How, M. J., Porter, M., Radford, A. N., Feller, K., Temple, S. E., R.L., C., ... Roberts, N. W. (2014). Out of the blue: the evolution of horizontally polarized signals in Haptosquilla (Crustacea, Stomatopoda, Protosquillidae). The Journal of Experimental Biology, 217, 3425-3431. 10.1242/jeb.107581

J Exp Biol Advance Online Articles. First posted online on 7 August 2014 as doi:10.1242/jeb.107581
Access the most recent version at http://jeb.biologists.org/lookup/doi/10.1242/jeb.107581

Out of the blue: The evolution of horizontally polarized signals in *Haptosquilla* (Crustacea, Stomatopoda, Protosquillidae)

Martin J How[1§], Megan L Porter[2§], Andrew N Radford[1§], Kathryn D Feller[3], Shelby E Temple[1], Roy L Caldwell[4], N Justin Marshall[5], Thomas W Cronin[3] and Nicholas W Roberts[1*]

1. School of Biological Sciences, University of Bristol, Tyndall Avenue, Bristol, BS8 1TQ, UK
2. Department of Biology, University of South Dakota, Vermillion, SD 57069, USA
3. Department of Biological Sciences, University of Maryland Baltimore County, 1000 Hilltop Circle, Baltimore, Maryland 21250, USA
4. Department of Integrative Biology, University of California, Berkeley, CA 94720, USA
5. Queensland Brain Institute, The University of Queensland, St Lucia, QLD 4072, Australia

*Corresponding author: nicholas.roberts@bristol.ac.uk
§ These authors contributed equally to this work.

Running title: Evolution of polarization signals

Keywords
stomatopod, mantis shrimp, polarization vision, signal evolution, sensory bias, multi-modal signal.

The polarization of light provides information that is used by many animals for a number of different visually guided behaviours. Several marine species, such as stomatopod crustaceans and cephalopod molluscs, communicate using visual signals that contain polarized information, content that is often part of a more complex multi-dimensional visual signal. In this work, we investigate the evolution of polarized signals in species of *Haptosquilla,* a widespread genus of stomatopod, as well as related protosquillids. We present evidence for a pre-

existing bias towards horizontally polarized signal content and demonstrate that the properties of the polarization vision system in these animals increase the signal-to-noise ratio of the signal. Combining these results with the increase in efficacy that polarization provides over intensity and hue in a shallow marine environment, we propose a joint framework for the evolution of the polarized form of these complex signals based on both efficacy-driven (proximate) and content-driven (ultimate) selection pressures.

INTRODUCTION

Polarization sensitivity is a common visual specialization that has evolved in both terrestrial and aquatic animals, and is particularly prevalent in invertebrates (Wehner and Labhart, 2006). On land, many insects use the celestial polarization pattern for navigation (Wehner, 1976; Rossel and Wehner, 1986; Labhart and Meyer, 1999; Dacke et al., 2003), while in the ocean, some crustaceans and cephalopod molluscs use polarization information to detect prey and possibly as a means of conspecific communication (Shashar et al., 1996; Cronin et al., 2003a; Chiou et al., 2007; Mäthger et al., 2009; Cronin et al., 2009; Chiou et al., 2011). In the context of communication, polarization often forms composite signals with other visual dimensions, such as hue and brightness (Cronin et al., 2003a; Cronin et al., 2009).

The term polarization is used to define several properties of light. The angle of polarization describes the predominant direction in which the electric field of the light oscillates, while the degree of polarization defines the extent to which waves oscillate at the same angle. Underwater, differential sensitivity to either angle or degree of polarization has several fundamental advantages over

other forms of visual information (Cronin et al., 2003a; Cronin et al., 2003b; Cronin et al., 2009; Shashar et al., 2011). For instance, in shallow, clear marine waters, the intensity and spectral composition of the down-welling light can vary dramatically, both as a function of the time of day, and because of environmental factors such as turbidity (Cronin et al., 2014). In such changing conditions, the polarization of light remains more constant than other visual dimensions over short ranges (Waterman, 1954; Cronin, 2001), which renders it a reliable provider of information (Shashar et al., 2011; Johnsen et al., 2011). Previous research in this field has focused on either the underlying retinal mechanisms of polarization sensitivity (for review see Horváth and Varjú, 2004; Roberts et al., 2011), or the optical mechanisms by which polarization and multi-component polarization/colour signals are produced (Chiou et al., 2005; Mäthger and Hanlon, 2006; Chiou et al., 2007; Mäthger et al., 2009; Cronin et al., 2009). In contrast, the evolutionary context of polarization signal content relative to the visual system of receivers is still very much unknown.

Stomatopod crustaceans are some of the best-studied species in terms of polarization vision. Electrophysiological studies have detailed the spatial variation of polarization sensitivity in the different photoreceptor classes in the eye (Kleinlogel and Marshall, 2006; Chiou et al., 2008). Optical measurements (Marshall et al., 1991; Chiou et al., 2008), optical modeling (Roberts et al., 2009) and molecular methods (Porter et al., 2009; Roberts et al., 2011) have provided additional information on the underlying mechanisms of polarization sensitivity. Optical techniques have also shown that many species of stomatopod produce visual signals that are either linearly or circularly polarized (Chiou et al., 2005; Chiou et al., 2008; Cronin et al., 2009). The stomatopod genus *Haptosquilla*

(family Protosquillidae) is known to use signals from the first maxillipeds for both sexual and agonistic communication (Dingle and Caldwell, 1969; Caldwell and Dingle, 1975; Chiou et al., 2011). A common feature of *Haptosquilla* first maxillipeds is the production of a conspicuous blue structural reflection (Chiou et al., 2005; Cronin et al., 2009). Fig. 1 illustrates the blue signal in four species: *Haptosquilla trispinosa*, *H. glyptocercus*, *H. stoliura* and *H. banggai*. In some species of the genus (e.g. *H. trispinosa*, *H. stoliura* and *H. banggai*), this reflection is also horizontally polarized (Chiou et al., 2005; Cronin et al., 2009).

Here we explore the potential evolutionary pathways of polarization communication in protosquillid stomatopods. First, we use experiments to investigate whether the behavioural responses to different forms of polarization signal content are species specific. We do this by exploiting the animal's innate behavioural responses to polarized looming stimuli presented on modified LCD monitors. We compare four representative protosquillid species: *H. trispinosa*, *H. glyptocercus, Chorisquilla tweediei* and *C. hystrix*. Second, and in the context of the signal's polarization content, we measure the threshold at which *H. trispinosa* are no longer able to discriminate between two different angles of polarization. Finally, we construct a phylogeny of protosquillid species to consider the evolution of the polarization properties of maxilliped signals.

RESULTS

Responses to polarized stimuli

H. trispinosa, H. glyptocercus and *C. tweediei* all showed a significantly greater probability of response to the horizontally polarized stimulus compared with a vertically polarized stimulus. (*H. trispinosa:* Wilcoxon Test: Z=2.93, d.f = 9, p =

0.002; Fig. 2A; *H. glyptocercus*: Z = 2.42, d.f = 9, p = 0.02; Fig. 2B; *C. tweediei:* Z = 2.77, d.f = 9, p = 0.004; Fig. 2C). *C. hystrix* also appeared to be more responsive to horizontally polarized light (Fig. 2D), but the small sample size (n=5) precluded statistical testing. There was no significant difference between *H. trispinosa, H. glyptocercus* and *C. tweediei* in their relative responses to the two stimuli (Kruskal-Wallis test: $\chi^2 = 2.90$, d.f. = 2, p = 0.24).

Level of discrimination between two angles of linearly polarized light

H. trispinosa showed little or no response to stimuli when the difference between the polarization angles of the stimulus and background was between 31.4 degrees and 20 degrees (Fig 3, Supplemental Table S1). At angles of 20 degrees or less, the animals rarely responded to the polarization stimulus; at values of 31.4 degrees and above, they displayed a consistent statistically significant response to the stimulus.

Presence of polarized signals

The 1st maxilliped reflections from *H. trispinosa*, *H. glyptocercus*, *C. tweediei* and *C. hystrix* are presented in the microscope images displayed in Figs 4A–D. Both *H. trispinosa* (Fig. 4A) and *H. glyptocercus* (Fig. 4B) showblue reflections from the maxillipeds compared with very weak, spectrally broad reflections from the *Chorisquilla* species (Figs 4C, D). Of the blue *Haptosquilla* reflections, *H. trispinosa* are horizontally polarized (Fig. 4A) whereas the reflections from *H. glyptocercus* are unpolarized (Fig. 4B).

Visual analyses of other species of *Haptosquilla* showed that *H. stoliura*, *H. banggai, H. pulchella, H. nefanda* and *H. hamifera* all have blue-reflecting 1st

maxillipeds, but only the reflections from *H. stoliura*, *H. banggai*, *H. pulchella* and *H. nefanda* are horizontally polarized. Within the rest of the Protosquillidae, five further species have been analyzed (*C. excavata*, *C. hystrix*, *C. tweediei*, *Echinosquilla guerinii*, and *Protosquilla folini*) with none possessing blue or blue and horizontally polarized 1st maxillipeds. Outside of the Protosquillidae, six other stomatopod species from nine genera and four families have been inspected for 1st maxilliped signal types. Of these species, only *G. smithii* possess blue signals and no other species possess either blue or horizontally polarizing signals (Fig. 5).

Phylogenetic analyses

Phylogenetic analyses of protosquillid relationships recapitulate previous studies (Barber & Boyce 2006; Porter et al., 2010) recovering the protosquillids (bootstrap percentages (BP) = 98), and in particular the genus *Haptosquilla* (BP = 89), as monophyletic (Fig. 5). Within the *Haptosquilla*, our phylogeny recovered two sub-groups of species that correspond to the two known types of 1st maxilliped signaling, either blue and unpolarized or blue and polarizing.

DISCUSSION

Our results provide the direct evidence that several species of stomatopod have an *inherent* (i.e. non-trained) behavioural response to a looming, linearly polarized stimulus. Moreover, all the protosquillid species tested displayed a greater probability of response to horizontally polarized stimuli compared with those that are vertically polarized. The measurements of the structural colour and polarization properties of the maxillipeds, in combination with the

comparative phylogenetic analyses, revealed that of these protosquillids, only the genus *Haptosquilla* displays the blue signals. Furthermore, it is only the sub-group of *Haptosquilla* including *H. trispinosa* that possesses the additional polarized signal dimension. In these species, the polarization of the signals is always orientated horizontally. Therefore, it is possible that the common behavioural predisposition towards horizontally polarized stimuli seen across the protosquillids could have biased the polarization content of 1st maxilliped signals to be horizontal in the *H. trispinosa* clade (Guilford and Dawkins, 1991; Endler and Basolo, 1998). A common question raised by the concept of sensory bias is why does the bias preexist? Whilst we can only speculate, the bias for a horizontal angle of polarization may come from the fact that this angle is most prevalent in reflections from objects and preferential sensitivity may have previously evolved to improve contrast discrimination (Temple, 2012).

H. trispinosa also displayed a threshold of between 21.4 and 30 degrees in their response to distinguishing between two angles of polarization. Such a coarse level of discrimination would improve the signal-to-noise ratio of a linearly polarized signal by effectively low-pass filtering any variation in the background. This threshold is an order of magnitude higher than measured in other species (fiddler crab, *Uca vomeris*, 3.2 degrees - How et al., 2012; cuttlefish, *Sepia plangon*, 1 degree - Temple et al., 2012) and is suggestive of tuning for high contrast signals compared with the current evidence that other crustacean and cephalopod polarization visual systems are used to resolve high levels of polarization detail.

The complex nature of stomatopod eye design (two hemispheres separated by a specialized midband) may place limitations on the amount of information that can be processed from the visual scene but in turn enhance the processing efficiency. Currently, it is thought that the two hemispheres are primarily involved in producing a two-dimensional representation of the visual scene, over which the midband is then scanned, rather like a line-scan sensor, to expand on the colour and polarization information (Land et al., 1990). The motion component of the LCD looming stimulus used in our experiment is therefore most likely to be stimulating responses in the stomatopod visual hemispheres, which elicit a visual saccade to the target, and presumably this would be followed by a subsequent visual scan of the target with the midband to fill in the remaining information. It is conceivable therefore, that much of the early visual information is simplified to speed up sensory processing (for an equivalent discussion for colour vision see Thoen et al., 2014). If so, the polarization discrimination responses we have measured specifically represent a property of the visual system in the dorsal and ventral hemispheres. However, the precise behavioural context should also not be ignored. It is quite possible that the measured discrimination threshold is specific to the task demanded of the animals. Further work is also still needed to investigate how the degree of polarization affects behavioural responses to such polarization signals.

Overall, our findings provide a framework for understanding the potential evolutionary pathway of the polarization properties of these maxilliped signals in stomatopods. Successful communication relies on information being sent through the environment in such a way that it will be received in its intended form, and be interpreted as to elicit a behavioural response in the intended

receiver (Parten and Marler, 2005). In this context, the selective pressures on signal evolution are both efficacy-driven and content-driven (Guilford and Dawkins, 1991; Hebets and Papaj, 2005). As described in the Introduction, polarization provides a reliable form of visual information, particularly in spectrally variable light environments, such as the conditions that these species of stomatopod inhabit. The increase in signal efficacy by the inclusion of this extra visual dimension is therefore fairly clear. The behavioural bias towards horizontal polarized light provides a further explanation for why the polarized content of the signals has evolved to be horizontally polarized. Together, the addition of polarization to the signal and nature of the bias suggest both the proximate and ultimate drivers respectively for the evolution of this complex signal.

Two questions for the future are: can manipulating the relative polarization contrast of the signal and the background influence the bias? Secondly, do the spectral and polarization dimensions act independently for purposes of information redundancy or do they combine in a functional way; for example, increasing the accuracy of receiver response as is described by an amplifier hypothesis of multi-component signals (Hasson, 1991; Candolin, 2003; Hebets and Papaj, 2005)? We suggest that future studies of combined polarization and colour signals in other animals should also carefully consider how these dual dimensions are viewed together by receiver visual systems under the correct environmental light conditions. Whilst it is not always easy to decompose complex signals and test the functions of individual components (Hebets and Papaj, 2005), the combined colour and polarization signals in

stomatopods represent an excellent behavioural system to investigate the function and evolution of signal complexity.

MATERIALS AND METHODS

Animals

To investigate the inherent ability of stomatopods to generate distinct behavioral responses to polarized stimuli, we collected 39 individuals of *H. trispinosa*, 10 individuals of both *H. glyptocercus* and *C. tweediei* and five individuals of *C. hystrix* from off-shore reefs near Lizard Island, Great Barrier Reef, Australia in August 2011 (Queensland–GBRMPA permit G12/35042.1). Animals were maintained before testing in a natural seawater flow-through marine aquarium facility at the Lizard Island Research Station (24–25°C, natural daylight illumination, and fed pieces of frozen shrimp). All procedures were approved by the Animal Ethics Committees of the University of Queensland (AEC, permit # QBI/223/10/ARC/US AIRFORCE (NF)).

Relationship between behavioural responses and polarization stimulus content

Individual stomatopods were placed in a 30 x 15 x 15 cm tank containing local beach sand. Each individual was placed inside an 8 mm diameter clear tube and restrained using a small amount of fishing line (Land et al., 1990; Cronin et al., 1991). The animal was positioned such that the eyes were forward of the front end of the tube (Fig. 6A). Directly above the animal was a video camera (Canon Legria FS20) that recorded its response to the presentation of the stimuli. On the

outside of the tank, and in front of the animal, was an LCD screen (Viglen LC552; 1280 x 1024 spatial resolution at 60 Hz); the eyes were at a distance of approximately 12 cm from the screen. By removing the front polarizer from the LCD screen and addressing the LCD with a grayscale value of either 0 (black) or 255 (white), the local output polarization could be controlled as vertical (V stimulus) or horizontal (H stimulus) respectively (Pignatelli et al., 2011). The stimuli expanded to cover 22.5° of the visual field angle in 1 s (taking into account refraction at the air / glass / water boundaries). The simple electro-optic control of the polarization of the light permitted not only dynamic control of the polarization, but most importantly an inherent zero luminance and chromatic contrast between the background and the looming stimulus. To check the polarization properties of the LCD, accurate broadband Stokes parameter measurements (Fig. 6B) were made using Glan-Thompson polarizers and a ¼ wave Fresnel–rhomb (Edmund Optics, York, UK), which permitted the computation of the polarization ellipse of each of the stimuli for any wavelength (Fig. 6C).

All animals received a balanced pseudo-randomized presentation of 10 H stimuli and 10 V stimuli, against a perpendicularly linearly polarized background. No more than three instances of the same stimulus were presented in a row. We randomly varied the time between successive stimuli, from 20 to 120 s, to minimize any effect of habituation. To determine whether the animal responded to the two stimulus types, we monitored the optokinetic response of the focal animal. We defined a positive response to the stimulus as a saccadic eye movement, in which one or both eyestalks were rapidly brought together (see Fig. 7 for an example). No such saccadic eye movements were observed in a 5 s

period before the onset of the stimulus or from 3 s after its presentation. Animals were scored by their number of responses out of the 10 presentations giving a probability of response to each stimulus type.

Discrimination threshold between two angles of linearly polarized light

A similar method was used to measure the polarization angular contrast sensitivity of *H. trispinosa*. Individual unrestrained animals were housed in a 20 x 20 x 30 cm aquarium partition in burrows positioned approximately 12 cm from the front wall. A different polarization LCD monitor (HP L1906; see How et al., 2012 for calibration details) to that described above, but with very similar properties, was positioned against the front wall. A looming circle stimulus expanded to cover 27° of the visual field angle in 1 s (taking into account refraction at the air / glass / water boundaries). The greyscale values addressed to the monitor were set to 0 (black) for the background and ranged between 0 and 255 for the stimulus, resulting in a stimulus that varied in the angle of polarization against a horizontally polarized background, with no corresponding changes in hue or light intensity. Stomatopod eye movements in response to the stimulus were recorded using a digital video camera (Sony HDR-SR11, Tokyo, Japan) mounted on the top edge of the front aquarium wall. Stimuli were generated automatically using MATLAB (r2011, Mathworks, Natick, MA, USA) and the whole experiment was conducted without experimenter intervention. Video recordings were synchronized to the stimulus by means of an audio signal conveyed by audio cable directly from the computer to the microphone port of the camera. Measures of saccadic eye movements were made in a 5 s period both before and after the stimulus presentation. Two independent groups (n=15 and

14 animals) were tested using two sets of stimuli (angles of 0, 0.5, 1, 5, 7, 9, 11 degrees and of 20, 31, 56, 70, 74 degrees respectively). The stimulus order was fully randomized and the interval between stimuli was randomized between 20 s and 60 s.

Polarization analysis of the maxilliped signals

Images of the maxillipeds of *H. trispinosa, H. glyptocercus, C. tweediei* and *C. hystrix* were taken though a Leitz compound microscope (Leica Micrsystems, Wetzler, Germany) using a 10x objective and Canon G9 digital camera (Canon, Melville, USA) mounted using a photo tube extension on the trinocular head. Spectral reflection data of the same four species were measured using an Ocean Optics halogen HL-2000 light source (Ocean Optics, Dunedin, USA) mount at the back focal plane of the eyepiece and illuminating the maxillipeds normally. The reflected light was collected at the back focal plane of the second eyepiece using a 1 mm diameter optic fibre connected to a QE65000 spectrometer (Ocean Optics, Dunedin, USA). Linear horizontal and vertical polarization filters were placed in the path of the reflected light inside the microscope to collect each respective polarized reflectance spectrum. Over several preceding years, the colour and polarizing nature of the 1st maxillipeds from 17 other representative species of stomatopods across the superfamily Gonodactyloidea have been assessed visually by viewing the maxillipeds thorough a rotatable linear polarizer.

Phylogenetic analyses

342 To investigate the potential evolutionary pathway of color and polarization
343 signals within the genus *Haptosquilla*, DNA sequences from both nuclear and
344 mitochondrial genes for all available species were either obtained from GenBank
345 or provided by P. Barber (Barber and Boyce, 2006), or were sequenced following
346 the methods of Porter et al., (2010) (Supplemental Table S2). Additional
347 representative stomatopod species from within the same family
348 (Protosquillidae) and superfamily (Gonodactyloidea) were included to provide
349 increased resolution and stability at deeper nodes within the phylogeny and to
350 use as outgroups. We used a concatenated matrix consisting of nucleotide
351 sequences from the cytochrome oxidase I (COI) and 16S mitochondrial genes,
352 and the 18S and 28S nuclear rDNA genes, although the number of sequences
353 available varied across species (see Supplemental Table S2 for full description of
354 data sources and gene representation).

355 Nucleotide sequences of the 16S, 18S, and 28S genes were aligned using
356 the E-INS-I strategy in MAFFT v6.0.0 (http://mafft.cbrc.jp/alignment/server/)
357 (Katoh et al., 2002; Katoh et al., 2005). The COI sequences were inspected for
358 evidence of pseudogenes (e.g. stop codons, indels not continuous with codons)
359 and then manually aligned using the translated amino acid sequences. The four
360 gene regions were then concatenated and the combined dataset was used to
361 reconstruct a phylogeny using Randomized Axelerated Maximum Likelihood
362 (RAxML) v.7.2.7 with rapid bootstrapping as implemented on the
363 Cyberinfrastructure for Phylogenetic Research (CIPRES) Portal v.2.0 (Stamatakis
364 2006; Stamatakis et al., 2008; Miller et al., 2009). Three partitions were
365 designated for the RAxML analysis: (1) COI codon positions 1 and 2; (2) COI
366 codon position 3; and (3) all of the ribosomal genes (16S, 18S, and 28S). All

partitions were analyzed with the GTR+gamma model, as this was the best-fitting model available in RAxML, according to the results of jModelTest v0.1.1 (Guindon and Gascuel 2003; Posada 2008).

Statistical analysis

All statistical analyses were conducted in R 3.0.2 (R Foundation for Statistical Computing). Response probabilities to either horizontally or vertically polarized looming stimuli were analysed using Wilcoxon Signed-Rank tests and differences between species were calculated using a Kruskal-Wallis rank sum test. The individual saccadic responses of *H. trispinosa* to different angular e-vector contrasts were analysed using a McNemar's test.

ACKNOWLEDGEMENTS

We are very grateful to the Lizard Island Research station staff for all their assistance, to Innes Cuthill for help with the statistical analysis, and to Paul Barber for providing sequence data.

FUNDING

Biotechnology and Biological Sciences Research Council (N.W.R., BB/G022917/1 and BB/H01635X/1), and the Air Force Office of Scientific Research (M.J.H, T.W.C, N.J.M, and N.W.R., FA8655-12-1-2112) supported this work. S.E.T was a recipient of a Yulgilbar Foundation Lizard Island Postdoctoral Fellowship.

REFERENCES

Barber, P. and Boyce, S. L. (2006). Estimating diversity of Indo-Pacific coral reef stomatopods through DNA barcoding of stomatopod larvae. *Proc. R. Soc. B.* **273**, 2053–2061.

Caldwell, R. and Dingle, H. (1975). Ecology and evolution of agonistic behavior in stomatopods. *Naturwissenschaften* **62**, 214-222.

Candolin, U. (2003). The use of multiple cues in mate choice. *Biol. Rev.* **78**, 575-595.

Chiou, T. H., Cronin, T.W., Caldwell R.L. and Marshall, J. (2005). Biological polarized light reflectors in stomatopod crustaceans. *Proc. SPIE* **5888**, 58881B.

Chiou, T. H., Mäthger L. M., Hanlon, R. T. and Cronin, T. W. (2007). Spectral and spatial properties of polarized light reflections from the arms of squid (*Loligo pealeii*) and cuttlefish (*Sepia officinalis L.*). *J. Exp. Biol.* **210**, 3624-3635.

Chiou, T. H., Kleinlogel, S., Cronin, T., Caldwell, R., Loeffler, B., Siddiqi, A. and Marshall, J. (2008). Circular polarization vision in a stomatopod crustacean. *Curr. Biol.* **18**, 429-434.

Chiou, T. H., Marshall, N. J., Caldwell, R. L. and Cronin, T. W. (2011). Changes in light-reflecting properties of signalling appendages alter mate choice behaviour in a stomatopod crustacean *Haptosquilla trispinosa*. *Mar. Freshw. Behav. Physiol.* **44**, 1-11.

Cronin, T. W., Marshall, N. J. and Land, M. F. (1991). Optokinesis in Gonodactyloid mantis shrimps (Crustacea, Stomatopoda, Gonodactylidae). *J. Comp. Physiol. A.* **168**, 233-240.

Cronin, T. W., Caldwell, R. L. and Marshall, J. (2001). Sensory adaptation: tunable colour vision in a mantis shrimp. *Nature* **411**, 547-548.

Cronin, T. W., Shashar, N., Caldwell, R. L., Marshall, J., Cheroske, A. G. and Chiou, T. H. (2003a). Polarization signals in the marine environment. *Proc. SPIE* **5158**, 85-92.

Cronin, T. W., Shashar, N., Caldwell, R. L., Marshall, J., Cheroske, A. G. and Chiou, T. H. (2003b). Polarization vision and its role in biological signaling. *Integr. Comp. Biol.* **43**, 549-558.

Cronin, T. W., Chiou, T. H., Caldwell, R. L., Roberts, N. and Marshall, J. (2009). Polarization signals in mantis shrimps. *Proc. SPIE* **7461**, 74610C.

Cronin, T. W., Johnsen, S., Marshall, J. and Warrant, E. J. (2014). *Visual Ecology*. Princeton New Jersey, Princeton University Press.

Dacke, M., Nilsson, D-E., Scholtz, C. H., Byrne, M. and Warrant, E. J. (2003). Animal behaviour: Insect orientation to polarized moonlight. *Nature* **424**, 33.

Dingle, H. and Caldwell, R. (1969). Aggressive and territorial behaviour of mantis shrimp *Gonodactylus bredini* Manning (Crustacea, Stomatopoda). *Behaviour* **33**, 115-136.

Endler, J. and Basolo, A. (1998). Sensory ecology, receiver biases and sexual selection. *Trends Ecol. Evol.* **13**, 415-420.

Guindon, S. and Gascuel, O. (2003). A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Syst. Biol.* **52**, 696-704.

Guilford, T. and Dawkins, M. (1991). Receiver psychology and the evolution of animal signals. *Anim. Behav.* **42**, 1-14.

Hasson, O. (1991). Sexual displays as amplifiers: practical examples with an emphasis on feather decorations. *Behav. Ecol.* **2**, 189-197.

Hebets, E. A. and Papaj, D. R. (2005). Complex signal function: developing a framework of testable hypotheses. *Behav. Ecol. Sociobiol.* **57**, 197-214.

How, M. J., Pignatelli, V., Temple, S. E., Marshall, N. J. and Hemmi, J. M. (2012). High e-vector acuity in the polarisation vision system of the fiddler crab *Uca vomeris*. *J. Exp. Biol.* **215**, 2128-2134.

Horváth, G. and Varjú, D. (2004). *Polarized light in animal vision: polarization patterns in nature*. Springer, U.K.

Johnsen, S., Marshall, N. J. and Widder, E. A. (2011). Polarization sensitivity as a contrast enhancer in pelagic predators: lessons from in situ polarization imaging of transparent zooplankton. *Phil. Trans. R. Soc. B* **366**, 655-670.

Katoh, K., Misawa, K., Kuma, K. I. and Miyata, T. (2002). MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Res*. **30**, 3059-3066.

Katoh, K., Kuma, K. I., Toh, H. and Miyata, T. (2005). MAFFT version 5: improvement in accuracy of multiple sequence alignment. *Nucleic Acids Res.* **33**, 511-518.

Kleinlogel, S. and Marshall, N. J. (2006). Electrophysiological evidence for linear polarization sensitivity in the compound eyes of the stomatopod crustacean *Gonodactylus chiragra*. *J. Exp. Biol.* **209**, 4262-4272.

Labhart, T. and Meyer, E. P. (1999). Detectors for polarized skylight in insects: a survey of ommatidial specializations in the dorsal rim area of the compound eye. *Microsc. Res. Tech.* **47**, 368-379.

Land, M. F., Marshall, J. N., Brownless, D. and Cronin, T.W. (1990). The eye-movements of the mantis shrimp *Odontodactylus scyllarus* (Crustacea, Stomatopoda). *J. Comp. Physiol. A.* **167**, 155-166.

Marshall, N. J., Land, M. F., King, C. A. and Cronin T. W. (1991). The compound eyes of mantis shrimps (Crustacea, Hoplocarida, Stomatopoda). I. Compound eye structure: the detection of polarized light. *Phil. Trans. R. Soc. B* **334**, 33-56.

Mäthger, L. M. and Hanlon, R. T. (2006). Anatomical basis for camouflaged polarized light communication in squid. *Biol. Lett.* **2**, 494-496.

Mäthger, L. M., Shashar, N. and Hanlon, R. T. (2009). Do cephalopods communicate using polarized light reflections from their skin? *J. Exp. Biol.* **212**, 2133-2140.

Miller, M. A., Holder, M. T., Vos, R., Midford, P. E., Liebowitz, T., Chan, L., Hoover, P. and Warnow, T. (2009-08-04). *The CIPRES Portals.* CIPRES. (Archived by WebCite at http://www.webcitation.org/5imQlJeQa). Accessed September 29 2010.

Partan, S. R. and Marler, P. (2005). Issues in the classification of multimodal communication signals. *Am. Nat.* **166**, 231-245.

Pignatelli, V., Temple, S. E., Chiou, T. H., Roberts, N. W., Collin, S. P. and Marshall, N. J. (2011). Behavioural relevance of polarization sensitivity as a target detection mechanism in cephalopods and fishes. *Phil. Trans. R. Soc. B* **366**, 734-741.

Porter, M. L., Bok, M. J., Robinson, P. R. and Cronin, T. W. (2009). Molecular diversity of visual pigments in Stomatopoda (Crustacea). *Vis. Neurosci.* **26**, 255-265.

Porter, M. L., Zhang, Y., Desai, S., Caldwell, R. L. and Cronin, T. W. (2010). Evolution of anatomical and physiological specialization in the compound eyes of stomatopod crustaceans. *J. Exp. Biol.* **213**, 3473-3486.

Roberts, N. W., Chiou, T. H., Marshall, N. J. and Cronin, T. W. (2009). A biological quarter-wave retarder with excellent achromaticity in the visible wavelength region. *Nat. Photonics*, **3**, 641-644.

Roberts, N. W., Porter, M. L. and Cronin, T. W. (2011). The molecular basis of mechanisms underlying polarization vision. *Phil. Trans. R. Soc. B.* **366**, 627-637.

Rossel, S. and Wehner, R. (1986). Polarization vision in bees. *Nature* **323**, 128-131.

Shashar, N., Rutledge, P. and Cronin, T. (1996). Polarization vision in cuttlefish - A concealed communication channel? *J. Exp. Biol.* **199,** 2077-2084.

Shashar, N., Johnsen, S., Lerner, A., Sabbah, S., Chiao, C. C., Mäthger, L. M. and Hanlon, R. T. (2011). Underwater linear polarization: physical limitations to biological functions. *Phil. Trans. R. Soc. B* **366**, 649-654.

Stamatakis, A. (2006). RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* **22**, 2688-2690.

Stamatakis, A., Hoover, P. and Rougemont, J. (2008). A rapid bootstrap algorithm for the RAxML Web servers. *Syst. Biol.* **57**, 758-771.

Temple, S. E., Pignatelli, V., Cook, T., How, M. J., Chiou, T. H., Roberts, N. W. and Marshall, N. J. (2012). High-resolution polarisation vision in a cuttlefish. Curr. Biol. **22**, R121-R122.

Thoen, H. H., How, M. J., Chiou, T. H. and Marshall, N. J. (2014). A new form of colour vision in Mantis shrimps. *Science* **343**, 411-413.

Waterman, T. H. (1954). Polarization patterns in submarine illumination. *Science* **120**, 927-932.

Wehner, R. (1976). Polarized-light navigation by insects. *Sci. Am. 235*, 106-115.

Wehner, R. and Labhart, T. (2006). Polarisation vision. In *Invertebrate Vision* (eds. Warrant E., Nilsson D. -E.), Cambridge University Press, Cambridge, UK. 291-348.

Figure Captions

Figure 1. **Illustrative examples, shown by arrows, of the conspicuous maxilliped signals**. (A) *H. trispinosa*, (B) *H. glyptocercus*, (C) *H. stoliura* and (D) *H. banggai*.

Figure 2. **Paired plots of the probability of response of each individual to the vertically and horizontally polarized stimulii**. Numbers of points (open

565 circles) at each probability represent the number of individuals that responded
566 with that probability. (A) *H. trispinosa,* (B) *H. glyptocercus,* (C) *C. tweediei,* and
567 (D) *C. hystrix*.
568
569 Figure 3. Responses of *H. trispinosa* (black dots) to differences between the angles of
570 polarization of the stimulus and the background (x-axis). The response data are fitted
571 with a hyperbolic tangent (dashed line). The background level of false positive
572 responses are represented for each stimulus type (white dots) and as an overall mean
573 (dotted line). McNemar's test was used to determine which response values differed
574 from the level of false positives (* = $p < 0.05$).
575
576 Figure 4. **Microscope images of the maxillipeds**. *H. trispinosa* (A), *H.*
577 *glyptocercus* (B), *C. tweediei* (C) and *C. hystrix* (D). Accompanying each plot are
578 the reflection spectra from the area denoted by the circle in each image. In the
579 spectral plots, open circles represent the horizontally polarized reflectivity and
580 open triangles represent the vertically polarized reflectivity. V and H in (A)
581 denote the vertical and horizontal directions respectively relative to the axes of
582 the maxillipeds.
583
584 Figure 5. **A maximum likelihood phylogeny of protosquillid species**
585 **relationships, rooted using representative species from the**
586 **Gonodactyloidea.** Branch support values represent bootstrap percentages.
587 Nodes representing the genus *Haptosquilla* and the family Protosquillidae are
588 indicated by 'H' and 'P', respectively. Where known, the presence or absence of
589 blue signals and polarizing signals on the 1st maxilllipeds has been mapped onto

the phylogeny. Species names in bold indicate those animals measured in this experiment, all of which have a bias to horizontally polarized stimuli, illustrating the occurrence across the two main genera of the Protosquillidae.

Figure 6. **Schematic diagram of the experimental apparatus.** (A) The tank setup in front of the LCD screen. (B) An example measure of the normalized Stokes parameters (P0–3) of the horizontally polarized stimulus as a function of wavelength. (C) An example of the vertical and horizontal polarization ellipses at 560 nm.

Figure 7. **Measurements of the behavioural saccadic response of the stomatopods.** (A) Time sequences of images from a video recording illustrating the typical saccadic eye movement response in *H. trispinosa* to a looming polarized contrast stimulus (horizontally polarized on a vertically polarized background). Each image is a single frame, approximately 0.2 s apart; the first two images show the eyes before the stimulus, the 3rd image shows the eye position 0.1 s after the stimulus onset, and the final image shows the eye position approx. 0.3 s after the stimulus onset. (B) The measured change in the angular separation of the eye stalks as a function of the onset of the looming polarized contrast stimulus. The numbers and filled points correspond to the numbered frames displayed in (A). The red line indicates the stimulus diameter as a function of time.

Figures

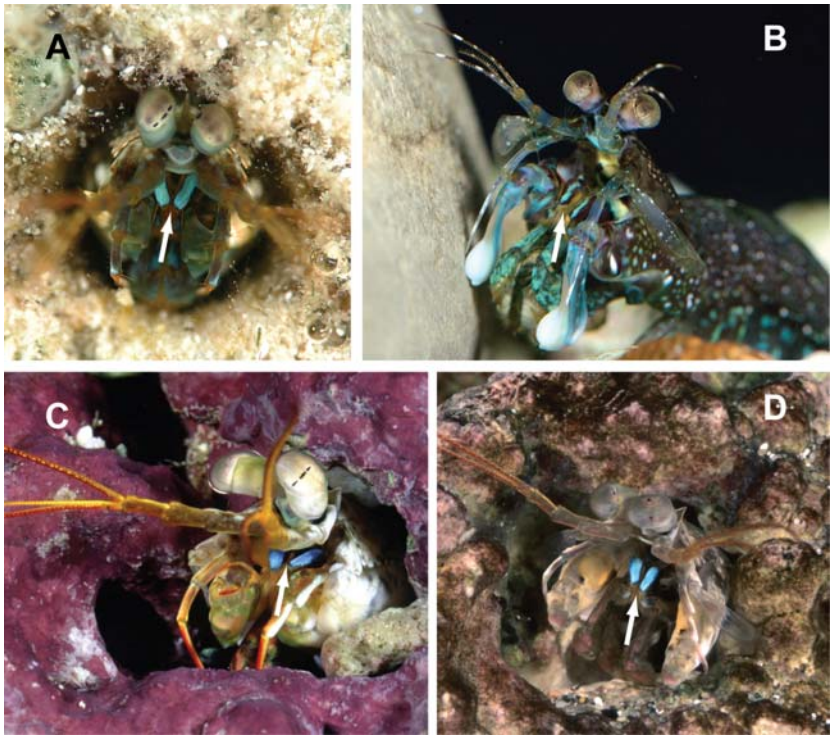


FIGURE 1

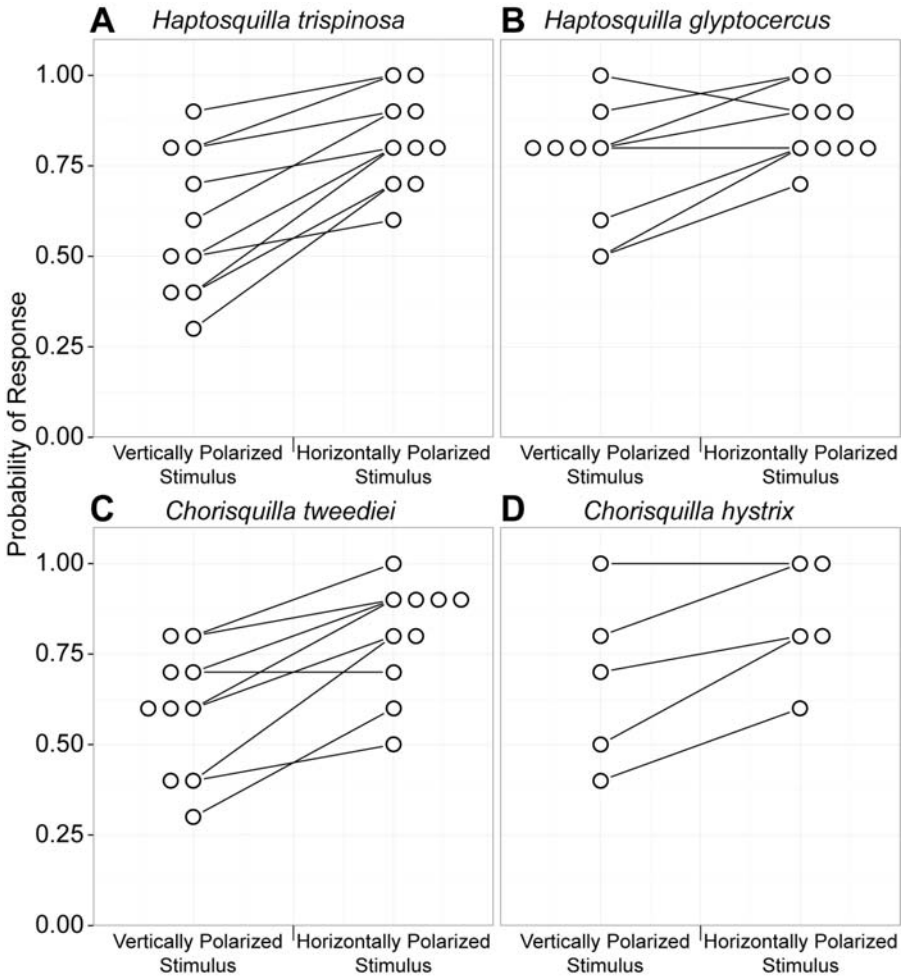


FIGURE 2

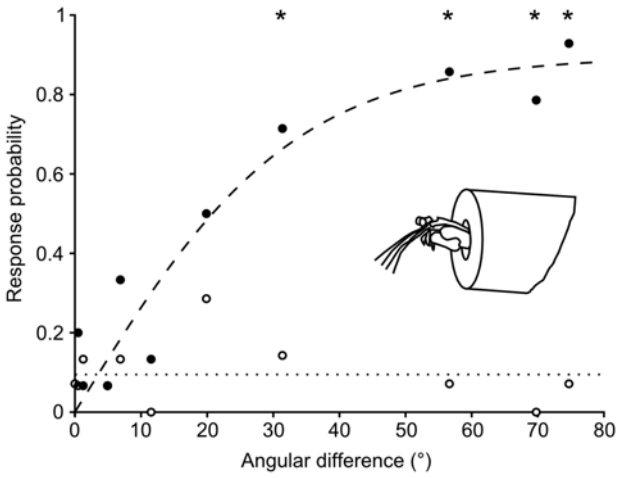
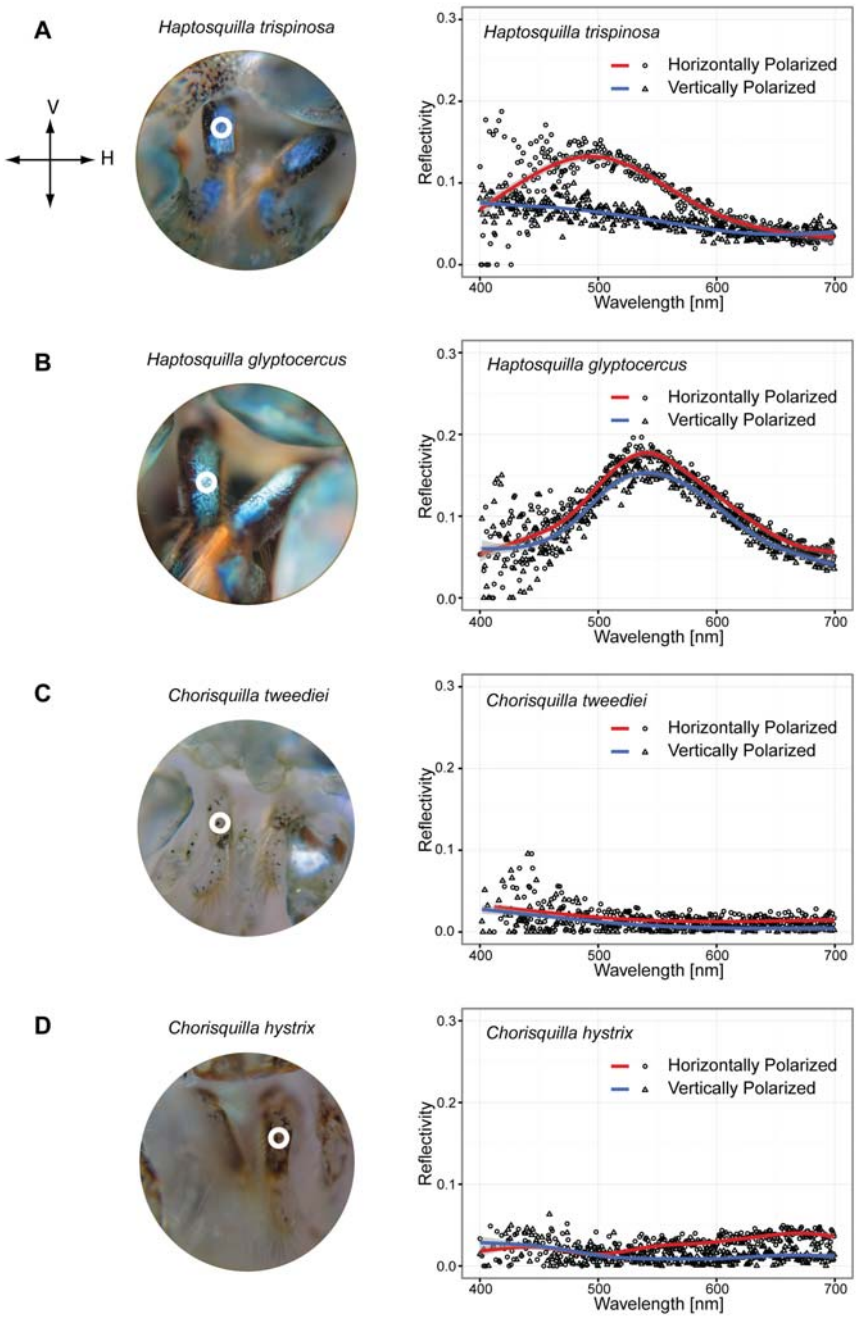


FIGURE 3



673
674 FIGURE 4
675

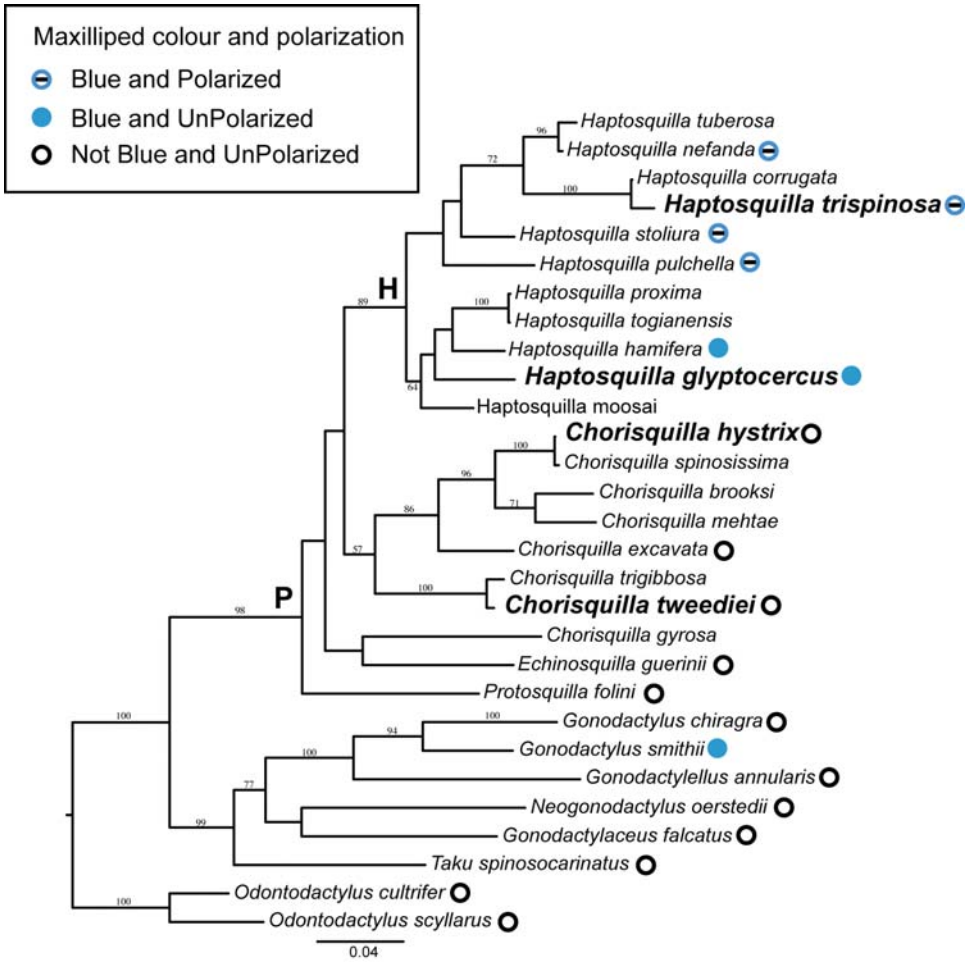


FIGURE 5

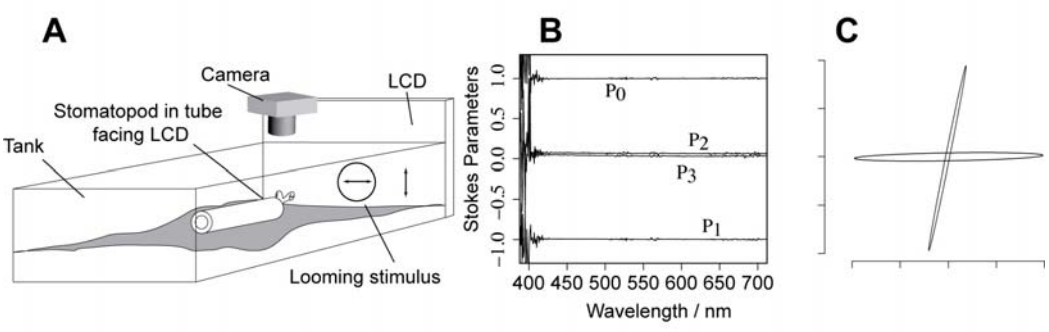
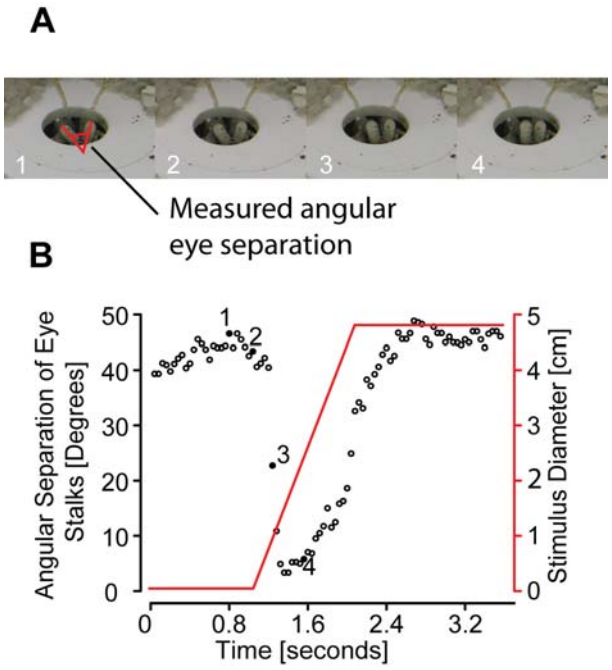


FIGURE 6



715
716
717
718
719 FIGURE 7
720
721