examined thus far (Sharkey et al., 2017), highlighting flower-visiting scarabs as an interesting group to study visual systems and signals in the context of anthophily.

Flower visitation is not always a predictor of an opsin duplication event; opsin duplications were absent from six flower-visiting lineages. It is possible that these species rely more heavily on olfaction than vision, and we may expect to see greater investment in these sensory structures (e.g., larger antennae), rather than vision (Bauer and Kredler, 1993; Stanger-Hall et al., 2018). Such sensory adaptations can be seen in the flabellate (fan-shaped) antennae of *Macrosiagon limbatum* (Ripiphoridae). Additionally, if odor was a primary cue in ancient cycads, as in modern species (Toon et al., 2020), lineages such as the false oil beetles (Oedemeridae) may have initially established olfactory rather than visual specializations that persisted after they transitioned to angiosperm hosts (Peris et al., 2017). A UV-green color channel system may be adequate to detect floral cues commonly attractive to beetles, e.g., white and yellow (Reverté et al., 2016), but wavelength sensitivity expansion may be advantageous to detect floral cues that fall outside this spectral range (e.g., pink or violet).

In this study we measured eye size as a proportion of the head (i.e., relative eye size), which has been used in prior studies of beetles to test relationships between visual investment and behavior (Bauer and Kredler, 1993; Stanger-Hall et al., 2018). This does not give a perfect estimation of eye volume due to the variation in eye shape across coleopterans with extreme morphological diversity. However, using relative eye size to examine the potential link between flower visitation, opsin copy number and investment in vision reveals some interesting findings. For the species examined in this study, eye size was found to be predicted by flower visitation behavior with obligate flower visitors having larger eyes as a proportion of the head. This suggests that flower-visitation or associated visual ecology may have driven selection for greater investment in vision. Similarly, flower visitors were also more likely to have a greater LW opsin copy number, suggesting that an expansion of long-wavelength sensitivity is advantageous for behaviors associated with flower visitation in the species we have examined. This is certainly true among beetles that prefer orange and red flowers and have dedicated red-sensitive photoreceptors, but red flower visitation is not commonplace among other species with LW duplications examined herein. Interestingly, among all species, LW opsin copy number was not correlated with eye size suggesting that it is not eye size alone that predicts the number of opsin duplicates. This may point to greater visual specialization in flower visiting beetles.

Our aim was to demonstrate that there is much more exciting work that needs to be done to better understand the evolution of beetle visual systems. This is particularly true for flower visitors, which have multiple origins within the majority of coleopteran superfamilies. Equally exciting is the large visual system diversity, morphological, molecular and certainly functional, among the anthophilous Coleoptera. In short, beetles represent a largely untapped area to study insect plant interactions at both the fine and coarse scales of evolution.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/, PRJNA718629.

AUTHOR CONTRIBUTIONS

CS conceived the study, ran transcriptomic and phylogenetic analyses, and wrote the manuscript. GP advised on beetle taxa, collected and analyzed eye size measurements. SB advised on beetle taxa and generated the species topology. All authors edited the manuscript and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.676369/full#supplementary-material

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