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SYMPOSIUM

Evasion of Predators Contributes to the Maintenance of Male Eyes in Sexually Dimorphic *Euphilomedes* Ostracods (Crustacea)

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Synopsis Sexual dimorphisms have long drawn the attention of evolutionary biologists. However, we still have much to learn about the evolutionary, genetic, and developmental drivers of sexual dimorphisms. Here, we introduce ostracods of the genus *Euphilomedes* (Myodocopida, Ostracoda, and Crustacea) as a promising new system in which to investigate why and how sexual dimorphisms evolve. First, we ask whether male-skewed selective pressure from pelagic predators may help explain a dramatic sexual dimorphism in which male *Euphilomedes* have compound eyes, but females do not. Manipulative experiments demonstrate that blindfolding reduces the survival rate of male *Euphilomedes* when they are exposed to predatory fish. Blindfolding of the female rudimentary eyes (rudiments) does not, however, similarly influence the survival rate of brooding females. Further, numerical estimates of sighting distances, based on reasonable extrapolations from *Euphilomedes*'s eye morphology, suggest that the eyes of male *Euphilomedes* are useful for detecting objects roughly the size of certain pelagic predators, but not conspecifics. We conclude that eyes do not mediate direct interactions between male and female *Euphilomedes*, but that differences in predation pressure—perhaps associated with different reproductive behaviors—contribute to maintaining the sexually dimorphic eyes of these ostracods. Second, through transcriptome sequencing, we examined potential gene regulatory networks that could underlie sexual dimorphism in *Euphilomedes*' eyes. From the transcriptome of juvenile male *Euphilomedes*' eyes, we identified phototransduction genes and components of eye-related developmental networks that are well characterized in *Drosophila* and other species. The presence of suites of eye regulatory genes in our *Euphilomedes* juvenile male transcriptome will allow us, in future studies, to test how ostracods regulate the development of their sexually dimorphic eyes.

Introduction

Sexual dimorphisms—morphological or behavioral differences between male and female conspecifics—are widespread among dioecious organisms. Sexual dimorphisms are categorized as primary, secondary, or ecological sex traits (Andersson 1994); primary sex traits are directly involved with the act of sexual reproduction, secondary sex traits contribute to reproductive success through non-sexual interactions between conspecifics, and ecological sex traits are involved in other interactions with the environment (e.g., Slatkin 1984; Hedrick and Temeles 1989; Shine 1989). Biologists have long asked why certain sexual dimorphisms exist, i.e., how asymmetries in selection drive phenotypic divergence between the sexes (e.g., Darwin 1871). It is generally assumed that primary and secondary sex traits are produced and maintained by sexual selection (Andersson 1994), as exemplified by *Calliphora* blowflies, in which males have relatively large eyes that are thought to be better-suited than are the small eyes of females for detecting mates under low light intensity (Petrowitz et al. 2000).

In contrast, ecological sex traits may evolve under at least two different sets of circumstances. First, sex-specific partitioning of resources may result in

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sexual dimorphisms, as demonstrated by certain hummingbirds in which feeding behavior and morphology of the beak differ between the sexes (Temeles et al. 2000, 2010). Second, the reproductive role hypothesis (RRH) predicts that sexual dimorphisms may evolve when males and females are segregated into different selective environments due to sex-specific reproductive roles (Lande 1980; Slatkin 1984). Ecological sex traits clearly may be influenced by sexual selection, but they also may be influenced by natural selection. Following the RRH, for example, sexual selection may act on behaviors that segregate males and females into different environments, and natural selection—acting differently within these environments—may be a primary driver of particular sexual dimorphisms.

A second critical question about sexual dimorphisms is how differences in developmental mechanisms between males and females produce dissimilar phenotypes from similar genotypes. Several mechanisms have been suggested. First, a single tissue may respond differently to male- and female-specific growth signals, as seen in mammalian gonad development (Estrada et al. 2003; Brennan and Capel 2004). A bipotential tissue can also be regulated by differential tissue growth or by remodeling. For example, developing horns in scarab beetles respond to the sex determination gene *doublesex*; this results in increased cell proliferation in males compared with females and, thus, sexually dimorphic horns (Moczek and Nagy 2005; Moczek et al. 2006, 2007; Kijimoto et al. 2012). Finally, sex-specific duplication of tissue may lead to sexually dimorphic traits, a possibility that researchers only recently have begun to explore (Rivera and Oakley 2009).

For several reasons, we chose ostracods of the genus *Euphilomedes* (Myodocopida, Ostracoda, and Crustacea) as a promising new system in which to investigate why and how sexual dimorphisms evolve. First, *Euphilomedes* have a dramatic sexual dimorphism whose function can be manipulated experimentally: adult males have compound eyes, but females do not. More specifically, adult males have two anterio-dorsal, lateral compound eyes with 20–33 ommatidia, depending on species. In contrast, most female *Euphilomedes* lack these eyes altogether (Kornicker and Harrison-Nelson 1997; Lum et al. 2008) and instead in their place have rudimentary eyes (rudiments) without ommatidia (Rivera and Oakley 2009). Distinct ommatidia indicate that males' eyes provide spatial vision, whereas a lack of ommatidia indicates that the females' rudiments cannot provide any spatial information whatsoever (although their sensitivity to light has not been tested). Second, differences in behavior may cause male *Euphilomedes* to spend more time in environments where spatial vision is potentially useful for detecting predators. *Euphilomedes* (and many other myodocopid ostracods) likely spend the majority of their lives buried in the sediment, relatively sheltered from pelagic predators such as fish, yet swim into the water column at night to mate (Macquart-Moulin 1999; Lum et al. 2008). Males likely swim on multiple nights, but it appears that females only leave the sediment to mate once during their lives (Cohen 1983; Kornicker 1978). Because male *Euphilomedes* almost certainly spend more time in the water column than do females, we hypothesize that the sexually dimorphic eyes of these animals are a trait that is driven, at least in part, by the need to visually detect pelagic predators.

Euphilomedes also constitutes a promising study system because their sexually dimorphic eyes develop in a manner well suited for comparative investigations of gene expression. Unlike many other arthropods, ommatidial development in *Euphilomedes* occurs exclusively in the stalked eyes of juveniles, making all stages of this developmental process experimentally accessible. Moreover, *Euphilomedes* exhibit XX/XO sex determination, ruling out a contribution of Y-linked genes in males' eye development: any genes found in the eye transcriptome of males will also be found in the female genome; only expression levels will differ between the two sexes (Rivera and Oakley 2009).

In this study, we use behavioral experiments and physical observations of the eyes to ascertain why *Euphilomedes* exhibit such drastic sexual dimorphism. Namely, we compare the abilities of male and female *Euphilomedes* to avoid pelagic predators after blindfolding. We also create rough numerical estimates of sighting distances, in part using information from other species, to evaluate whether male *Euphilomedes* may use their compound eyes to visually identify potential competitors, mates, and/or predators. Finally, we begin to address how sexually dimorphic eyes develop in *Euphilomedes* by generating a transcriptome (i.e., a sequenced set of genes expressed by a tissue) for juvenile males' eyes. From our transcriptome, we identify candidate genes from regulatory networks that may be involved in differential eye development. By following multiple approaches to examine why and how compound eyes are found in male *Euphilomedes*, but not in female conspecifics, we build a strong case that these ostracods are a promising system for the integrative study of the evolution of sexually dimorphic traits.

Methods

Predation trials for male and female *Euphilomedes*

We studied two species of *Euphilomedes*: *Euphilomedes chupacabra* (Lum et al. 2008) from Puerto Rico and *Euphilomedes morini* (Kornicker and Harrison-Nelson 1997) from CA. We examined two species for practical reasons: *E. morini* is available to us locally, which allowed us to develop behavioral protocols over a longer time. After developing protocols, we conducted field work at a site known to have abundant male *E. chupacabra* (Lum et al. 2008), which allowed for larger sample sizes. The eyes of males of the two species are very similar in ommatidial number (*E. morini* = 16, *E. chupacabra* = 20), and the rudimentary eyes of females also are similar between species (Kornicker and Harrison-Nelson 1997; Lum et al. 2008). We used previously published protocols to collect *E. chupacabra* and *E. morini*; see Supplementary Methods for details.

In a series of laboratory experiments, we tested whether blindfolded *Euphilomedes* are more likely to be consumed by predatory fish than are individuals without impaired vision. These experiments involved three treatment groups: unaltered, control, and blindfolded (Fig. 1; for blindfolding procedures, see Supplementary Methods). We conducted predation trials with *E. chupacabra* during September 2010 at the Isla Magueyes Marine Laboratory (La Parguera, Puerto Rico); from November 2009 through August 2010, we performed a second round of predation experiments, this time using *E. morini* at UC—Santa Barbara (for a full description of these trials, see Supplementary Methods). For all trials, we placed eight individuals from each treatment group into a tank, along with a single zooplanktivorous fish. In all of our experiments on predation, we siphoned out all of the sand from an experimental tank, filtered the sand through a 500-μm sieve that retained the ostracods, then counted the number of surviving individuals from each experimental group (i.e., unaltered, control, and blindfolded).

From March to July 2010, we ran predation experiments with brooding female *E. morini* (which lack the compound eyes found in males). As in our trials with males, our treatments consisted of unaltered, control, and blindfolded individuals. For the control females, we glued a square of ribbon to the carapace over their brood; for the "blindfolded" females, we glued ribbon over their lateral rudiments. We then followed the experimental procedures described above for the predation trials with males. Finally, we attempted a series of mating experiments to test whether the presence of functional eyes in males influences mating success. These experiments were unsuccessful, but will be revisited in the future (see Supplementary Methods for details).

Eye morphology and sighting distances in *Euphilomedes*

To estimate the visual acuity and sensitivity of the eyes of male *Euphilomedes* ostracods, we studied the morphology of the eye in *E. morini* (which has eyes morphologically similar to those of *E. chupacabra* [Kornicker and Harrison-Nelson 1997; Lum et al. 2008]). To do so, we fixed specimens in a seawater-buffered 3.7% formalin solution for 4 h, then rinsed and stored them in sterile (autoclaved) phosphate buffered saline. Using a Leica CM1900 cryostat microtome (Leica, Solms, Germany), we made sections that we then mounted on glass slides using Hydro-Matrix solution (MicroTech Lab, Graz, Austria). We viewed these sections with either an Olympus dissecting scope (Melville, NY) or the 10 or 40× objectives of an Olympus BX61 compound microscope and obtained images with a Microfire digital camera operated via PictureFrame software (Optronics, Goleta, CA). Certain images were subsequently processed using Helicon de-convolution software (Helicon Soft, Kharkov, Ukraine) and/or Picture Publisher (Micrografx Inc., Richardson, TX).

From our images of sectioned eyes of male *E. morini*, we calculated inter-ommatidial angle ($\Delta\varphi$), an estimate of visual acuity (Land and Nilsson 2002). To find this value, we used ImageJ to measure the angle between adjacent ommatidia, a technique appropriate for apposition compound eyes like those of *E. morini*. Next, we estimated sensitivity (S), a measure of an eye's ability to gather

Fig. 1 The three treatments of male *Euphilomedes* used in the predation experiments. Above, ostracods are shown as (**A**) unaltered, (**B**) control, and (**C**) blindfolded.

photons, using a formula for eyes operating under broad-spectrum light (Warrant and Nilsson 1998):

$$S = \left(\frac{\pi}{4}\right)^2 A^2 \Delta\rho^2 \left(\frac{kl}{2.3 + kl}\right),$$

where A is the pupil diameter; $\Delta\rho$ is the acceptance angle of the rhabdoms; l is the receptor length; and k is the absorption coefficient. Employing methods from Land and Nilsson's (1990) study of the deep-sea ostracod *Macrocypridina*, we used sections of eyes from *Euphilomedes* to estimate our parameters as: $A = 21\,\mu m$; $\Delta\rho = 20^\circ = 0.35$ radians; and $l = 26\,\mu m$. We used data from Bruno et al. (1977) to estimate that $k = 0.0067\,\mu m^{-1}$, a value commonly used to calculate the sensitivity of rhabdomeric photoreceptors from arthropods' eyes (Warrant and Nilsson 1998).

To evaluate whether male *Euphilomedes* use their eyes to visually identify predators, conspecifics, or both, we used the following formula to estimate the maximum distance at which an ostracod with eyes similar to those of *E. morini* can detect objects of different sizes:

$$d = \frac{p}{\tan(\Delta\varphi)},$$

where d is the distance between viewer and object, p is size of the object, and $\Delta\varphi$ is the inter-receptor angle of the viewer in degrees (Land and Nilsson 1990). For our estimates of sighting distance, we made several assumptions: first, the object being viewed contrasts highly with the background, and neither emits light nor stimulates light production by nearby organisms such as dinoflagellates (Fleisher and Case 1995); second, the scene is bright enough that visual resolution is not compromised by low photon counts; and, third, *E. morini* is able to detect objects as small as its smallest value of $\Delta\varphi$. Please note that our calculations involve inter-receptor angle ($\Delta\varphi$), as in Land and Nilsson's (1990) work on *Macrocypridina*, not maximum resolvable spatial frequency, as in many papers concerning sighting distance in fish (e.g., Brokovich et al. 2010).

Preparation, sequencing, and assembly of transcriptomes

We used Illumina Ultra High Throughput Sequencing to collect new transcriptome data from the isolated eyes of juvenile male *Euphilomedes carcharodonta*. To produce cDNA for sequencing, we first dissected the stalked compound eyes from the bodies of juvenile *E. carcharodonta* instars III-V collected from Pillar Point Harbor, Half Moon Bay, CA (see Rivera and Oakley 2009 for collection protocol). We identified male juveniles by examination of the endopodite of the second antenna (Kornicker and Harrison-Nelson 1997). To extract RNA from *E. carcharodonta* eyes, we homogenized the eyes in the organic solvent TRIzol (Invitrogen) using plastic pestles and stored the homogenate at −80°C. Next, we removed trace DNA with the Ambion TURBO DNA-'free' kit (Invitrogen) and quantified RNA yield with a Qubit Fluorometer (Invitrogen). Next, we generated cDNA using the SMARTer cDNA synthesis kit (Clontech). To reduce sequencing artifacts due to poly-T tracts, we used modified 3′-primers for first-strand synthesis: 5′-AAG CAG TGG TAT CAA CGC AGA GTA CTTTTTTCTTTTTT-3′. For second-strand synthesis, we used the protocol outlined in the SMARTer cDNA kits and 21 cycles of amplification (a number chosen through a series of optimization procedures). Next, we purified the amplified cDNA using the Agencourt AMPure XP kit (Beckman Coulter) and again quantified yield by Qubit. Finally, we shipped 2 µg of cDNA to the UC Davis Genome Center for sequencing with their HiSeq2000 Illumina platform (Paired End 80 cycles). To prepare our reads from *E. carcharodonta* for assembly, we filtered out reads with all but three identical bases and then further filtered reads by specifying that 90% of their bases have quality scores equal to or greater than 20. We assembled our transcriptome by first passing reads through Trinity, with default settings and a minimum contig length of 150 bp, then passing the resulting contigs through iAssembler with default settings.

Transcriptome annotation

We used two separate methods to search juvenile males' eye transcriptome for orthologs of known phototransduction, retinal determination, and pro-neural network genes. Our first method relied on PhyML tree-building using a modified version of methods previously described (Rivera et al. 2010). Briefly, for each gene of interest, we first performed tblastn searches of the *E. carcharodonta* transcriptome using *Drosophila* sequences as bait (Table 1). We used *Drosophila* protein sequences to search the UniRef50 database, retaining the top 50–100 hits. We then translated our *E. carcharodonta* hits, identified earlier from our transcriptome, using CLC Main Workbench (CLCbio). For each gene family, we aligned *E. carcharodonta*, *Drosophila*, and UniRef50 hits using Muscle implemented in Seaview. We then used the resulting alignments to build phylogenetic trees, using PhyML implemented in Seaview assuming a JTT model of protein evolution.

Table 1 Presence of eye-gene family members in juvenile eye transcriptome of *Euphilomedes carcharodonta*

Gene	No. contigs found	Gene	No. contigs found	Gene	No. contigs found
Engrailed	4	Six1/2 (Sine oculis)	0	Trp	5
Hedgehog	4	Six4	0	Opsin	4
Dac	3	Vsx	0	Gprotein receptor kinase 1	3
Decapentaplegic	3	Wnt	0	Gq-alpha	3
Eya	3	Atonal	0*	Gq-beta	3
Notch	3	Calphotin	0*	Gq-gamma	3
EGFR	2	Elav	0*	Phospholipase C	3
Munster (Pph13)	2	Fernandez (Dan)	0*	PKC	3
Ocelliless (Otd)	2	Lozenge	0*	Retinal degeneration B	3
Pax6 (Ey/Toy)	2	Phyllopod	0*	Arrestin	2
Optix	1	Prospero	0*	DAGK	2
Eyg	0	Rough	0*	Gprotein receptor kinase 2	1
Glass	0	Teashirt	0*	Calnexin	1**
Rx	0	Tiptop	0*	Retinal degeneration C	0*
				Lazaro	0*
				Pinta	0*

Left columns are developmental genes, right columns are phototransduction genes. Asterisks denote that Evolutionary Placement Algorithm was not performed; either there were no tblastn hits for the gene family with an e-value above 0.01 (single asterisk) or a tree was generated via standard ML analysis (double asterisks, see "Methods" section for details). Gene families without representatives in the transcriptome are highlighted in gray.

Sequences from *E. carcharodonta* falling within a clade defined by known gene family members were considered to be members of that gene family. In our second, complementary, method, we searched our transcriptome for orthologs of well characterized eye-related genes using an Evolutionary Placement Algorithm (Berger et al. 2011). Our methods will be described in detail in a forth-coming paper, but a brief account follows here. First, we used functionally characterized exemplars of phototransduction, retinal determination, and proneural network proteins (e.g., those from *Drosophila melanogaster*) and the blastp algorithm to search predicted protein databases from the genomes of 23 metazoans, 1 choanoflagellate, and 2 fungi (species and sources listed in Supplementary Table S1). We used a set of these sequences to build reference trees for our genes of interest by using MAFFT for alignment and RAxML for estimating gene phylogenies (with WAG as a protein model and 100 bootstrap replicates, followed by a maximum-likelihood search for the best tree). Next, we searched our transcriptomes using blast searches with the same queries as above, identified potential orthologs, and placed these "hits" on our reference trees using the Evolutionary Placement Algorithm (Berger et al. 2011), which places unknown genes into a pre-calculated gene phylogeny (reference trees). We used the resulting trees to annotate our transcriptomes by identifying genes as orthologs of known genes if they fall in an expected position based on the gene phylogeny. Primary transcriptome data are available in the Dryad repository (doi:10.5061/dryad.277g0).

Results

Predation experiments

Our predation experiments demonstrate that blindfolded male *Euphilomedes* have a significantly lower survival rate than do control individuals when both are exposed to predatory fish. In trials with *E. chupacabra*, we recovered fewer blindfolded males than control males ($P_{dir} = 0.016$; Fig. 2A) and there were significantly more experimental trials in which a greater number of control males were recovered than were blindfolded males ($P = 0.006$; Fig. 2A). Similarly, fewer blindfolded male *E. morini* survived the predation trials than did control individuals ($P_{dir} = 0.043$; Fig. 2B). The relationship between unaltered and control individuals was less straightforward, however. There was no significant difference between the numbers of unaltered and control *E. chupacabra* males that we recovered ($P_{dir} = 0.630$; Fig. 2A), but we did recover more unaltered *E. morini* males than control males ($P_{dir} < 0.001$;

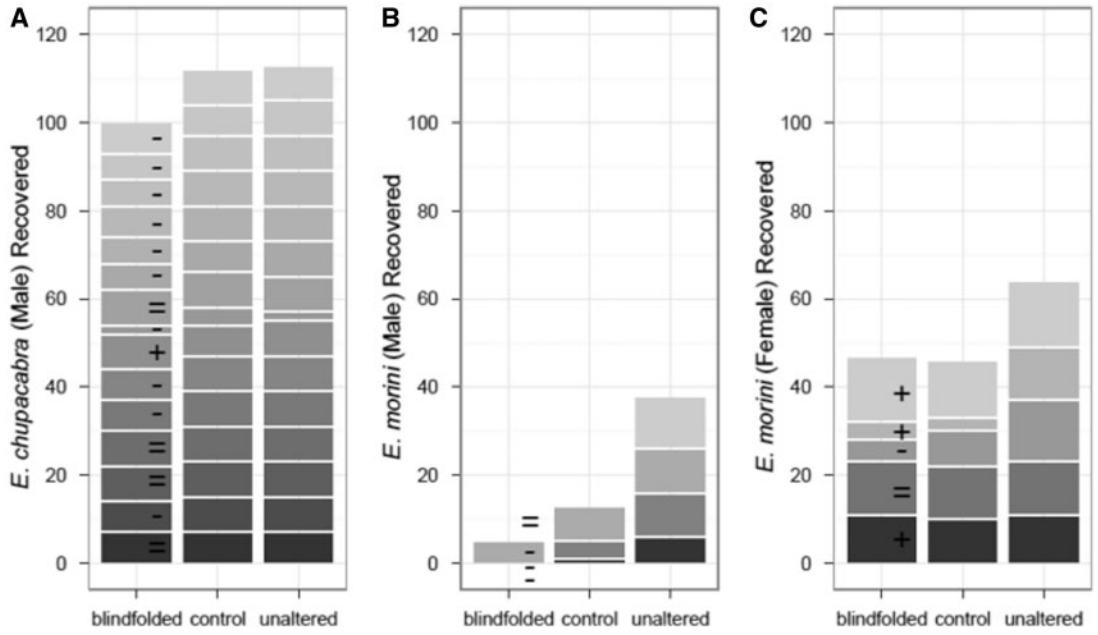

Fig. 2 The results of predation trials involving *Euphilomedes* ostracods. For each trial, equal numbers of unaltered, control, and blindfolded individuals were placed in a tank with one predatory fish. Surviving ostracods were then counted. Each gray shade within each plot represents an individual trial. (**A**) In *E. chupacabra*, blindfolded males had a lower survival rate than did control males ($P_{dir} = 0.016$) and there was no difference between the number of surviving control and unaltered males ($P_{dir} = 0.630$). (**B**) In male *E. morini*, blindfolding also decreased survival rate relative to control conditions ($P_{dir} = 0.043$), but for this species control animals had a higher mortality rate than did those left unaltered ($P_{dir} < 0.001$). (**C**) In female *E. morini*, there was no difference in the survival rate of blindfolded and control individuals ($P_{dir} = 0.630$), but significantly more unaltered females survived the predation trials than did control females ($P_{dir} < 0.001$). In the "blindfolded" columns in (A), (B), and (C), +, −, or = indicate how the survival rate of blindfolded ostracods compared with control individuals within each trial.

Fig. 2B). Blindfolding did not influence survival rates in *Euphilomedes* females as it did in males; we found no significant difference between the numbers of blindfolded and control *E. morini* females that survived our predation trials ($P_{dir} = 0.630$; Fig. 2C). We also noted that significantly more unaltered females survived the predation trials than did control females ($P_{dir} < 0.001$; Fig. 2C). Finally, we found that brooding *E. morini* females suffered lower mortality rates than did adult males in our predation experiments; across all trials, a significantly greater proportion of unaltered females survived than did unaltered males ($P < 0.0001$; Fig. 3).

Visual acuity and sighting distances in *Euphilomedes*

Next, we asked whether male *Euphilomedes* can visually identify predators, conspecifics, or both. We calculated that the compound eyes of male *E. morini* have a minimum inter-ommatidial angle ($\Delta\varphi$) of 8°, indicating that these eyes provide an angular resolution of about 8° (equivalent to a maximum resolvable spatial frequency of about 3.6 cycles per radian). Further, we found that the sensitivity (S) of the eyes of *E. morini* is 2.3 $\mu m^2 \cdot$steradian, meaning that each photoreceptor gathers 2.3 photons when viewing a scene with a standard radiance of R. If we assume realistic levels of uncertainty for our morphological measurements, S probably falls between 2 and 5 μm^2 sr for *E. morini*, well within the expected range for an eye adapted to the shallow, coastal environments where *Euphilomedes* are abundant (Land and Nilsson 2002). Given the above values for $\Delta\varphi$ and S and assuming bright, clear water, we found that an ostracod with eyes similar to those of *E. morini* would be able to detect conspecifics 1.7 mm long at a range of 12 mm or less and predators 100 mm long, such as the juvenile fish used in our predation experiments, at a maximum distance of 710 mm. We intend these values as rough estimates of the distances at which *Euphilomedes* may detect conspecifics and predators; a more complete model with a full set of empirically-derived parameters is beyond the scope of this article, but will be pursued in the future. Because our calculations assume a best-case scenario for angular resolution in *Euphilomedes* and the best possible conditions for viewing, it is likely that we are over-estimating the maximum distances at which *Euphilomedes* can detect conspecifics and predators.

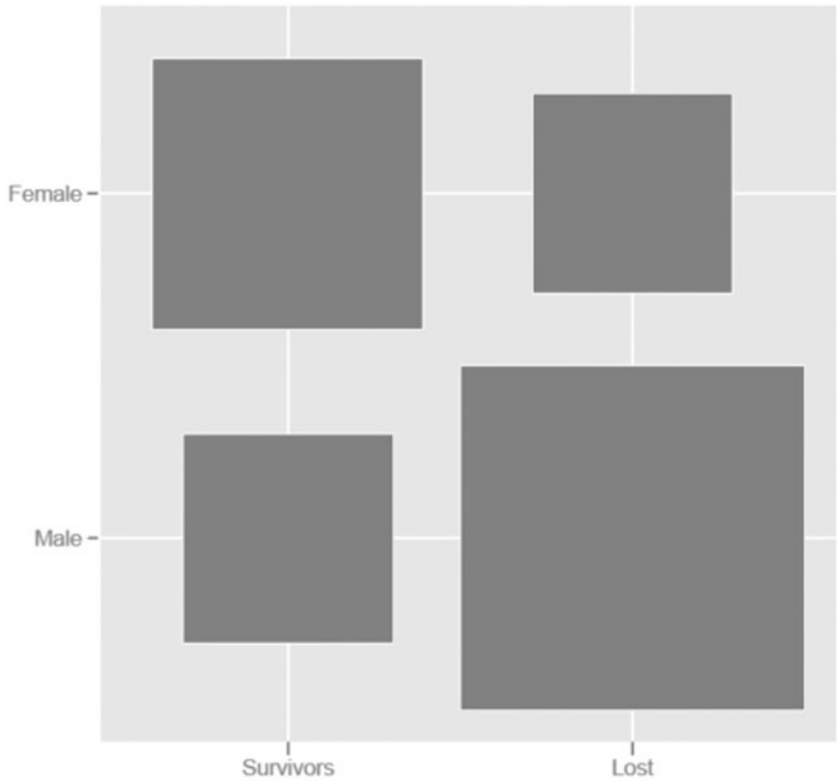

Fig. 3 A comparison between survival rates in unaltered male and female *Euphilomedes* ostracods. In these experiments, we separately placed males and brooding females in tanks with one predatory fish and counted the number of surviving ostracods. The area of each gray square is proportional to the number of animals recovered from each category. We found, in a two-tailed Fisher's exact test, that brooding *E. morini* females suffered significantly lower mortality rates than adult males: across all trials, a significantly greater proportion of unaltered females survived than unaltered males ($P < 0.0001$).

Transcriptome analysis

We performed blast searches and phylogenetically informed analysis to search juvenile *E. carcharodonta* males' eye transcriptome for members of 43 gene families involved in rhabdomeric phototransduction or eye development in *Drosophila* (Bao and Friedrich 2009). Of these 43 targets, we identified members of 23 eye-gene families in *E. carcharodonta* juvenile males' eyes (Table 1; Supplementary Fig. S2). These include most core members of the so-called retinal determination network (i.e., Pax6, Eya, Dac, and Optix) as well as non-core members of this network (Hh and Dpp) and the proneural gene Notch. Also of note is the presence of nearly every phototransduction gene expected for the rhabdomeric-type photoreceptors employed by *Drosophila* eyes.

Discussion

Euphilomedes constitute a promising model for investigations into both the "how" and the "why" of the evolution of sexual dimorphism. Our previous study identified these ostracods as an attractive system for studying eye development and this study brings the additional tools of experimental behavioral studies and transcriptomics (Rivera and Oakley 2009).

The eyes of *Euphilomedes* are an ecological sex trait

Manipulative experiments and numerical estimates of visual abilities suggest that the eyes of *Euphilomedes* ought to be considered ecological sex traits, not secondary sexual characteristics. First, through a series of manipulative laboratory experiments, we demonstrated that selection, in the form of pelagic predators, acts differently on the visual abilities of male and female *Euphilomedes*. We placed ribbon on the carapace of *Euphilomedes* males either over the eye (blindfolded) or on their dorsal aspect (sham). Because we recovered more sham-treated than blindfolded males after exposure to predators, we conclude that vision plays a role in evasion of predators. However, addition of a ribbon alone seems to have an effect on evasion of predators as unaltered males and females had a higher rate of recovery than did the sham controls after exposure to predators. Finally, the two species of *Euphilomedes* that we tested were differentially affected by sham treatment,

possibly due to differences in experimental protocol and/or differences in the local predators to which they were exposed (see Supplementary Materials for details).

Blindfolded male *Euphilomedes* are more likely to be consumed by predatory fish than are males without impaired vision, but blindfolding does not similarly influence the survival rates of brooding female *Euphilomedes*. Thus, we conclude that male *Euphilomedes* use their compound eyes (lacking in females) to detect pelagic predators. We suspect that male *Euphilomedes* encounter such predators during the multiple nights they spend searching for mates in the water column. We assume that blindfolding did not alter the time males spent in the water column, an assumption supported by our sham controls and which could be tested in future experiments with infrared imaging. We also found that unaltered, brooding female *E. morini* had higher survival rates across our predation trials than did unaltered males. It is probably not surprising that the females, which appear to lack spatial vision, are relatively unaffected by blindfolding. Our preferred explanation for this is that the brooding females, which had no mating-related motivation to leave the relative safety of the sediment, spent more time in the sediment at the bottom of the experimental tanks than did the males and were thus more likely to avoid the pelagic predators we introduced. Future research could also examine survival of blindfolded virgin females, with a prediction that their survival rate would not differ from that of sham controls. However, obtaining virgin, sexually mature females is technically challenging.

Through rough estimates of sighting distances, it appears unlikely that the eyes of male *Euphilomedes* are employed for male–male competition or mate choice, indicating that these organs ought to be considered an ecological sex trait, not a secondary sex character. It is likely that the eyes of male *Euphilomedes* allow these animals to detect predators at ecologically relevant distances. However, males' eyes almost certainly lack the optical ability necessary to distinguish between males and females. Higher acuity vision would be necessary for males to resolve sex-related details, such as females' body orientation or the slight differences in body shape that distinguish male and female conspecifics. Without vision, what sensory modalities are then used in recognition and choice of mates by *Euphilomedes*? Other ostracod species use luminescent displays (Rivers and Morin 2008), but *Euphilomedes* do not exhibit bioluminescence. Little is known about non-visual mating cues in ostracods, but it is possible that they use chemical signals as do other crustaceans (e.g., lobsters [Skog 2009], blue crabs [Kamio and Derby 2011], mantis shrimp [Mead and Caldwell 2011], and copepods [Yen et al. 2011]).

The reproductive role hypothesis

Ecological sex traits, such as the eyes of *Euphilomedes* ostracods, may be explained by either sex-specific resource partitioning or the RRH. We find that the RRH provides a better explanation than does resource partitioning for why male *Euphilomedes* have compound eyes but females do not. In cases in which competition for resources has produced sexually dimorphic traits, e.g., head sizes in terrapins (Tucker et al. 1995), head shapes in cottonmouth snakes (Vincent et al. 2004), and bill shapes in certain hummingbirds (Temeles et al. 2000, 2010), male and female conspecifics evolve distinct feeding structures that allow them to take advantage of different diets. *Euphilomedes* ostracods are deposit-feeders, not visually-guided predators or scavengers, so spatial vision will not help males exploit food resources unavailable to females. Indeed, it is common in sarsielloid ostracods, including many *Euphilomedes*, for adult males to have reduced feeding appendages (mandible, maxilla and fifth limb), and perhaps some adult male *Euphilomedes* simply do not feed (Fenwick 1984; Kornicker 1981). Our work with *Euphilomedes* constitutes (to our knowledge) the first time that the RRH has been tested by manipulative experiments. Although previously published research shows a positive relationship between eye size and predation pressure in the amphipod *Gammarus minus* (Glazier and Deptola 2011) and sexually dimorphic sensory abilities in many species are known to be correlated with males and females facing different predation pressures (Lau et al. 2007; Meyer-Rochow and Lau 2008; Yager 1990), to our knowledge none of these possibilities have been tested empirically.

Consistent with our experimental results and with the predictions of the RRH, observational data suggest that sex-specific reproductive behaviors cause male *Euphilomedes* to spend more time in the plankton than do females. Additionally, brooding female myodocopids possess spermatophores that allow multiple fertilizations from a single mating (Cohen 1983; Gerrish and Morin 2008) and may act as sperm plugs that prevent females from mating multiple times (Cohen and Morin 1990). Along with previous authors who have studied a diversity of myodocopid ostracods (Baker 1977; Cohen and Morin 1990; Fenwick 1984), we find that the

majority of *Euphilomedes* in sediment samples are females and that nearly all individuals collected from the water column are males. To explain the female bias in sediment samples, we suspect that an initial sex ratio of 1:1 at birth becomes skewed due to the numbers of males that are preyed upon during their time in the plankton (Baker 1977), as also proposed elsewhere (Fenwick 1984).

Although the meager optical abilities of *Euphilomedes* raise significant doubts about the ability of sexual selection to act directly upon the morphological dimorphism of the eye, sexual selection may influence evolution of the eyes in these ostracods indirectly. For example, competition for mates may influence the amount of time *Euphilomedes* males spend in the water column; the longer an individual male spends in the water, the higher are his chances of mating. Generally, the benefits of planktonic swimming may be advantageous to males when the increase of mating success due to swimming outweighs the increase in risk of predation. Therefore, we hypothesize that sexual selection, mediated by intraspecific interactions, is a primary driver of the time males spend in the plankton. Despite being unable to visually identify females, male *Euphilomedes* could use spatial vision for mating-related activities such as the detection of habitats (e.g., particular substrates) where females tend to be relatively abundant. Bioluminescent males in the family Cypridinidae are known to signal to females over stereotyped, species-specific microhabitats (Cohen and Morin 1990), so similar behaviors in *Euphilomedes* are plausible. These considerations highlight the likelihood of a complex interplay between sexual and natural selection in the evolution of sexual dimorphism in the possession of eyes in *Euphilomedes*.

Evolution of eyes in *Euphilomedes*

Although this study is focused on factors maintaining eyes in males and not in females in modern populations, another interesting consideration is the historical question of whether females lost eyes or males gained eyes during the evolutionary history of *Euphilomedes*. Developmental/ontogenetic data suggest *Euphilomedes* males gained eyes while available phylogenetic data indicate females lost eyes (Rivera and Oakley 2009). Ontogenetically, the eyes of *Euphilomedes* males develop in subadults, much later than the embryonic development of eyes in other myodocopids, i.e., heterochrony. Eyes of *Euphilomedes* males also develop from a novel two-part structure that may originate developmentally by field-splitting and evolutionarily by furcation, the ''duplication'' of structures. Both heterochrony and the novel two-part structure are consistent with a gain of eyes in *Euphilomedes* males. The possibility of gaining a compound eye during evolution may seem surprising, but genes regulating eye development in most organisms are largely pleiotropic and thus would likely have been present in an eyeless ancestor. In this way, only interactions between these genes would need to be tweaked to rebuild an eye (Oakley 2003; Syme and Oakley 2011).

Our current study makes significant inroads into this thorny issue by examining genes involved in regulating eye development and phototransduction in the *E. carcharodonta* transcriptome. We find that several well-known genetic networks have key members represented in juvenile males' eye transcriptome (Table 1). These include the phototransduction signaling network (Table 1, right column), the proneural network (exemplified by Notch), and the retinal determination network (Pax6, Eya, Dac, and Optix). Because the transcriptome was made using dissected eyes, these genes are very likely expressed in the developing ommatidia. This suggests that at least some of the genetics underlying eye development in *Drosophila* is conserved in *E. carcharodonta* males. The ontogeny of gene expression in eyes is of particular interest—does the release from quiescence of eye-development seen in males of instars III–V correspond to an upregulation of eye-development genes at this time? Which genes are expressed only in males' eyes but not in females' rudiments? Once these genetic questions are answered, future work can compare eye development *E. carcharodonta* to eye development in other ostracod species, both dimorphic and non-dimorphic. With an understanding of the evolution of eye genetics with regards to dimorphism, we will be able to test hypotheses about loss and gain of eyes in this group. For example, do non-dimorphic species express genes in a pattern similar to that in male *E. carcharodonta* (suggestive of loss of eyes in females) or does *E. carcharodonta* have significant differences in order or timing of deployment of eye genes (suggestive of regain of eyes in males)? This study provides the transcriptome data for beginning this type of analysis of expression.

Author contributions

R.I.L. and T.H.O. designed the behavioral experiments, R.I.L. and V.R.L. performed the behavioral experiments, and R.I.L. and T.H.O. analyzed behavioral experiments. D.I.S. was responsible for the

analysis of ostracods' visual acuity and sighting distances. A.S.R., D.I.S., and B.C.-Z. performed transcriptome preparation and analysis. D.I.S., T.H.O., A.S.R., and R.I.L. wrote the paper.

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Supplementary data

Supplementary Data available at *ICB* online.

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