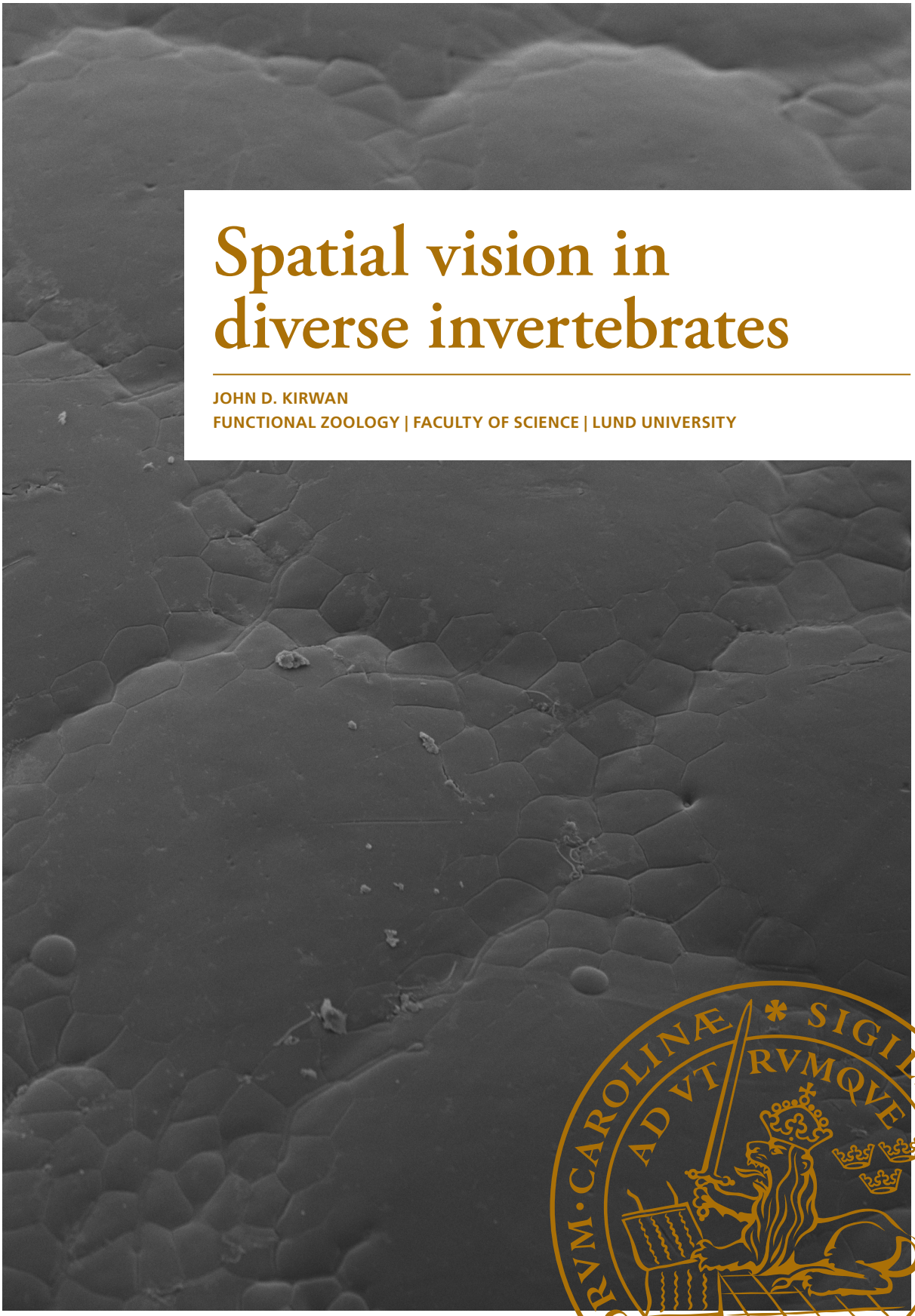
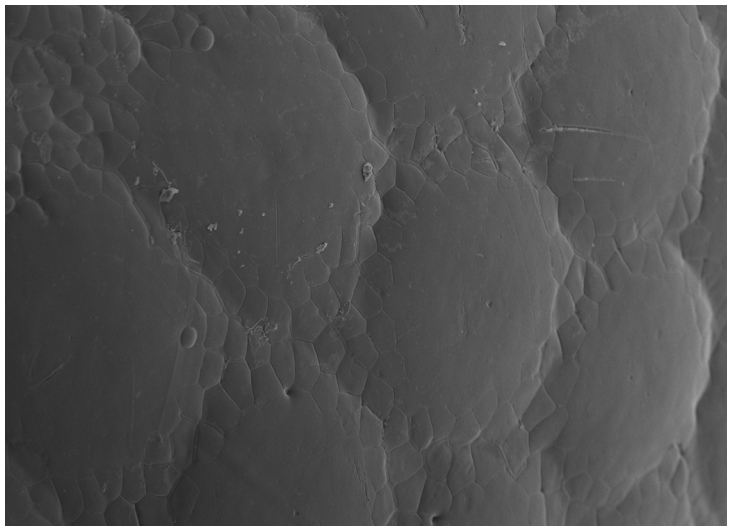


Spatial vision in diverse invertebrates

JOHN D. KIRWAN
FUNCTIONAL ZOOLOGY | FACULTY OF SCIENCE | LUND UNIVERSITY



Scanning electron microscopic (SEM) image of the eye of the millipede *Cylindroiulus punctatus*. This compound eye comprises thirty-three large facets, which overlay corneal lenses made from modified cuticle.

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LUND
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Abstract

Light-detection provides incomparabaly rapid information at a range of time scales and, consequently, directional photoreception is found in almost every major metazoan clade. Conversely, a much smaller cohort have evolved sophisticated high resolution vision, which facillitates complex tasks via the detection of fast or small objects,. such as courtship and high-speed pursuit of prey. A few such species are the focus of most vision research. In contrast, low resolution image-forming vision is little investigated and, in some cases, whether vision is present has not been directly evidenced.

To ameliorate this, I tested the image-forming capability (spatial resolution) of a variety of eye types from diverse animal groups: the camera eye of a velvet worm, *Euperipatoides rowelli*, (paper I), the dispersed visual system of a diadematid sea urchin, *Diadema africanum*, (paper II), which lacks discrete eyes, the cup eye of a planarian flatworm, *Schmidtea lugubris*, (paper III) and the compound eye of a millipede, *Cylindroiulus punctatus*, (paper IV).

For each study animal, I used behavioural experiments in which the animals responded to dark visual cues to test whether they could response to visual stimuli of different sizes. The animals were placed in circular arenas under bright light and the direction they moved in relation to the stimulus was recorded. As a negative phototaxis response is present in each species, attraction towards the stimulus, if it could be resolved provided a measure of resolution.

The angular sensitivity of an eye required to see a given visual signal was modelled (making assumptions about their contrast sensitivity; discussed in paper I).. I compared the efficacy of the various visual stimulus types for this purpose in paper II. To account for mutiple effects and low response rates to stimuli I apply logistic regression models using Bayesian inference in papers III and IV , to make more robust estimates which better express uncertainty. I also used imaging techniques, including x-ray tomography and transmission electron microscopy, to relate the visual performance of these animals to their visual systems.

We found that these animals each had coarse vision with a spatial resolution of 20-30° to for the velvet worm *E. rowelli*, 29-69° for the sea urchin *D. africanum* (in respect of object taxis), 63° for the millipede *C. punctatus* and 73° for the flatworm *S. lugubris*. These modest proposed ranges of spatial resolution are nonetheless sufficient for the visual tasks employed by these animals.

Key words: vision, invertebrates, spatial resolution, behaviour, visual ecology

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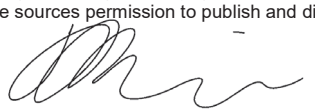
Signature  Date 2018-05-02

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To my friends and family

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List of Papers and Manuscripts

I. **Kirwan, J.D**., Graf, J., Smolka, J., Mayer, G., Henze, M.J. and Nilsson, D-E. Low-resolution vision in a velvet worm (Onychophora). Journal of Experimental Biology DOI 10.1242/jeb.175802 (*In press*).

II. **Kirwan, J.D**., Bok, M.J., Smolka, J., Foster, J.J., Hernandez, J.C., and Nilsson, D-E. A sea urchin (*Diadema*) uses low-resolution vision to find shelter and deter enemies. Journal of Experimental Biology DOI 10.1242/jeb.176271 (*In press*).

III. **Kirwan, J.D**. and Nilsson, D-E. Coarse spatial vision in the free-living planarian flatworm *Schmidtea lugubris*. Manuscript.

IV. **Kirwan, J.D**. and Nilsson, D-E. Where thy dark eye glances: coarse spatial vision in the julid millipede *Cylindroiulus punctatus*. Manuscript.

Author contributions

I. DEN and MJH designed the experiments with input from GM. JG performed most of the experimental work and first analyses under supervision of MJH, JS, and DEN. JS and MJH wrote scripts to visualize and evaluate the data. JDK tested experimental designs, carried out the final analyses, and drafted the manuscript based on a thesis by JG. JDK and MJH finalized the figures. MJH, JS, GM, and DEN edited the manuscript, and all authors read and approved the final version.

II. All authors contributed to project conceptualization. Animal collection was carried out by JCH, MJB and JDK. JDK performed most of the experimental work with help from MJB and under the supervision of DEN and JCH. Environmental light measurements were carried out by MJB. Experimental analysis was carried out by JDK with help from JS and JJF. All authors edited the manuscript and read and approved the final version.

III. The idea was conceptualized and the experiment was designed by JDK with input from DEN; data were acquired and analysed by JDK; the article was drafted by JDK and critical revision was performed by DEN.

IV. The idea was conceptualized and the experiment was designed by JDK with input from DEN; data was acquired and analysed by JDK; the article was drafted by JDK and critical revision was performed by DEN.

Popular thesis summary

The ability to detect light and which direction it is coming from is extraordinarily useful for animals. As a result, this ability (directional photoreception) is found in almost every major evolutionary group of animal, even among those considered simple, such as corals or roundworms. On the other hand, only a few kinds of animal have in the course of their evolution developed truly excellent eyes, which can rapidly identify objects of their own size at distance and be used for sophisticated tasks, such as courtship and high-speed pursuit of prey. These remarkable high-resolution eyes, such as those of humans, birds, octopus and jumping spiders, are the subject of much fascinating research into what can be achieved with a given eye and how evolution moulds eyes for differing tasks.

However, there are many kinds of animals which do not possess these high-resolution eyes but are possibly still capable of forming a visual image and using this information to influence their behaviour. These low-resolution eyes have not received the same attention and, in many cases, we do not have strong evidence whether they can truly be used to see and, if so, how well.

To ameliorate this, I tested the image-forming capability (spatial resolution) of a variety of eye types from diverse animal groups: the camera eye of a velvet worm (paper I), the dispersed visual system of a diadematid sea urchin (paper II), which lacks discrete eyes, the cup eye of a planarian flatworm (paper III) and the compound eye of a millipede (paper IV).

For each study animal, I used behavioural experiments in which the animals responded to dark visual cues to test whether they could response to visual stimuli of different sizes. The animals were placed in circular arenas under bright light and the direction they moved in relation to the stimulus was recorded (in addition to a further 'alarm response' experiment in paper II). As all of these animals will typically move towards a dark space under bright light, if they can resolve it, this provided a measure of what visual stimulus an animal could observably resolve.

The angular sensitivity of an eye required to see a given visual signal was modelled, from which I was able to measure the acceptance angle which would be needed by these animals to detect the stimuli (making assumptions about their contrast sensitivity; discussed in paper I). I compared the efficacy of the various visual stimulus types for this purpose in paper II. Partly because these were novel experiments and it can be difficult to induce these animals to respond to visual signals reliably, I introduced statistical approaches in papers III and IV, which are not widely used in sensory biology, to improve my measurements and make them more robust. I also used imaging techniques, including x-ray tomography

and transmission electron microscopy, to relate the visual performance of these animals to their visual systems.

I found that these animals each had coarse vision with a spatial resolution of 20-30° to for the velvet worm *Euperipatoides rowelli*, 29-69° for the sea urchin *Diadema africanum* (in respect of object taxis), 63° for the millipede *Cylindroiulus punctatus* and 73° for the flatworm *Schmidtea lugubris*. These modest proposed ranges (as compared to approximately 0.02° for the human eye) of spatial resolution are nonetheless sufficient for the visual tasks employed by these animals and to warrant the continued use of resolving vision across evolutionary time.

Populärvetenskaplig sammanfattning

Förmågan att uppfatta ljus och vilken riktning det kommer från är utomordentligt användbart för djur. Därför är det inte förvånande att denna förmåga återfinns hos i stort sett varje större djurgrupp, även bland de som anses enkla, såsom koraller och spolmaskar. Endast några få djurgrupper har dock, under evolutionens gång, utvecklat verkligt bra ögon som snabbt kan identifiera objekt av djurens egen storlek på avstånd och som kan användas för sofistikerade uppgifter, såsom att hitta partner eller bytesdjur. Så högpresterande ögon, hittar man nästan bara hos ryggradsdjur, bläckfiskar, spindlar och insekter, där de är fokus för ett stort intresse av hur evolutionen anpassat ögon och synsinnet för olika uppgifter.

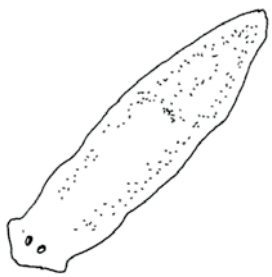
Däremot finns det många olika djur med enklare ögon som inte ger ett detaljrikt seende utan endast en grov bild som djuren använder för att styra sina beteenden. Dessa lågupplösande ögon har inte fått samma vetenskapliga uppmärksamhet och vi vet inte mycket om vad dessa ögon kan se och vad de används till.

För att förbättra detta testade jag synskärpan (den spatiala upplösningen) hos ett urval ögontyper från olika djurgrupper, kameraögat hos en klomask (manus I), det utspridda synsystemet hos en sjöborre (manus II) som saknar egentliga ögon, gropögat hos en plattmask (manus III) och fasettögat hos en tusenfoting (manus IV).

För alla de olika djurarterna använde jag beteendeexperiment där djuren fick möjlighet att röra sig mot mörka strukturer som kunde göra olika stora för att testa deras synförmåga. Djuren placerades i runda arenor i stark belysning, och deras rörelseriktning i relation till stimuli noterades. Eftersom alla dessa djur i starkt ljus normalt rör sig mot mörka områden som de kan urskilja med synen, gav detta experiment ett mått på djurens synskärpa. Hos sjöborrar använde jag även ett försvarsbeteende som utlöses av rörliga objekt för att på samma sätt mäta synskärpan.

Synskärpan som behövdes för att se en given visuell signal beräknades även med en matematisk modell. Från detta kunde jag räkna ut hur liten vinkel varje pixel i djurens ögon måste vara för att de skall kunna upptäcka signalen (givet vissa antaganden om deras kontrastkänslighet, diskuterad i manus I). Jag jämförde effektiviteten av olika typer av visuella stimuli för att detta ändamål i manus II. Eftersom sättet att mäta synskärpa var nytt och det ibland kan vara svårt att få dessa djur att reagera på visuella stimuli på ett pålitligt sätt så introducerade jag statistista metoder i manus III och IV som inte tidigare använts inom sinnesbiologi. Dessa metoder stärker mina resultat och göra dem mer robusta. För att relatera djurens beteende till deras synsystem använde jag mig även av röntgen-tomografi och transmissionselektronmikroskopi,

Jag fann att djuren hade en låg synskärpa och uppskattade deras spatiala upplösning till 20-30° för klomasken *Euperipatoides rowelli*, 29-69° för sjöborren *Diadema africanum*, 63° för tusenfotingen *Cylindroiulus punctatus* och 73° för plattmasken *Schmidtea lugubris*. Som jämförelse är vår egen synskärpa ca 0,02°. Dessa blygsamma uppskattade synförmågor är ändå tillräckliga och nödvändiga för de beteenden som djuren faktiskt har och förklarar varför de gynnas av evolutionen.



Achoimre coitianta tráchtais

Tá an cumas chun solas a bhrath agus an treo as a dtagann sé thar a bheith úsáideach d'ainmhithe. Mar thoradh air sin, faightear an cumas seo (fótaghabháil treorach) i mbeagnach gach mór-ghrúpa éabhlóideach ainmhithe, fiú amháin ina measc siúd a mheastar a bheith simplí, mar shampla coiréil nó cruinnphéisteanna. É sin ráite, níl ach cúpla cineál ainmhí, a d'fhorbair súile den scoth le linn a gcuid éabhlóide, ar féidir leo rudaí a méid féin a aithint go tapa i bhfad uathu agus iad a úsáid le haghaidh tascanna sofaisticiúla, mar shampla suirí agus creach a leanúint ag luas ard. Tá na súile ard-réitigh seo de chuid, mar shampla, an duine, na n-éan, an ochtapais agus an damhán alla léime, mar ábhar taighde an-suimiúil ar an méid is féidir a bhaint amach le súil ar leith agus an chaoi a mhúnlaíonn éabhlóid súile le haghaidh tascanna éagsúla.

Mar sin féin, tá go leor cineálacha ainmhithe nach bhfuil na súile ard-réitigh sin acu ach b'fhéidir go bhfuil siad fós in ann íomhá a aithint agus úsáid a bhaint as an bhfaisnéis sin le tionchar a bheith aici ar a n-iompraíocht. Ní bhfuair na súile íseal-réitigh seo an aird chéanna agus, i go leor cásanna, níl aon fhianaise láidir againn a léiríonn gur féidir iad a úsáid go fírinneach chun feiceáil agus, más amhlaidh, cé chomh maith.

Chun é seo a fheabhsú, thástáil mé cumas íomhá-chruthaitheach (taifeach spásúil) chineálacha éagsúla súile ó ghrúpaí ainmhithe éagsúla: súil ceamara na péiste veilbhite (páipéar I), córas radhairc scaipthe an chuán mhara diadematid atá gan súile scoite (páipéar II), súile cupán leithphéist phlánárach (páipéar III) agus comhshúil an mhílechosaigh (páipéar IV).

I gcás gach ainmhí staidéir, d'úsáid mé turgnaimh iompraíochta ina raibh freagairt na n-ainmhithe do leideanna amhairc dorcha á dtástáil chun spreagadh amhairc de mhéideanna éagsúla a aithint. Cuireadh na hainmhithe in áiteanna ciorclacha faoi sholas geal agus taifeadadh an treo inar bhog siad i ndáil leis an spreagadh (chomh maith le turgnamh eile 'freagra aláraim' i bpáipéar II). Os rud é go dtarraingíonn gach ceann de na hainmhithe seo go hiondúil i dtreo spás dorcha faoi sholas geal, más féidir leo é a réiteach, thug sé seo tomhas ar an spreagadh amhairc a d'fhéadfadh ainmhí a bhrath.

Múnlaíodh an cumas taifigh a bhí ag teastáil chun comhartha amhairc áirithe a fheiceáil, as a raibh mé in ann an cumas taifigh a bheadh ag teastáil ó na hainmhithe seo chun na spreagthaigh a bhrath a thomhas (ag úsáid barúlacha maidir lena n-íogaireacht chodarsnachta; pléite i bpáipéar I). Rinne mé comparáid idir éifeachtúlacht na gcineálacha éagsúla spreagthaí amhairc chun na críche seo i bpáipéar II. Toisc go raibh na turgnaimh nuálach, go pointe áirithe, agus gur féidir leis a bheith deacair na hainmhithe seo a spreagadh chun comharthaí amhairc a fhreagairt go hiontaofa, thug mé isteach cur chuige

staitistiúil i bpáipéir III agus IV, nach n-úsáidtear go forleathan sa bhitheolaíocht céadfach chun mo thomhais a fheabhsú agus iad a dhéanamh níos daingne. D'úsáid mé teicnící íomháithe freisin, lena n-áirítear tomagrafaíocht x-ghathaithe agus micreascópacht tarchur leictreonach, chun feidhmíocht amhairc na n-ainmhithe sin a cheangal lena gcórais amhairc.

Fuair mé amach go raibh taifeach spásúil íseal ag na hainmhithe seo go léir le réiteach spásúil de 20-30° ag an bpéist veilbhite *Euperipatoides rowelli*, 29-69° ag an gcuán mara *Diadema africanum* (i ndáil le fótachóidis), 63° ag an mílechosach *Cylindroiulus punctatus* agus 73° ag an leithphéist phlánárach *Schmidtea lugubris*. Mar sin féin, tá na raonta modhúla seo de taifeach spásúil dóthanach do na tascanna amhairc le na bhfuil siad á n-úsáid ag na hainmhithe seo le gur fiú fís réitigh a chaomhnú.

I. Light detection and vision

Photoreception

Photoreception is light-detection by an organism, which in animals utilizes specialized cells termed photoreceptor cells (hereafter, PRCs) and in its sophisticated incarnations gives rise to image-forming vision. Photoreception and vision have obvious advantages for an organism, providing incomparably rapid and directed information about the environment at every scale (Land and Nilsson, 2012). Photoreception occurs because of phototransduction events within the PRCs. In animals, this occurs when a photon of visible light interacts with visual pigment: a chromophore (in most cases, 11-cis retinal) bound to an apoprotein, which thereby changes conformation. This initiates the phototransduction cascade, resulting in a change in electrical potential in the cell and the conversion of a light stimulus to an electrical response.

Two kinds of apoprotein are used in photoreception: cryptochromes and 7-transmembrane domain (7TM) proteins, both of which are found in animals. In addition, it is proposed that a dTRPA1-dependent photochemical pathway in the fruitfly *Drosophila melanogaster* can respond directly to blue or UV light without such a visual pigment (Guntur et al., 2015). Cryptochromes are flavoproteins, which are ordinarily used in UV damage repair, activated by short wavelength (blue to UV) light but have been co-opted for light-detection roles (Mei and Dvornyk, 2015). These are used for a variety of roles from maintaining circadian rhythms in animals to photomorphogenesis in plants. Three kinds of 7TM proteins (also known as G-protein coupled receptors: GPCRs) are used for photoreception: Type I opsins, Type II opsins and gustatory receptors. Upon a change in conformation triggered by the absorption of a photon, the 7TM protein interacts with a G-protein, which precipitates the intracellular signalling cascade. Both Type I and Type II opsin proteins binds the chromophore retinal and they are structurally similar to one another but they nonetheless share very little sequence similarity (Ernst et al., 2014). Thus, whether these proteins have a shared origin within this diverse family or whether the light-detecting role of 7TM proteins evolved convergently remains an open question.

Type I (microbial) opsin is found in all three domains of life but not in animals and has been shown to be involved in phototaxis (Ernst et al., 2014). Type II

(animal) opsins, hereafter referred to as opsins, are found only in animals (and have not been identified in the sponges). They are the most important class for photoreception and vision in animals and a variety of classes have been identified (Porter et al., 2012). Gustatory receptor apoproteins involved in light detection have so far been identified in the nematode *Caenorhabditis elegans* (Liu et al., 2010) and the fruitfly *D. melanogaster* (Xiang et al., 2010), where they respond to blue or UV light and are used for light avoidance.

Photoreceptor cell types have conventionally been split into two divisions, based on the cell extrusions used: ciliary and rhabdomeric, with the former originally thought to be unique to the deuterostomes and the latter in protostomes (Eakin, 1965). These cellular extrusions (modified cilia and microvilli, corresponding to ciliary and rhabdomeric cells respectively) are used to increase the photoreceptive surface area: the region of membrane, upon which visual pigment is bound. However, this view and the evolutionary implication – a simple divergence of photoreceptor cell types in deuterostome versus protostome lineages has been overturned by detailed investigation of cell morphology, molecular characterization and the identification of numerous exceptions (e.g. Lawrence and Krasne, 1965; Ullrich-Lüter et al., 2011). Most of what is known about PRCs is described from vertebrate cell types, especially the rods and cones of the mammalian cephalic eyes and from the retinal photoreceptors of arthropods, especially pancrustaceans.

Visual systems

A tremendous diversity of eye designs is found in the animal kingdom. The most sophisticated eyes are limited to a few groups (vertebrates, certain arthropods and molluscs) and these groups, justifiably, are extensively studied. Nonetheless, the greatest diversity of form and function is not restricted to these groups, nor is the greater part of underlying molecular components or of differing degrees of visual system complexity. The main eye designs which have been characterized in animals are summarized by Land and Nilsson (2012b). These range from very simple eye designs (sometimes termed ocelli or eyespots) from single PRCs lacking any kind of shielding pigments to those which are partly surrounded by pigmented supporting cells (and thus can detect directional information), both of which are almost ubiquitously found in the various animal phyla. Also common are the increasingly large and more complex pit eyes and eyecups, which capture more light than simpler, unmodified eyespots and provide more detailed directional information. Conversely, designs are found, which agglomerate photoreceptors around an extrusion rather than an invagination of tissue to provide the same optical function. More sophisticated designs can include lenses,

mirrors, or other optics, which gather or even focus light. Eyes of increasing complexity opt for one of two basic designs: camera and compound eyes. The former is a concave structure, which allows light onto the PRCs from a single aperture and optics, whereas compound eyes are convex structures, composed of multiple optical systems, which collect light arising from different directions through many separate sets of apertures and optics to the underlying photoreceptors.

An alternative is possible to these aggregated visual systems, in which the light detecting components of the visual system are integrated into a small number of structures, forming eyes. Dispersed visual systems, in contrast, are distributed across a part the body surface (being found either in dermal or neural tissue) and have been reported in the tissues of several different clades, such as the shell of chitons (Speiser et al., 2011), the skeleton of sea urchins (Yerramilli and Johnsen, 2010) and in the trunk region of errant polychaetes (Backfisch and Rajan, 2013). Ramirez et al. (2011) have developed a classifications scheme for dispersed PRC types and found that they are typically used for tasks not requiring spatial vision (other than in the echinoderms) and predominantly lack light gathering and focusing specialisations.

As is typical of sensory systems, the development of visual systems relies on the expression of ancient and conserved, and often highly pleiotropic homeotic transcription factors. Some of the same suite of master control genes involved in eye positioning and development have homologs across the animal kingdom. This is true of *Pax6*, a homeotic transcription factor which is encoded by *eyeless* in *Drosphila melanogaster* and the homologues of which are crucially important in eye morphogenesis (Gehring, 1996) and other aspects of nervous system development. In addition, orthologues of the *six1-2* transcription factor family are associated with eye development in numerous phylogenetic groups across Bilateria (Pineda et al., 2000; Seimiya and Gehring, 2000) and Cnidaria (Graziussi et al., 2012; Stierwald et al., 2004), amongst other roles.

Properties of vision

The simplest light detector will function as a radiance detector and identify changes in light intensity falling upon the photoreceptors. Biological light-detectors must capture enough photons of visible light in order to extract relevant information concerning their environment. While photoreceptors can respond to individual photons, more are required to provide sufficient signal to limit the effect of noise (principally, stochastic photon arrival and thermal activation of the receptor). Thus, where photon numbers are limiting, photoreceptors, and ultimately eyes, must increase their quantum catch. The sensitivity of eyes found

in nature varies by orders of magnitude and even within the same visual system due to adaptive mechanisms. At the limit of an animal's sensitivity, a sensitivity threshold is reached: the lowest radiance which can be detected by the complete visual system. Precise psychophysical experiments have found human vision to be capable of detecting minute numbers of photons (Hecht et al., 1942) and even single photons (Tinsley et al., 2016). Luminance relates the perceived brightness of light by an organism, which is a function of the true radiance but also the sensitivity of the visual system to light of specific wavelengths.

The introduction of multiple PRCs allows for the simultaneous detection of light transmitted from different directions. If these inputs are interpreted separately rather than simply summed, spatial resolution is possible. This describes the ability to distinguish features in space, as opposed to merely detecting broad changes in luminance. If short spans of time are considered instantaneously (or rather if the environment of the animal is relatively static over the period), then even a single PRC can provide spatial resolution, if its orientation is changed by scanning.

Spatial resolution can be measured by determining the period of the finest sinusoidal grating which can be resolved by an animal (Campbell and Robson, 1968) and thus is represented in cycles per degree (cpd). For low resolution visual systems (<1 cpd), such as those considered in this thesis, the reciprocal value, in degrees can be given (which can be counterintuitive as the numerical value is inversely related to performance). This is because sinusoidal gratings comprise a single spatial frequency which is the reciprocal of the sine period. High spatial resolution requires many photoreceptors and a small aperture to partition light based on its angle of incidence (as well as focusing optics). This reduces the light available to individual PRCs and especially in dim light conditions photon catch becomes limiting, resulting in a trade-off between resolution and sensitivity. This trade-off can be modulated in a single individual, optimized to the environmental light level, both anatomically and physiologically.

Contrast describes the difference in radiance found across a visual scene or in a feature. It can be defined in several ways, which are relevant for differing circumstances (Johnsen, 2012). Michelson contrast (modulation) describes contrast between two areas or an object on a background, when the object is a significant portion of the scene (e.g. peaks versus troughs of a sine wave grating). It is defined as:

$$C = \frac{L_o - L_b}{L_o + L_b} \quad (1)$$

where L_o is the radiance of the object and L_b is the radiance of the background. Weber contrast is used to describe the contrast between small objects and a larger background and originates from studies of human psychophysics (Fechner, 1860). It is defined as follows:

$$C = \frac{L_o - L_b}{L_b} \quad (2)$$

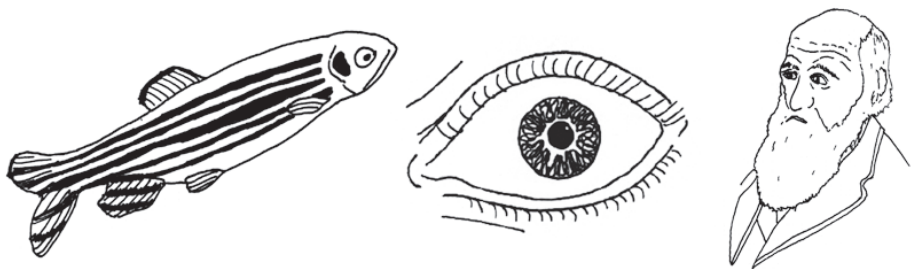
It differs from Michelson contrast only in that it assumes that the object radiance makes a negligible contribution to the total radiance of the scene. (These two contrast values may however differ considerably when background and object illumination are very similar.) When visualizing a scene rather than simply detecting a change in overall luminance, sensitivity to contrast plays a role. The contrast threshold refers to the lowest contrast which can be detected, which is formulated as the Michelson contrast expressed as a percentage. The reciprocal of contrast threshold is called the contrast sensitivity and can be more intuitive as larger values scale positively with improved performance. Spatial resolution is dependent on contrast sensitivity and this relationship is described by a contrast sensitivity function (CSF). Contrast sensitivity peaks at a spatial frequency and declines at progressively higher and lower frequencies.

Temporal resolution refers to the rate at which an animal's visual system can respond to a change in light intensity. The ultimate limitation of temporal resolution is physiological and relates to the rate at which the PRCs can recycle photopigments and be ready for subsequent photoactivation events. This attribute is frequently measured physiologically by means of a critical flicker fusion frequency test (Landis, 1954): increasingly frequent sinusoidal light flashes are directed at the eye until they can no longer be resolved from one another, resulting in a cessation of PRC activation. This threshold marks the temporal resolving limit at a specified light intensity. Temporal resolution also suffers in dim light; temporal summation can occur which sacrifices temporal resolution to provide sufficient spatial information.

The amount of information which can be acquired by even a modest eye can be overwhelming and vastly outstrip the suite of potential behavioural responses of an individual animal (Land and Nilsson, 2006) or the capacity of a small brain to process it. Consequently, visual systems are organized on the basis of matched filters (Wehner, 1987), neural and anatomical adaptations which restrict the information perceived by the animal to that which is relevant for the visual tasks which it requires. This includes limiting attributes such as the field of view and spatial and spectral resolution in a way, which may lower overall performance but nonetheless optimize it for the species' ecology and resource allocation.

Photons of differing wavelength will be absorbed or not by a visual pigment according to a probability distribution unique to that pigment and its bound

chromophore. Even structurally similar visual pigments can have very different absorption characteristics for specific wavelengths of light. There is generally a close association between the spectral properties of the visual system and the environment and ecology of a given species, in order to maximize quantum catch at the most relevant spectral range. For instance, diurnal terrestrial or surface-dwelling aquatic animals may benefit from broad-spectrum photoreception whereas those deep in the photic zone are sensitive to blue wavelengths as an adaptation to the heavy attenuation of both long and short wavelengths in water. By employing multiple visual pigments expressed separately in different PRCs spectral differences can be detected and colour vision is possible. This can also potentially be achieved by the use of optical filters. However, expressing multiple visual pigments does not necessitate image-forming colour vision or even spectrally-sensitive photoreception and multiple visual pigments can be used to mediate spectral tuning and broaden the range of spectral absorbance (Dalton et al., 2014). In addition, the polarization profile of light can be detected by many animals, as a consequence of the inherently polarization-sensitive dichroic nature of visual pigments (Cronin et al., 2003). Linearly polarized light can be distinguished by a variety of animals and some can even detect circularly polarized light (Warrant, 2010).



II. Visual evolution

Visual evolution in animals

The question of the origin of eyes and vision has been at the crux of our understanding of macroevolution since the proposal of descent with modification (Darwin, 1859). Since then, our understanding of eye function and evolution has increased greatly, most recently with the addition of molecular genetic tools (Fernald, 2006). Newly applied tools such as cell specific transcriptomics (Kanter and Kalisky, 2015), connectomics (e.g. Randel et al., 2014) and more widely-applicable genetic manipulation (Hsu et al., 2014) hold great promise to characterize visual systems in many non-model species, which can provide clues as to their origin. Previously, analyses of the evolution of vision and visual systems have been hampered by a reductive view of what constitutes vision. Analyses of visual evolution within phyla have often involved reconstruction of ancestral state from a binary character of 'eyes present' or 'eyes absent' (Oakley and Speiser, 2015). A single evolution of the vision in animals has been posited (Gehring, 2005) based on the antiquity of the developmental genetic architecture of eyes. Conversely, manifold evolution of utterly different visual systems has been suggested, based on the diversity of PRC morphology (Von Salvini-Plawen and Mayr, 1977). Considering eyes not as binary units (which necessitates a single evolutionary trajectory) but recognizing that they are modular and comprise multiple components, which may appear at different time points, provides a more coherent framework in which to consider the evolution of photoreception and vision (Oakley, 2003).

A major question within evolutionary developmental biology concerns the phenotype and genotype of the ancestors of ancient metazoan lineages, particularly, the last common ancestor of the bilaterian animals: Urbilateria. Regarding its visual system, this entails comparison of the (cerebral) eye morphology, cell types and phototransduction molecules of extant bilaterians (Arendt and Wittbrodt, 2001) to infer which components are likely to be truly ancestral.

A discussion of simple eyes and their evolution should heed the fact that most animals have biphasic life histories, undergoing indirect development with the larval stage developing from an egg. The larva, often planktonic in the case of

marine animals, undergoes a radical morphological, behavioural and ecological transformation and metamorphoses into a juvenile, which develops into a sexually-mature adult (Holstein and Laudet, 2014). This multiplicity of morphology and ecotype affects visual systems, which can be divergent or absent in one or both of the phases. The nereidid *Platynereis dumerilii* exhibits a pair of simple eye cups in the trochophore larva and two pairs of camera type eyes in the juvenile and adult stages (Arendt et al., 2002). There is a period of overlap around the settlement metamorphosis in which both eye types are present and they differ in their cell fate and genetic expression profile (both express *six1/2*but adult eyes do not express *Pax6*). The authors suggest that the bilaterian larval eye gene regulation network is highly conserved, which implies that the larval eye may be primitive within Bilateria.

It is difficult to glean much direct evidence of early eye evolution from paleontological data because (apart from the limited extent to which simple eyes fossilize). The earliest *lagerstätten* of fauna with complex sensory systems were laid down in the Cambrian, at which point most of the 'crown group taxa' (those including extant phyla) appear already equipped with visual systems. Many of the earliest known animal fossils (especially prior to the Cambrian) are trace fossils, which usually consist of tracks or burrows rather than petrified bodies (Budd, 2015). While lacking phylogenetic information, these fossils are used to infer a great deal about the ecology of the animals which made them, including their means of locomotion, feeding and important sensory modalities. Thus, an understanding of the kinds of visual tasks carried out by simple eyes of extant animals – and the visual performance these are associated with – can improve our understanding of the visual ecology of the early fauna.

Opsins

A number of authors have attempted to come to grips with the ever expanding cacophony of opsins which have been discovered (Fig. 1), how they interrelate and what their respective roles may be (e.g. Oakley and Speiser, 2015; Porter et al., 2012; Ramirez et al., 2016). In many cases, opsins are identified in genomic and transcriptomic analyses by the identification of 7TM proteins exhibiting the signature lysine residue in transmembrane domain VII. All GPCRs investigated carrying this residue exhibit conformational change on photon absorption by the chromophore though not all are explicitly involved in the phototransduction cascade.

Opsin protein-coding genes across animal groups have been classified into four divisions based on phylogenetic relatedness of their coding sequence (Ramirez et al., 2016): (i) canonical visual opsins, (ii) chaopsin, (iii) xenopsin and (iv)

tetraopsin. Most important for vision is this first group: the canonical visual opsins. This includes the two best known opsin groups, C-opsins (Gt-coupled opsins) and R-opsins (Gq-coupled opsins) are those associated with ciliary and rhabdomeric PRCs respectively; they bind different classes of G protein and use different phototransduction cascades. The R-opsin cascade utilizes TRP channel signalling and thus results in an electrical depolarization event, in contrast to the hyperpolarization event induced by cAMP channels in the C-opsin cascade (Plachetzki et al., 2007). C-opsins, which include vertebrate visual opsins, release the bound retinal chromophore following photoactivation and an enzymatic pathway is used to restore the chromophore to its original confirmation (Lamb and Pugh, 2004). R-opsins (which include the classical 'invertebrate opsins' as well as vertebrate melanopsin) do not unbind the chromophore with photon absorption and are capable of restoring the original conformation by the absorption of another photon (Autrum et al., 1979).

Cnidops are the opsin class found in Cnidaria (Bielecki et al., 2014; Plachetzki et al., 2007) and possibly Ctenophora and are members of the Xenopsins (group III). This class is associated with cAMP signalling with a Gs cascade in the ciliary PRCs of Cnidaria (Koyanagi et al., 2008). Group IV, tetraopsins are the least understood class and probably represent several classes with differing roles, which may become better resolved with more widespread opsin sequencing. They include RGR opsins and peropsin, which are expressed in the retinal ganglion cells and Müller cells of vertebrates and do not bind a G-protein but may function as retinal photoisomerases or transporters (Shichida and Matsuyama, 2009) as well as the enigmatic neuropsins, UV/violet-sensitive opsins with a role in entraining circadian rhythm (Buhr et al., 2015). In *Platynereis dumerilii*, *Go-opsin1* is expressed alongside R-opsins in rhabdomeric PRCs of the larval eyes. It forms a cyan visual pigment with a spectral sensitivity peak at 498 nm, which is used to increase the spectral range of larval phototaxis (Gühmann et al., 2015). Further work and more widespread sequencing will help identify more opsins, unravel their function and their relationships and improve our understanding of the ancestral visual pigments and how these influenced vision.

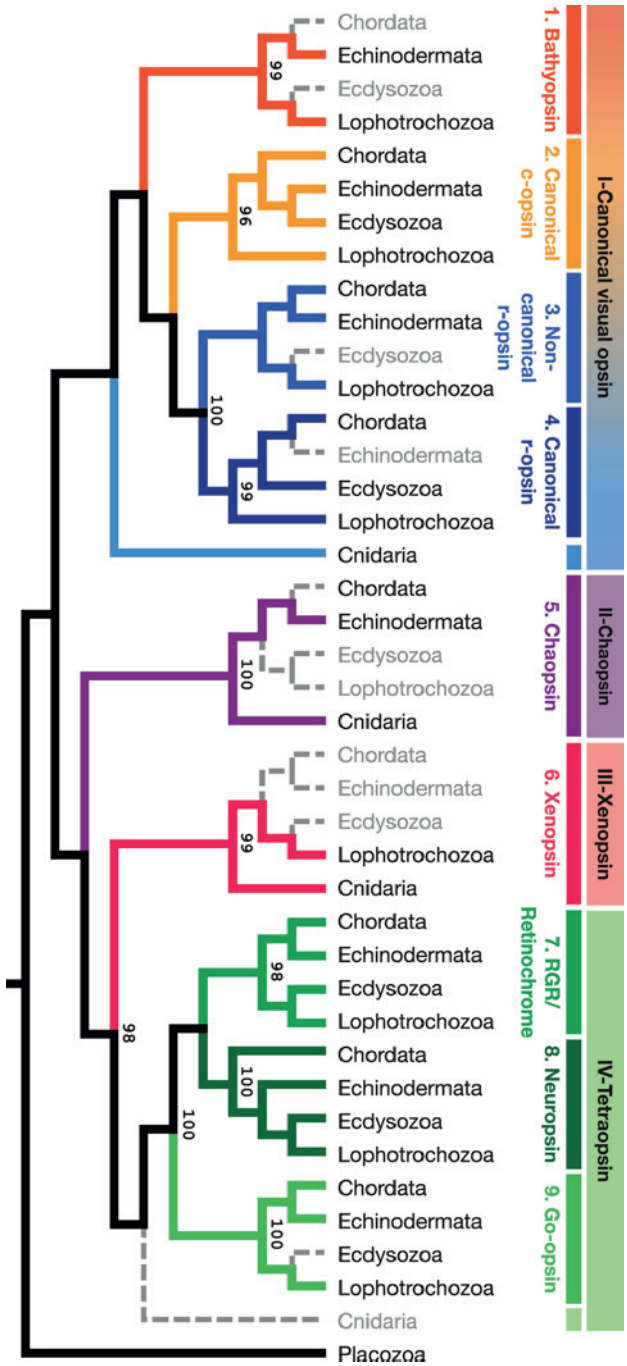


Fig. 1: Phylogenetic relationships of known opsin clades. Modified from Ramirez et al. (2016); licensed under CC BY-NC 4.0: https://creativecommons.org/licenses/by-nc/4.0/ . Values at each node represent bootstrap percentage values derived from ultrafast bootstrap approximation (UFBoot); values greater than 95 are considered well supported.

A functional classification of visual systems

Whilst we have learnt much by investigating morphology and genetics, selection acts directly upon visual behaviours and (given the fitness cost of neural tissue and sensory systems) in the absence of a considerable fitness reward selection will act strongly against even ancient visual systems, as in blind cave fish for instance (Protas et al., 2007). It is therefore highly unlikely that the performance of an eye will be greater than is absolutely required by its repertoire of visual tasks. In contrast, selective pressure can act rapidly on a macro-evolutionary scale, as indicated by modelling of the gradual modification of a photoreceptive tissue (Nilsson and Pelger, 1994). Thus, it is essential to consider not only the cellular and genetic mechanisms at work but also the likely sensory functions. Sensory tasks refer to behavioural and physiological responses, which are systematic and induced by specific sensory information (Nilsson, 2009). The interplay between the evolution of visual systems and visually-guided behaviour is considered by Nilsson (2009).

A functional classification of visual systems has been proposed (Fig. 2) which avoids uniting eyes and eye cups by phylogeny, cell-type or developmental history but is instead concerned with their functional role (Nilsson, 2013). As eyes encounter the same optical and biological constraints, this system finds clear parallels in convergent eye types (Fig. 3). In this system, light-detecting behaviour is divided into four classes: (I) non-directional and (II) directional photoreception and (III) simple low resolution and (IV) sophisticated high-resolution vision. These classes have clear morphological correlates. Only a photopigment is required for class I whereas shielding pigment is required for class II. Class III, the first true resolving vision requires extensive packing of photopigment into cellular extrusions to increase the photon catch in order to be sufficiently sensitive. Class IV represents high-resolution vision as is found, for instance, in most vertebrates and many insects. This class requires optical structures, such as lenses, to gather sufficient light and focus this light to achieve high acuity. This system also recapitulates ecological and behavioural complexity of visual tasks. Only slow and undirected sensory tasks, such as entraining circadian rhythms or vertical migration in the water column are possible with class I photoreception. Class II photoreception allows for faster and directed behaviours such as directional phototaxis and shadow detection. With the appearance of class III vision, faster and more precise visual tasks such as habitat seeking and predator avoidance can occur. Class IV image-resolution allows for the most complex and fastest tasks, including social behaviour. Whilst phylogeny does not reveal a clear progression of visual systems, the classes are progressive in the sense that the emergence of a given class takes place through a stepwise evolution of the preceding classes (e.g., a resolving eye is adapted from a directional photodetector). This is achieved by co-opting components of

the existing genetic architecture and morphology. Nonetheless, the macroevolutionary changes can occur in a graded manner, because each of the anatomical prerequisites of the class II, III and IV vision can facilitate the preceding class and be present therein. For instance, photopigment packing to increase quantum catch is commonly present in class II directional photoreceptors, though it is not an absolute optical requirement. The same is true of light-gathering and focusing optics, which may be present in class III low-resolution eyes.

This classification escapes the difficulties that have plagued a purely phylogenetic approach to understanding visual evolution, which occur partly because of widespread and systemic convergent evolution of forms. Rather, this classification is concerned with the essentially optical problems that drive convergent evolution of eye types or, conversely, the adoption of very different solutions in related groups. It also circumvents the insoluble problem of how many times did eyes independently evolve by reflecting the modular evolution of visual systems, given that they are complex traits.

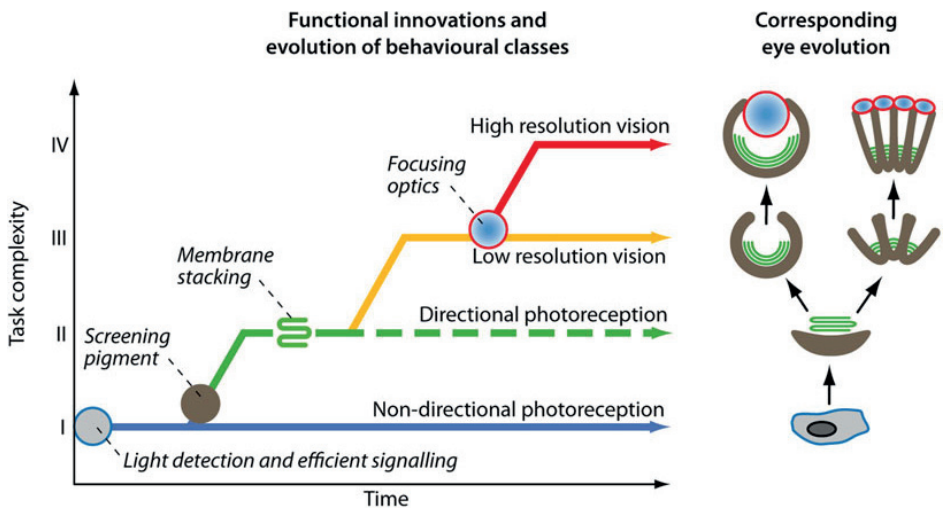


Fig. 2: A functional classificiation of visual systems
Increasing complexity of visual tasks have emerged alongisde functional innovations in the evolution of eyes (Nilsson, 2013); licensed under CC BY-NC-SA 4.0: https://creativecommons.org/licenses/by-nc-sa/4.0/ .

III. Phylogenetic distribution of light-detection systems

Organismic light detection

Light-detection is truly ancient and widespread. Phototaxis has been observed in both eubacteria and archaea (Armitage and Hellingwerf, 2003; Scharf and Wolff, 1994). Opsins have been identified only in the metazoan animals, to the exclusion of the choanoflagellate *Monosiga brevicollis* and are also absent from the Porifera, though are found in the Ctenophora (Porter et al., 2012). Furthermore, no phototaxis has been identified in the choanoflagellates or other non-animal holozoans (Jékely, 2009). Perhaps the most fascinating and sophisticated photoreceptive organ outside of Metazoa is found amongst the dinoflagellates, aquatic single-celled eukaryotes of the Alveolates clade (Burki, 2014), which often use both photosynthesis and prey capture. In one family, Warnowiaceae, an *ocelloid* is present, which appears to serve the function of an intracellular camera type eye (Hayakawa et al., 2015). Moreover, even the freshwater cyanobacterium *Synechocystis* meets the most basic definition of vision (Nilsson and Colley, 2016). The cell surface of this microbe focuses light onto the opposite point from a light source, which the animal then avoids via taxis.

Metazoan phylogeny

Our understanding of animal interrelationships (and therefore evolutionary history) has been utterly transformed in the last two decades due to improved molecular and statistical tools, combined with fossil and developmental data. The monophyly of Metazoa (animals) is not in dispute and this clade is the sister group to the choanoflagellates (Dunn et al., 2014). Metazoa can be divided into five distinct clades: Ctenophora (comb jellies), Porifera (sponges), Placozoa (Placozoans), Cnidaria (jellyfish, polyps, corals et al.) and Bilateria; the relationships between these groups are depicted in Fig. 3. The position of some groups such as the Ctenophora is disputed (Borowiec et al., 2015; Giribet, 2015; Jékely et al., 2015; Pisani et al., 2015; Whelan et al., 2015). Bilateria (the

majority of species and the clade which is primitively and predominantly bilaterally symmetrical in body axis of the adult phase) is further composed of five distinct clades: Acoelomorpha, Deuterostomia, Chaetognatha, Ecdysozoa and Spiralia. All of these clades, except Bryozoa, and many subclades (most of the classical phyla) are thought on the basis of fossil evidence to have been present in the early Cambrian. Below, some aspects of the visual systems and their functions are considered in the various animal groups, giving priority to the simpler eyes.

Nonbilaterians

In spite of being equipped with mesoderm and muscle cells, Ctenophora (comb jellies) are proposed as the sister clade to all remaining metazoa based on molecular phylogenomic evidence (Ryan et al., 2013); their phylogeny has implications for our understanding of the evolution of photopigments (Feuda et al., 2014; Porter et al., 2012). Studies have found that opsins found in ctenophores are of the cnidops clade, whereas others suggest that they are C-opsins (Feuda et al., 2014). Unique ciliary photoreceptors, have been putatively identified in the ctenophores but a definite light response has not been demonstrated (Horridge, 1964). Opsins have not been identified in the genome of any member of Porifera (sponges) but this group nonetheless display light sensitivity in larvae (Leys et al., 2002) via pigment cup eyes, which is mediated by a cryptochrome (Rivera et al., 2012). Placozoa is represented by a single known extant species, *Trichoplax adhaerens* Schultze, 1883, which is marine, flattened and only a few millimetres in length (Minot, 1883). Placozoans lack a nervous system and photoreceptive structures have yet to be identified. This group have ‘placopsins’, proteins which are the sister-group to known animal opsins (Feuda et al., 2012). However, placopsins lack the lysine residue required for phototransduction and are therefore not visual pigments. These genes code for proteins which lack the lysine residue involved in phototransduction and probably have no photoreceptive function.

Cnidaria is the clade other than its putative sister group Bilateria, which exhibits sophisticated visual systems and resolving vision (Nilsson et al., 2005). The major phylogenetic relationships within the cnidarians are still not well resolved but there is support for the traditional sister group relationship between Anthozoa and Medusozoa (Zapata et al., 2015). Although previously considered ‘primitive’ and basal within Metazoa, partly due to the absence of mesoderm, there is increasing phylogenetic evidence that Cnidaria is the sister group to the Bilateria. Two opsin groups are present in Cnidaria, C-opsins and cnidops (Bielecki et al., 2014). Low-resolution vision is present in a single cnidarian clade, Cubozoa (Nilsson et al., 2005). They bear external sensory structures, called rhopalia, which carry numerous eyes of diverse type. These include lens

eyes, which are the most sophisticated visual organs of a nonbilaterian and are of a comparable quality to bilaterian lens eyes. One species, *Tripedalia cystophora* lives among mangrove roots, where it forages showing phototactic attraction to beams of light permeating the canopy (Stewart, 1996), which is strongly influenced by photosensory information from the rhopalia. The lens eyes of *T. cystophora* , which are typical of Cubozoa, have a full-width at half maximum (hereafter, half-width) of the receptor field of approximately 20° (Nilsson et al., 2005). This results from an under-focused lens, which could theoretically achieve an angular sensitivity of 1° if in focus. These lens eyes provide the animal with low resolution (class III) vision, which is also slow (8-10 Hz) for an active swimmer in bright light (O'Connor et al., 2010). They may represent adaptations to increase sensitivity, without increasing signal noise. These animals rely on visual cues from the lens eyes to steer away from mangrove roots to avoid predation but stay in their proximity in order to find food using visual cues (Garm et al., 2011; Petie et al., 2011). The low spatial and temporal resolution can be explained by the specific performance requirements of visual steering in this animal, i.e. a matched filter for the specialist sensory task. Two distinct cnidops opsins have been identified as being expressed in the rhopalium in this species, without finding orthologues outside the Cubozoa (Bielecki et al., 2014) and the lens crystallin also appears to be independently evolved (Kozmik et al., 2008; Piatigorsky et al., 1989).This visual guidance system represents the only extant example of a gain of class III vision in nonbilaterian animals.

Xenoacoelomorpha: acoels *et al.*

This group of elegantly simple animals comprises the acoelomorphs and the peculiar Xenoturbellida. There is a continuing dispute over the affinity of Xenoacoelomorpha, which some regard as a basal lineage of deuterostomes (Philippe et al., 2011; discussed by Telford et al., 2015), though recent phylogenomic evidence rejects this (Cannon et al., 2016; Rouse et al., 2016). Acoelomorphs have no larval form and a miniature adult is instead hatched (Hejnol and Martindale, 2008). Some of the simplest body plans of all bilaterians are found in the Xenoacoelomorpha and it has been argued that Urbilateria, the last common ancestor of Bilateria, may have shared similarities with xenoacoelomorphs (Cannon et al., 2016). Acoelomorpha contains the Acoela (acoel flatworms) and the Nemertodermatida (about 10 species of small, parasitic worms). The anatomy of acoel flatworm ocelli were investigated in four acoel genera (Yamasu, 1991) and it was found that these structures consisted of extremely simple concave pigment cell containing platelets and several photosensory cells – one in close contact with the pigment cell and the others not.

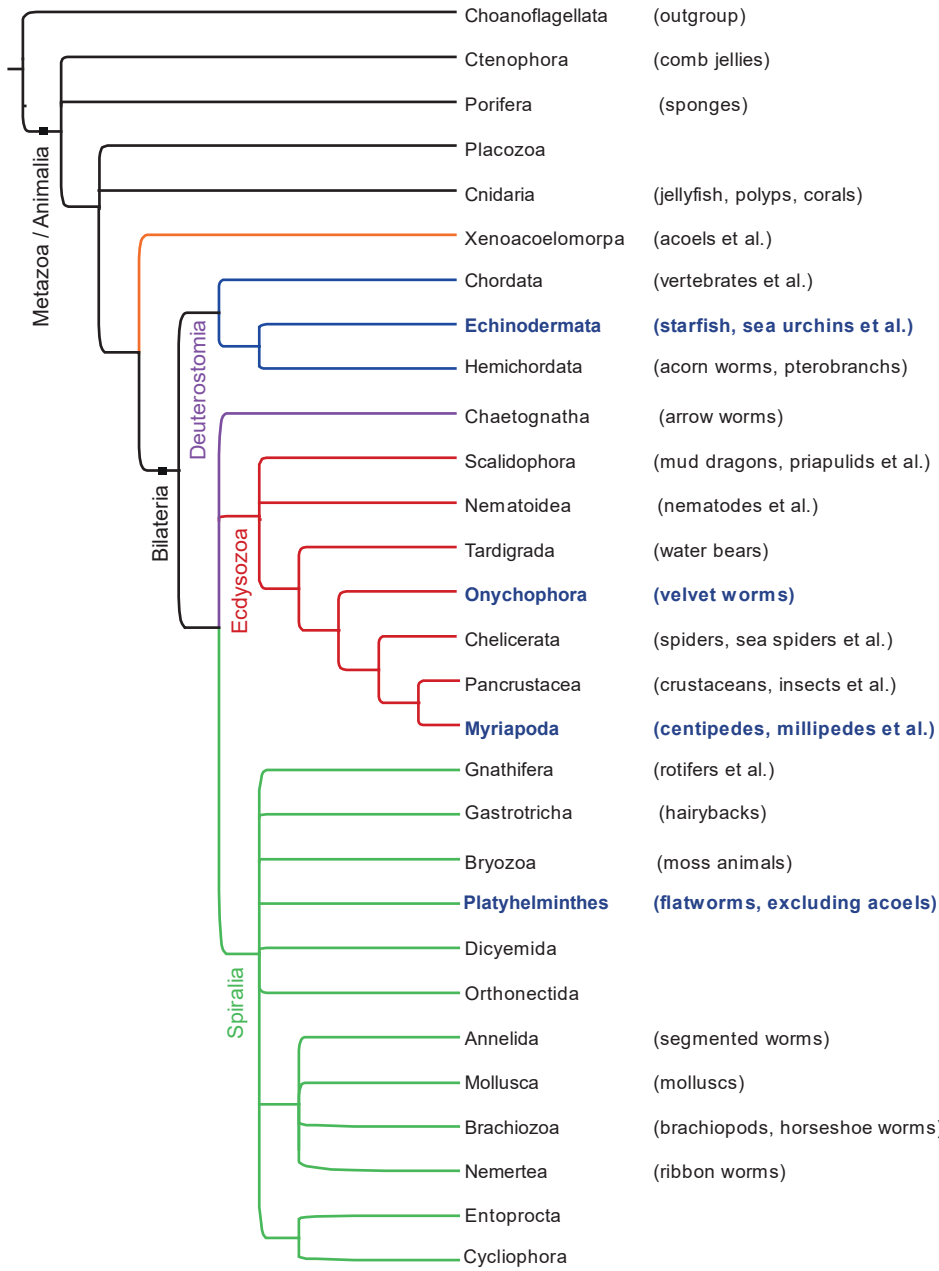


Fig. 3: Rooted cladogram of the major lineages within Metazoa
Polytomies indicate an unresolved branch. Outgroup of the Metazoa (animals) is Choanoflagellata. The phylogeny based on Dunn et al. (2014) and is compiled from multiple studies utilizing genomic data. The names of the clades here investigated are indicated in blue.

These cells (differ from the PRCs of Platyhelminthes in that they) lack either rhabdomeres or cilia and have platelets in the pigment cell. Ablation of these

ocelli in *Praesagittifera naikaiensis* demonstrated them to be photoreceptive. Photoreceptive sensory structures have not been observed in the Nemertodermatida (Rieger et al., 1991). The six known species of Xenoturbellida, are deep-water mud-dwellers found in the North Sea and Skagerrak and the Pacific (Rouse et al., 2016). The basics of their ecology and life-history are a matter of continued interest, although based on gut contents they appear to feed on bivalves (Nakano, 2015). They do not possess eyes or any known photoreceptors (Rieger et al., 1991).

Deuterostomes: Ambulacraria and Chordata

Ambulacraria comprises Echinodermata (echinoderms) and Hemichordata (hemichordates), which in turn comprises the Enteropneusta (acorn worms) and the species poor Pterobranchia. Echinodermata are typically characterized by derived radial symmetry in the benthic adult phase, which develops from a bilaterally symmetrical planktonic larva as well as their tube feet, which are used for a variety of functions, including locomotion. This group consists of four extant classes: Echinoidea (sea urchins and sand dollars), Holothuroidea (sea cucumbers), Asteroidea (starfish), Ophiuroidea (brittlestars) and Crinoidea (sea lilies). Recent analyses support a sister group relationship of Echinozoa (Echinoidea + Holothuroidea) and of Asterozoa (Asteroidea + Ophiuroidea), with Crinoidea as the basal clade (Reich et al., 2015; Telford et al., 2014). Amongst the echinoderms, true eyes have been reported in starfish (Smith, 1937) and a single known species of holothurian, *Opheodesoma spectabilis*, (Yamamoto and Yoshida, 1978). Garm and Nilsson (2014) found that the compound eye of the starfish *Linckia laevigata* is used for slow and low-resolution visual tasks, being the first behavioural evidence of such in Asteroidea. It has been proposed for the brittlestar *Ophiocoma wendtii* that calcite crystals in the exoskeleton act as microlenses overlaying dispersed photoreceptor cells (Aizenberg et al., 2001). These structures are absent in species which are light-indifferent.

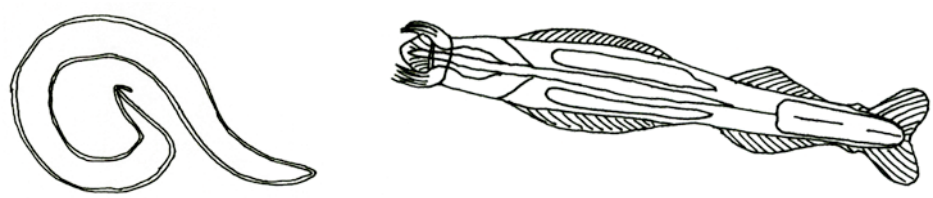
Phylogenetic analysis of genomic and transcriptomic data from echinoderms and hemichordates has identified representatives of all of the major bilaterian opsin clades in Ambulacraria, as well as two further opsin clades, which on current evidence, are restricted to echinoderms within the deuterostomes: echinopsins-A and echinopsins-B (D'Aniello et al., 2015). The relationships of these echinopsin clades to other opsins is not resolved, though they do not appear to be closely related to one another; no rhabdomeric opsin has been identified in the hemichordates.

Low resolution vision is reported in sea urchins, which lack the ocelli of starfish but instead use dispersed photoreceptors upon their exoskeletal test. Visually-

guided locomotion depends on the central nervous system and is ablated by the removal of the oral nerve ring (Yoshida et al., 1984). Lesser et al. (2011) found R-opsin expression throughout the tube feet of the green sea urchin (*Strongylocentrotus droebachiensis*), with increasing expression in regions less exposed to light. They also found PAX6 expression, whose orthologues control eye development in many bilaterian clades, in the distal portion of the tube foot, within pigmented areas including a series of calcite ossicles, which may function as a light collector. On this basis, they regard the tube feet as photosensory organs. Using in-situ hybridisation against the R-opsin *Sp-opsin4* and *Pax6* orthologues in the congeneric *S. purpuratus*, *Sp-opsin4* was found to be co-localized with *Pax6*, in two groups of non-pigmented rhabdomeric PRCs in the animal's tube feet (Ullrich-Lüter et al., 2011). Given that orthologues of the R-opsin *Sp-opsin4* are expressed in the ocelli of starfish, a visual role is suggested: it is proposed that *Sp-opsin4* is responsible for visually-guided phototaxis in these animals; within rhabdomeric PRCs, possibly using the calcite skeleton in lieu of shielding pigment. Using antibodies against the *S. purpuratus* opsin, *Sp-opsin1*, C-opsin pigments (allied to vertebrate visual C-opsins) have been found expressed in the spines, pedicellariae, locomotory and buccal feet and some portions of epidermis of a number of urchins as well as in the spines of starfish and brittlestars (Ullrich-Lüter et al., 2013). A close association was found between these expression patterns and the distribution of the nerve network (suggesting communication with the central nervous system) and the calcite skeleton.

Opsins of all major classes are present in a non-visual brittlestar *Amphiura filiformis* Delroisse et al (2014) and light detection seems to be mediated by opsins at least, in spines, tube feet and in the radial nerve cord whereas only non-visual opsins have been identified in larvae (Delroisse et al., 2015). Positive phototaxis is observed in adult starfish (Oviatt, 1969). In sea urchins it is predominantly negative. The brittlestar, *Ophioderma brevispinum*, by Johnsen and Kier (2009) are sensitive to polarized light and respond with shade seeking behaviour (Johnsen and Kier, 2009). It has long been observed that echinoderms can respond phototactically to light stimuli, typically moving towards a dark stimulus. More recently, it was realized that spatial visual might be present in some phototactic sea urchins and the resolution of visual stimuli by a number of species has been reported based on behavioural experiments (Blevins and Johnsen, 2004; Yerramilli and Johnsen, 2010). Resolution of 10º is reported, suggested to be facilitated by occlusion from spines (Yerramilli and Johnsen, 2010). In paper II, we measure the resolution of another urchin species, the diadematid *Diadema africanum*, and find a poorer spatial resolution than has been measured in *S. purpuratus*. We calculate the angular sensitivity we expect from resolving the signals used and compare this to morphological measurements of the putative receptors.

Hemichordates are of interest to evolutionary biologists due to their phylogenetic position and as they are considered to have retained a number of plesiomorphic characters: those representative of the primitive, ancestral state (Braun et al., 2015). The eyespots of the tornarian larva of the enteropneust *Ptychodera flava*, are simple ocelli with a single PRC each which exhibit both cilia and microvilli and these animals were found to be positively phototactic (Brandenburger et al., 1973). The other major clade of Deuterostomia, the Chordata includes the most sophisticated visual systems of all in terms of spatial resolution, which is reported to be in the range of 140c/deg for *Aquila audax* (Reymond, 1985). The extant species are divided between the Cephalochordata (lancelets) and another clade consisting of the highly derived Tunicata (tunicates) which are secondarily simplified in their adult phase and the vertebrates (Lowe et al., 2015). Tunicate larvae have simple pigmented eye-spots located in the brain and both positive and negative phototaxis have been demonstrated (Kusakabe and Tsuda). Vertebrates have a pair of camera type eyes in adults and in larvae where present (though there are some exceptions which have lost eyes and a few with simple median eyes). Whilst rhabdomeric PRCs are absent in vertebrates, the lancelet *Amphioxus* has both ciliary and rhabdomeric PRCs, the latter used in non-ocular PRCs for non-visual roles (Koyanagi et al., 2005). These cells express the cephalochordate homologue of vertebrate melanopsin, which in its structure and photochemistry (it is Gq coupled) resembles r-opsins. The PRCs of the frontal eyes of *Amphioxus* contain simple ciliated cells, far less sophisticated than those of vertebrates. However, in opsin and developmental transcription factor expression, as well as in the (off-responding) type of phototransduction cascade, these resemble the vertebrate ciliary PRCs of vertebrate lateral eyes (Vopalensky et al., 2012).



Chaetognatha: arrow worms

Chaetognaths are an enigmatic clade, with an uncertain phylogenetic affinity within the protostomes, i.e. their relationship to the Spiralia and Ecdysozoa (Ball and Miller, 2006; Dunn et al., 2014), though a basal position has been suggested, which is consistent with a number of deuterostome traits. Chaetognaths are highly abundant, small, direct-developing and mostly pelagic, marine predators. They are an ancient clade, and have putatively been identified in the early Cambrian fossil record (Chen and Huang, 2002). The visual system of Chaetognaths was investigated using transmission electron microscopy (Eakin and Westfall, 1964) and may have a role such as orientation or maintaining vertical position in the water column. Predatory behaviour can be induced by vibrations of specified frequency and amplitude, whereas other vibrations instigate an escape response – suggesting that predation is highly somatosensory rather than visual (Horridge and Boulton, 1967; Newbury, 1972).

Ecdysozoa: arthropods, nematodes *et al.*

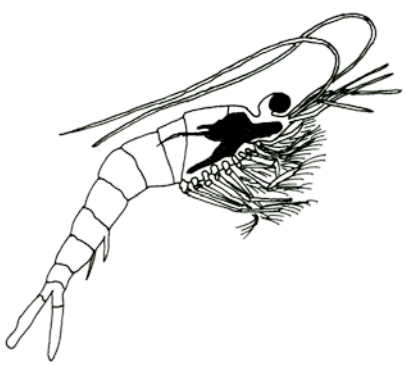
Ecdysozoa comprises the Panarthropoda (Arthropods, Onychophora and Tardigrada), Nematoida (Nematoda and Nematomorpha) and the Scalidophora (Kinorhyncha, Priapulida and Loricifera), with no agreement on the interrelationships of these three subclades. They include the majority of all recorded animal species, owing primarily to the inclusion of the insects and other arthropods, with nematodes also believed to be highly speciose. Within Arthropoda, the phylogenetic position of Myriapoda has remained ambiguous, although increased taxon sampling of transcriptomic data from Myriapoda has provided support for Mandibulata, i.e. a sister group relationship with Pancrustacea to the exclusion of Chelicerata (Rehm et al., 2014).

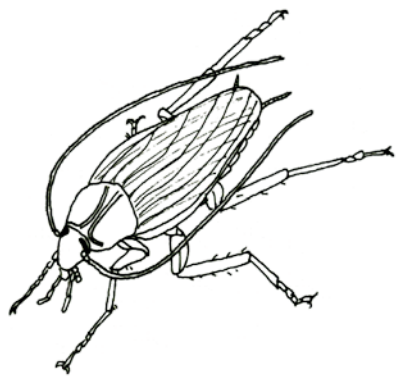
Arthropoda is the most species rich of all phyla and this group boasts some of the best performing eyes and complex visual behaviour and the fastest temporal resolution of all animals. The superlatively species-rich Pancrustacea showcase an incredible diversity of variations of the compound eye type. Adults of this group typically have two lateral compound cephalic eyes and often much smaller ocelli on the top of the head. Much of what is known about vision in this group has been learnt from the important model system *Drosophila melanogaster*, a fruitfly (see Borst, 2009). The unusual compound ocelli of myriapods have further confounded the enigma of arthropod eye evolution with eyes which may be reduced pancrustacean type eyes or akin to those of chelicerates. In paper III, we evaluate the visual capabilities of a millipede, *Cylindroiulus punctatus*, and provide the first behaviour estimates of spatial resolution for this group.

In two species of Onychophora, it has been found that R-opsins are expressed in the eye and C-opsins in the optic ganglion and brain, the latter of which may

serve a photoreceptive rather than visual function (Beckmann et al., 2015). The discovery of only a single opsin (onychopsin, sister to the R-opsins) in a survey of distantly related onychophoran species was initially suggestive of a single opsin being present in Panarthropoda which later diverged in Arthropods (Hering et al., 2012). However, five opsins have been identified within the genome of the tardigrade *Hypsibius dujardini*, constituting an R-opsin, three C-opsins, and a Group 4 opsin, which suggests that the these major groups of opsins were all present in the Panarthropoda ancestor, although only the R-opsin is implicated in vision (Hering and Mayer, 2014). In paper I, we investigate the visual system of the onychophoran *Euperipatoides rowelli* and demonstrate that it uses resolving vison with its taxis towards dark objects.

By comparison, little investigation has been carried out on photoreception in the remaining Ecdysozoa. According to Neuhaus (1997) it has frequently been proposed that the cephalic sensory organs of Nematoida and Scalidophora (referred to therein collectively as Nemathelminthes) have a photosensory function, this has nowhere been demonstrated. Nonetheless, photosensitivity is considered the most plausible function for the ciliary cephalic organs of these groups. In the most important model system of these taxa, the nematode *Caenorhabditis elegans*, pigmented PRCs are not present. Amongst Kinorhyncha but not elsewhere (Neuhaus and Higgins, 2002), pigmented eyespots have been reported in the genus *Echinoderes*, which disappear upon fixation (Zelinka, 1928).





Spiralia: molluscs, annelids, platyhelminthes *et al.*

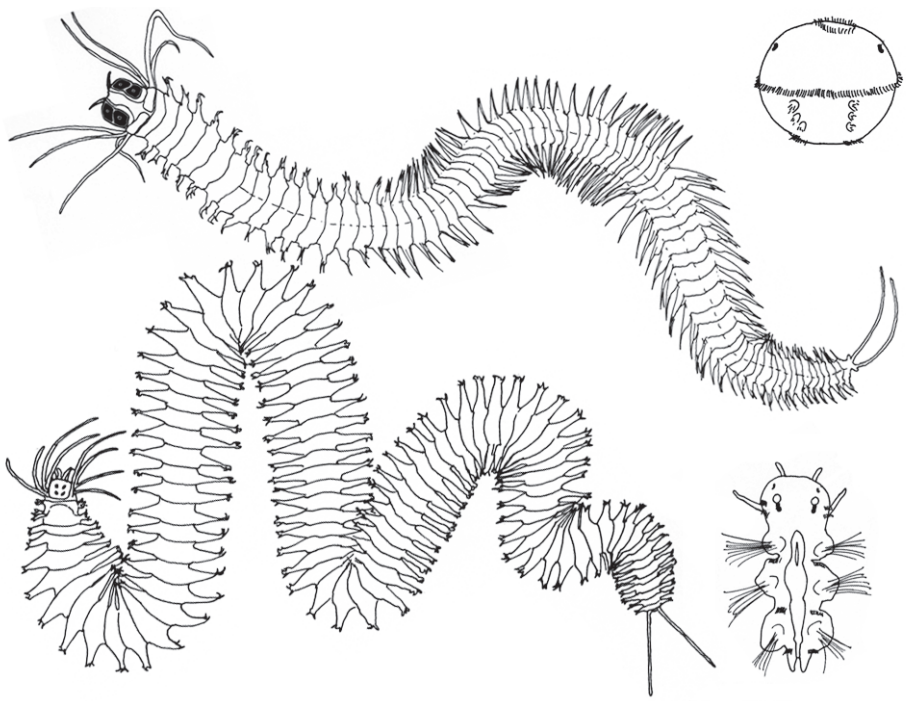
Apart from the molluscs, annelids and platyhelminthes flatworms, this group includes several other clades, such as brachiopods, nemerteans and phoronids, the phylogenetic interrelationships of which are not well resolved (Dunn et al., 2014) and in which sophisticated resolving eyes do not occur. Recent phylogenomic and transcriptomic analyses suggest that within Spiralia, the clade Lophotrochozoa *sensu stricto* includes annelids, molluscs, nemerteans, brachiozoans and possibly bryozoans and ectoprocts (Laumer et al., 2015; Struck et al., 2014). Furthermore, Platyhelminthes is likely sister to Gastrotricha, forming Rouphozoa: a sister clade to Lophotrochozoa. The remaining taxa may form a clade: Gnathifera (Laumer et al., 2015).

Numerous kinds of eye are found amongst the molluscs. The eyes of cephalopods share many characteristics with fish, as a consequence of convergent evolution of high resolution vision in aquatic environments (Land and Nilsson, 2012). The largest known eyes are found in the deep sea squid, which (based on anatomy and optics) appear to be used for detection of bioluminescence, triggered by the approach of their sperm whale predators (Nilsson et al., 2012). A variety of diffuse visual systems are present in bivalve molluscs, including mirrored forms in scallops (Land, 1965). Species of ark clams (Arcacea) respond rapidly to shadows and looming visual stimuli by closing the shell using an array of hundreds of lensless compound eyes and thousands of miniscule cup eyes on the mantle edge, operating not for resolving vision but as an optical 'burglar alarm' (Nilsson, 1994). Some chitons have evolved focusing lens optics through multiple camera eyes within their dorsal shell, which exhibit spatial vision (Speiser et al., 2011). They use this modality to respond to potential predators by clasping the rocky substrate, such that their shell simultaneously provides physical and sensorial defensive capabilities (Li et al., 2015).

Annelids represent a large, diverse and ancient clade. No indisputable fossil evidence is found prior to the Ordovician (Budd and Jackson, 2015) though recent evidence suggests they may have diverged in the lower Cambrian or earlier (Parry et al., 2015; Weigert et al., 2014). Transcriptomic analyses using extensive taxon sampling place most annelids within the *Pleistoannelida*, which comprises the sister clades Errantia (most errant polychaetes) and Sedentaria, to the exclusion of several notable groups, including the Amphinomidae (fireworms and allies), which had previously been considered to be Errantia (Weigert et al., 2014). Annelids also display an incredible diversity of eye types, sometimes even within the same individual (Nilsson, 1994). The most impressive eyes in terms of optics are found in the Errantia, especially amongst the pelagic Alciopidae and Tomopteridae (Verger-Bocquet, 1984). The marine ragworms (Nereididae) and some related taxa typically exhibit two pairs of large cephalic eyes, which in *Platynereis* each include a spherical lens eyes with

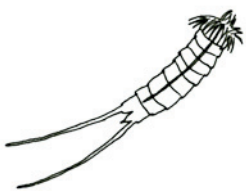
several thousand PRCs and a pupillary response (Brökelmann et al., 1966; Fischer, 1963). Most of the remaining errant polychaetes exhibit less sophisticated eyes (Verger-Bocquet, 1984). Amongst Sedentaria, which includes the predominantly tube or burrow dwelling sedentary polychaetes as well as clitellates (earthworms and leeches), most groups lack sophisticated eyes capable of image-formation. Nonetheless, many sedentary polychaetes use prominent eyes, including compound eyes. Clittelates exhibit only phaosomes, simple dispersed PRCs, which are derived from rhabdomeric PRCs (Döring et al., 2013) and, in some cases, pairs of simple ocelli.

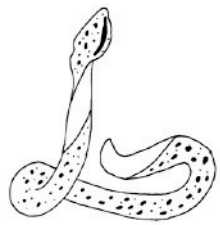
Some of the most peculiar and exceptional optical systems are found amongst the fan worms, Sabellida and Serpulidae (Bok et al., 2016; Bok et al., 2017; Nilsson, 1994), which respond to looming stimuli by retracting into their tubes. *Sabella melanostigma* can have over 200 compound eyes, of about 60 ommatidia, each that are arranged in pairs on the tentacular crown. From anatomical measurements, their ommatidial lenses have acceptance angles of around 10° (Nilsson, 1994). Responses to gratings of differing spatial frequencies suggest that the fan worm *B. melanostigma* has a maximal visual acuity (as measured by its response to looming stimuli) of approximately 7.5°. They use these eyes as an optical alarm system, which are comparable to those of the ark clams and constitute an additional subclass, class IIb according to the schema of Nilsson (2013).



The Platyhelminthes phylogeny has witnessed substantial revision in the last two decades, particularly the loss of the acoel flatworms which are distantly related (Laumer and Giribet, 2014; Ruiz-Trillo et al., 1999; Sempere et al., 2007). This group exhibit an impressive diversity of eye types for an, in some respects, morphologically simple clade. They include species lacking pigmented photoreceptors, those with superlatively simple eyes including only a single PRC (Sopott-Ehlers et al., 2001) and far more complex eyes which comprise many photoreceptors in a variety of orientations and designs. Polycladida (polyclad flatworms) are an early branching clade (Laumer and Giribet, 2014), which are notable for their often large size, motility and the presence of multiple, sometimes hundreds of simple eyes located in eye-patches. In contrast to most platyhelminthes, many polyclads undergo a 'Müller's' larval stage, which may possess simple cerebral pigmented ocelli (Eakin and Brandenburger, 1981) and exhibit positive and negative phototaxis (Johnson et al., 2003). Many platyhelminthes are parasitic and have secondarily lost much of their morphological complexity, including sensory function. Large paired eyes are present in some Platyhelminthes, such as many triclad 'planarians'. We explore the visual capabilities of one such species, *Schmidtea lugubris*, in paper IV.

Bryozoa undergo a brief, swimming larval stage (prior to the sessile adult) which exhibits positive and negative phototaxis (Burr, 1984). Ciliary PRCs expressing C-opsins have been characterized in larvae of the brachiopod *Terebratalia transversa* (Passamaneck et al., 2011). Rotifers have eye cups and ocelli – typically one eyecup in the brain and two ocelli in the rotary apparatus (Clément and Wurdak, 1984), with the exception of the parasitic acanthocephalans (Clément, 1993). Gastrotrichs are known to possess pigmented eye cups in some species (Remane and Bronn, 1936; Todaro and Leasi, 2013). In addition, it has been suggested that the ciliary anterior head sensory organ, which resembles the cephalic sensory organ of kinorhynchs (Liesenjohann et al., 2006), is also photoreceptive (Teuchert, 1976).





V. Quantifying resolving vision

Performance in the spotlight: measuring resolution

Advances in molecular methods and imaging have shed light on the occurrence and provenance of visual systems. It is also important to understand how effective these organs are and which conditions warrant them. The physiological or behavioural response to differing visual signals can be used to measure visual performance characteristics, including spatial resolution. Behavioural experiments have the advantage of explicitly showing what an animal *in vivo* can demonstrably achieve, rather than a theoretical measure. This provides a somewhat conservative metric, a lower bound of spatial vision performance which can demonstrably occur, in contrast to the upper bound of visual capability provided by an optical model of the visual system.

A prerequisite for behavioural assays of sensory performance is to be able to demonstrate a response to some stimulus. It is highly advantageous if this response is robust, i.e. that at some level of the stimulus the response can be elicited very reliably. Determination of the optical resolving power of visual systems via behavioural responses has been applied to a wide variety of species exhibiting high-resolution vision, such as numerous birds (summarized by Mitkus, 2015), mammals and arthropods.

The experiments conducted here all involved the detection of a visual stimulus which included a dark target region. In each of the papers in this thesis, this involved eliciting an object taxis response towards dark features. Additionally, in paper II, it took advantage of an alarm response to a dark spot on a bright field. Taxis is whole body locomotion in response to a stimulus and light-dependent taxis (phototaxis) is a widespread behaviour, which can be light-attractant (positive) or avoidant (negative). Object taxis not dependent on the overall luminance but on localised differences in luminance and can, thus, be used to assay spatial vision. Specifically, our taxis experiments involved testing the detection of varying arc widths of a stimulus by the orientation of animals to that stimulus over a series of independent trials. In order to distinguish these two forms of light dependent taxis (and thus evidence vision), it was necessary to use novel stimulus types. Experiments were carried out at high light intensities comparable to the light environment of the animal and by providing a high

contrast in the stimulus relative to the remainder of the visual scene in order to make the stimuli as salient as possible.

Mixed signals: defining the visual stimulus

To define what spatial resolution an animal is capable of, rather than simply show that an animal responds to light, an appropriate stimulus must be chosen. The stimulus ought to be of high contrast against the background to show the best resolution possible, as signal detection depends also on contrast. Importantly, the fewer spatial frequencies, which comprise the stimulus, the more precise will be the estimate (Paper III). The ideal visual stimulus comprises a continuous sine wave grating (Campbell and Robson, 1968), which comprises a single spatial frequency, which is the reciprocal of the sinus period. Such stimuli are employed in optomotor experiments, in which an animal is surrounded by a rotating sine wave pattern and change of orientation in response to the wave is observed. As mentioned above, conventionally, resolution is given by the smallest period of the wave that can be detected, in cycles per degree (cpd) but for very poor resolution the reciprocal of this value, in degrees, is convenient.

However, a continuous wave grating is not feasible for all experimental paradigms and often a stimulus, which is located in one direction, is needed. One alternative is to use a simple rectangular function, as a black bar or spot on a white background – a widely used visual stimulus, which we apply in paper II and III. A problem with this type of stimulus is that it necessarily changes the luminance in its locality (being, in this case, darker than the background). This means that, theoretically, a simple directional photodetector, with an aperture much wider than would allow resolution of the stimulus could detect it.

This can be mitigated by using a wavelet (a single period wave oscillation) stimulus, which is isoluminant, i.e. it has the same average luminance as an equivalent region of the background. Consequently, for most experiments we used stimuli derived from isoluminant wavelets. In paper I, a dual bar stimulus comprising a Haar wavelet was used for several treatments. This stimulus comprises a black target adjacent to an equivalent white region against an intermediate grey background.

Collectively, the discrete stimuli types derived from step functions present other challenges, as discussed in Paper II. They result in an additional range of high frequencies and local high contrast as a consequence of the stimulus edge. To avoid this, two isoluminant stimuli derived from continuous wavelets were used in this thesis. In paper I, a stimulus derived from a piecewise sine was used. For papers II-IV, a novel visual signal was applied comprising a difference of Gaussians (DoG) function. The function was defined as follows:

$$\Gamma_\sigma(x) = \frac{1}{\sigma\sqrt{2\pi}}\, e^{-\frac{(x)^2}{2\sigma^2}} - \frac{1}{2\sigma\sqrt{2\pi}}\, e^{-\frac{(x)^2}{4\sigma^2}} \quad (3)$$

where σ is the variance of the primary Gaussian and the variance of the secondary Gaussian is twice σ. The value of *x* is a numeric scale used to indicate the horizontal position on the printed images with the stimulus centre as the origin. Using wavelet stimuli, which comprise a range of spatial frequencies, it is not definitively clear which frequency has been detected. However, this is mitigated by using a narrowband continuous wavelet, such as the DoG (discussed in paper II).

Defining the width of the continuous wavelets, which is necessary to decide the resolution, is not self-evident. One possibility is to define the width based on the dark target, by analogy to bar or spot stimuli. Two such measures are the zero-crossing of the dark minimum (the points at which the stimulus is equally bright to the background) and the half-width (full-width at half maximum) of the dark target. The period of the wavelet (corresponding to the maxima of the white flanks) is another measure. This has the advantage of corresponding to how resolution is defined according to a sine wave (and, consequently, to other estimates of resolution). In paper I, we describe the stimulus width using the half-width of the dark target (which provided a comparison with the bar stimuli). However, in the remaining papers we opted to use the period of the wavelet, to be more readily comparable with other spatial resolution estimates. The period of the piecewise cosine stimulus used in paper I is three times the width of the dark target and, consequently, the (piecewise cosine) stimulus widths and spatial resolution measures from this first paper can be tripled to arrive at a metric comparable to the remaining papers.

Defining orientedness from animal tracking data

In each of our taxis experiments, trials were recorded and later tracked to determine the headings taken by animals in relation to the stimulus (Fig. 4). A response metric can be derived from experiments which track animal movements to assess if the animal is oriented (and in a specified direction) such as taxis towards a sensory stimulus, in several ways. The tortuosity of individual tracks can be measured, under the assumption that a more oriented animal will follow a more parsimonious route (Benhamou, 2004), as can its speed of progress. An explicit measure of orientedness determines the direction faced by an animal or the position or direction (bearing) of its track or destination. These latter cases can result in circular data, expressed as angles, which can be more challenging to interpret than data with linear support. One of the most widely-used one-

sample tests of uniformity is the Rayleigh test, which assesses the value of the mean resultant length (ρ) to determine if the data are uniformly distributed. If ρ is large, the null hypothesis of uniformity is rejected. The related V-test (Durand and Greenwood, 1958) assesses uniformity of the sample data against the special case of clustering towards a prespecified direction and is useful in testing for aggregation towards a sensory stimulus.

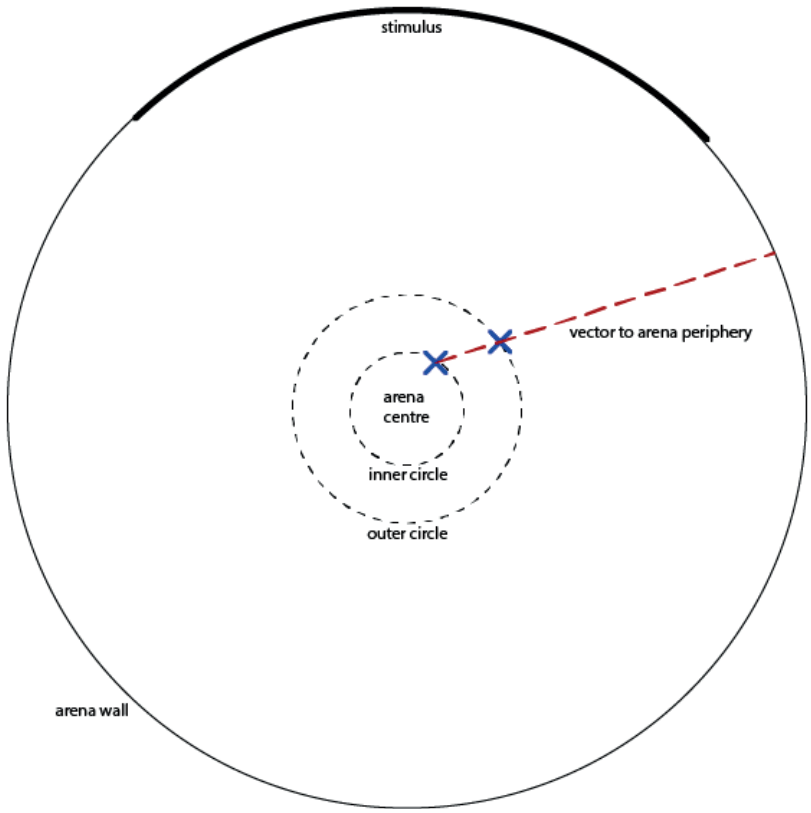


Fig. 4: Determination of animal heading
Diagram indicating how the direction of travel of the animal from the central region of the arena is determined for papers II-IV. The blue crosses indicate where the animal has reached two circles, equivalent to predetermined distances from the arena centre.

Have you thought about modelling? Inference of visual detection from orientation behaviour

Although the data generated in the circular experiments are directional, the ultimate property of interest (orientation) depends on a binary choice. One way

to treat these data is to discretize them based on a sensible criterion to distinguish between paths that are likely oriented and those which are not. We have done this for each set of circular arena experiments, by demarcating a sector of the arena with its midpoint at the midpoint of the stimulus target. The sector width was chosen to be wider than the half-width of widest stimulus target in each experiment. The sector width is 90° for Paper I, 60° for paper II and 72° for the subsequent papers. Headings which fall within the sector are considered successful orientation and those which don't are not. This introduces an arbitrary distinction between points at either side of the sector edge but it avoids making the precise headings of disoriented animals informative (e.g. whether an animal heads 90° or 180° away from the stimulus target) as would occur with circularly distributed data. As the alarm response experiment in Paper II already yields a binary outcome, all of the behavioural experiments were treated of as binary response data, which ought to arise from a binomial distribution.

An alternative to null hypothesis testing is to model the parameters which generated the data using a likelihood model. One solution to the challenges of avoiding pseudoreplication without sacrificing too much statistical power is the use of linear mixed models. In papers I and II we have used maximum likelihood estimation methods to estimate the parameters which resulted in the distribution of data. Moreover, as opposed to preceding methods which take account of repeated measures of individuals or groups, linear mixed models can take into account all sources of variation in a single model simultaneously (Sorensen and Vasishth, 2015). A varying effect for the variation of individuals is included for the statistical models in all of the papers.

Applying an absolute threshold model of signal detection allows us to define a critical value of stimulus, below which a salient visual cue is no longer recognised. When estimating signal detection from behaviour, statistical modelling can facilitate this by characterising the evidence and consequent uncertainty of your estimate. Modelling the relationship between the response of an animal to a stimulus (in this instance, via increased concentration towards the stimulus target) with respect to a change in stimulus intensity (here, via a change in stimulus arc angle) can be investigated using the psychometric function $\psi(x)$ (Fig. 5). This important psychophysical tool is an implementation of the generalized linear model, which can be modelled according to a variety of distributions depending on the relationship and the outcome data (e.g. a Bernoulli or binomial distribution applied to binary outcome data or a beta distribution for proportion data bounded between 0 and 1). In Papers I and II, logistic regression models are implemented using maximum likelihood estimation.

In the case of proportions, the psychometric function can be formulated as:

$$\psi(x; \alpha, \beta, \gamma, \lambda) = \gamma + (1 - \gamma - \lambda) \cdot F(x; \alpha, \beta) \quad (4)$$

where γ is the base rate (the rate at which animals will respond when no stimulus has been provided), λ is the lapse rate (the maximal rate at which an animal will respond to any relevant value of the stimulus) and $F(x;\alpha,\beta)$ is a function bounded between 0 and 1, which describes the performance of the animal, relative to stimulus intensity and is typically a sigmoid (Wichmann and Hill, 2001).

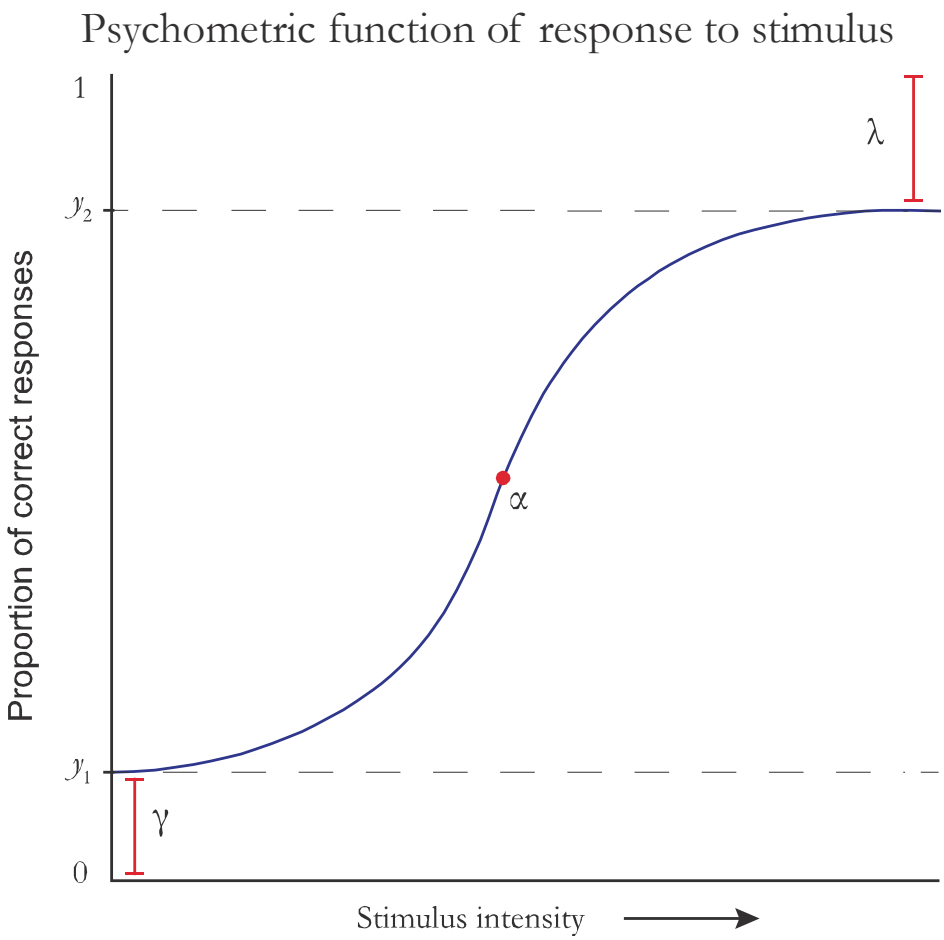


Fig. 5: The psychometric function
The psychometric function to identify the stimulus intensity required to instigate a behavioural response.

A slope and intercept are estimated for this curve, from which a detection threshold can be defined in a number of ways. A useful measure is the inflection point, as it is not dependent on an arbitrary interval and it is appropriate when the lapse rate and base rate are known (or can be estimated). Ideally, as

mentioned above, a robust behavioural response would be assayed but this is not always possible for species and taxon.

Bayesian inference

The lapse and base rates can be found from the upper and lower asymptotes of the resultant response curve respectively. These values can be difficult to estimate with likelihood models. Moreover, likelihood models can fail to converge correctly when multiple effects are included and can suffer from issues such as separation in logistic regression models. Therefore, in Papers III and IV, we used logistic regression models using Bayesian modelling. Bayesian inference methods are highly flexible and allow for the hierarchical modelling of data, with respect to many predictors. Mixed effects models can provide more accurate predictions than other available methods for future observations of data (Gelman, 2006). Bayesian inference is a form of statistical inference based on an application of Bayes theorem, which uses probabilistic modelling to incorporate prior knowledge as well as data. Using probabilistic modelling, the probability of many different hypotheses are assessed in the context of the data.

Generally speaking, Markov chain Monte Carlo (MCMC) is used to carry out Bayesian updating. Using these methods, Bayes theorem is typically formulated as follows:

$$p(\theta|y) \propto p(\theta) \cdot p(y|\theta) \tag{5}$$

where y is the outcome, θ is the model parameter (which results in the data), $p(\theta|y)$ is the posterior probability, $p(\theta)$ is the prior probability and $p(y|\theta)$ is the likelihood. The posterior probability is the distribution of probability of the hypothesis given the outcome (the data). The prior probability is the distribution of probability estimates of the hypothesis before the data are observed. The likelihood is the estimated probability of observing the data, given the model, which is derived using maximum likelihood estimation.

MCMC is computationally intensive and, therefore, it was not until recently that increased computing power has made Bayesian modelling feasible for widespread use. The Stan language (Carpenter et al., 2016) facilitate Bayesian probabilistic modelling using Hamiltonian Monte Carlo, which is much more effective in sampling the parameter space than the methods which preceded it. Stan is computationally fast, extremely flexible and can effectively estimate models with many parameters. The inclusion of prior knowledge is the key advantage but also the most problematic aspect of Bayesian inference.

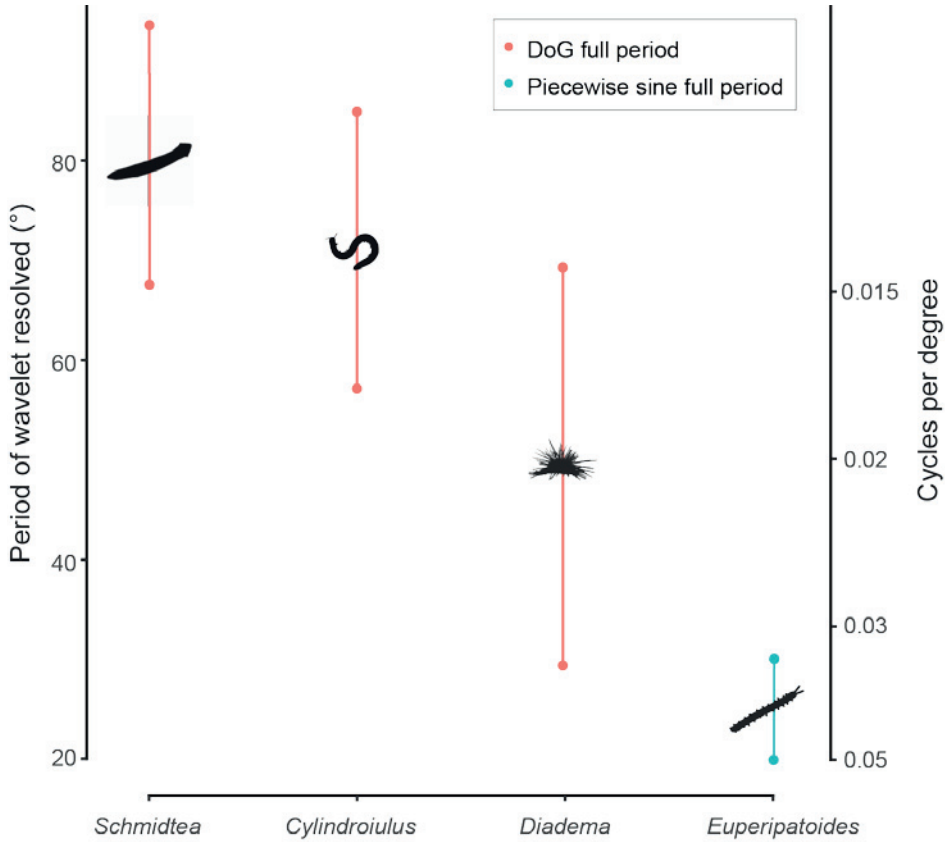


Fig. 6: Summary of signal resolution experiments
Ranges of resolution identified in the behavoural experiments in this thesis.

The prior, represented as a probability distribution for a given quantity in the model, such as a parameter to be estimated, can be derived from previous or preliminary data, or from other evidence. Thoughtfully-applied, appropriate priors make for a more realistic model and can increase the efficiency of the analysis; they do so by ruling out unreasonable possibilities while not ruling out sensible possibilities. The fit of each model is then assessed by several independent measures to ensure that it has converged. Bayesian methods excel with regard to mixed effects models (also known as hierarchical linear or multilevel models), which can take account of random effects and help avoid pseudoreplication.

Morphology and visual ecology

Knowing the resolution of the eye for a particular signal (Fig. 6), we can infer which kind of receptor could theoretically correspond to this value. The resolving power of a photoreceptor is a consequence of its angular sensitivity function, which describes the profile of light entering the receptor aperture from different directions. Angular sensitivity typically has a Gaussian-like shape (Götz, 1964; Tunstall and Horridge, 1967) and it is described by its half-width, which is known as the acceptance angle ($\Delta\rho$).

In paper I, a method is described to derive the widest possible acceptance angle which could provide a given resolution, knowing the characteristics of the stimulus and its width. This is achieved by progressive Gaussian blurring of the stimulus to simulate the information transfer to the receptor with increasingly wide acceptance angle. This results in decreasing contrast remaining in the image of the stimulus. To determine the acceptance angle, a threshold contrast ought to be known (at this spatial resolution) as both spatial resolution and contrast sensitivity contribute to stimulus detection. Lacking contrast sensitivity measures we posited a plausible range of values of contrast sensitivity based on other species (outlined in paper I) and proposed a range of acceptance angle values from this (Fig. 7). The predicted acceptance angle from behavioural responses ought to be in the range of the theoretical limit found from morphological and optical measurements. Specifically, we would expect the behaviourally predicted value to be slightly wider than the theoretical limit, as there can be further information loss in the visual system (and imperfect motivation of the animals). It should usually not be much wider, however, as natural selection ought to act against the perpetuation of superfluously fine optics in a visual system which does not avail of them (given the inherent resource cost and trade-off with sensitivity and, sometimes, other performance measures). In paper I, the theorized $\Delta\rho$ is referred to as spatial resolution (in reference to the resolving power of the optical component of the visual system), whereas the resolving limit identified in the behavioural assays is termed the detection threshold. In the subsequent papers (and consistent with the nomenclature used in this introduction) spatial resolution is used specifically to refer to the estimate of the stimulus period that can be resolved from the behavioural detection assay.

The attributes thus far considered, acceptance angle and its consequent resolution, apply to the ability of a single theoretical receptor to detect narrow stimuli. However, to make use of this measurement, this luminance information must be compared to neighbouring regions of space. If a detectably different contrast is identified between the two regions, image formation is possible. Comparative measurements be achieved by scanning: changing the orientation of the receptor and sampling from different points, over a short span of time.

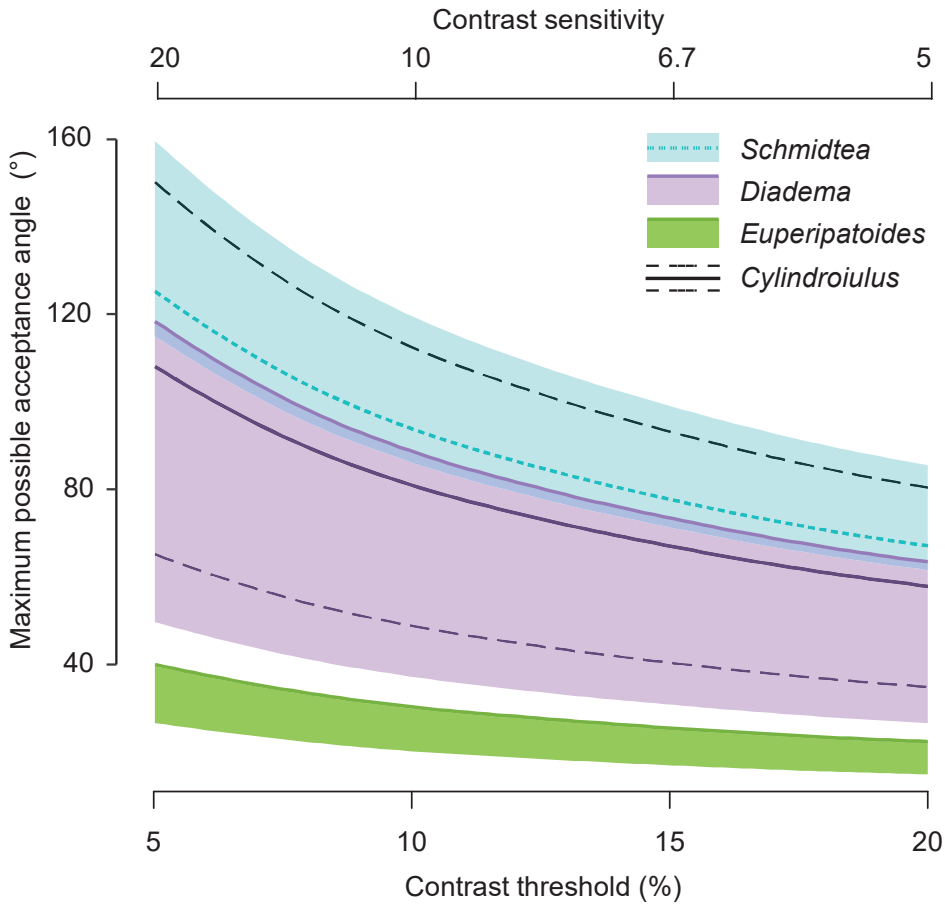


Fig. 7: Summary of angular sensitivity ranges for the four species investigated.
For each species, a spline is plotted, which represents the principle estimate of spatial resolution for this species. For each species a range is also plotted, represented by a shaded area or, in the case of the millipede *Cylindroiulus punctatus*, by the region between two dashed black lines. This range represents the possible range of $\Delta\rho$ given thebounds of the spatial resolution estimates for this species. Between the ranges of *Schmidtea* and *Diadema* there is a darkened region which represents the overlap of these ranges. For *Schmidtea* and *Diadema*, the principle estimate is the upper bound of the range. As detection is dependent on both resolving power and contrast sensitivity and as the contrast sensitivity is not known for these species, these ranges were estimated across an ecologically plausible range of contrast threshold values (5-20%). Contrast threshold is the reciprocal of contrast sensitivity expressed as a percentage.

Such a simple system, using only two eyespots as receptors, is exhibited by the trochophore larva of the nereid *Platynereis dumerilii* to maintain its swimming course (Jékely et al., 2008). This directional photoreception is not truly visual; a visual system does not rely solely on scanning but involves the simultaneous sampling and integration of light from multiple directions (Nilsson, 2013). Simultaneous sampling introduces another component to vision, the sampling density: the (angular) distance separating adjacent receptors, which is analogous to pixel density of a camera sensor. This can be determined from the interreceptor

angle: the angular difference between the apertures of neighbouring receptors. This interreceptor angle can be found from adjacent receptors within a lens eye (or paired eyes, each integrating as a simple receptor). An analogous value, the inter-ommatidial angle, can be found between the neighbouring receptors of a compound eye. According to the Nyquist theorem, to the detect a signal of a given frequency by sampling, a sampling frequency (ν_s), the reciprocal of sampling density, of twice the signal frequency must be present.

In each of the papers, we consider how the morphology of the eyes (or putative receptors in the case of paper II) relate to the behavioural estimates we have made. In addition, the role of the eye for the ecology of the animal is considered. Eyes are an expensive liability as are the neurons typically required to integrate and enact a response to the information they provide. Each of the species investigated in this thesis is predominantly night-active, which lends itself to sensitive vision that may be 'traded-off' against resolution. In addition, each of the species uses additional sensory modalities (chiefly, chemoreception and mechanosensation) to interact with the environment. Vision is, possibly, not the most crucial sensory modality for any of the species here studied, in many behavioural contexts. Nonetheless, in order for a given visual system to be of value for the animal's fitness, and thus not to be lost, it must provide valuable information for some set of circumstances, which other sensory modalities do not. Vision does this by providing incomparably fast and accurate directional information at all scales (Land and Nilsson, 2012).

Concluding remarks

It is indeed surprising that spatial resolution, one of the basic aspects of vision, has been so little explored in many important animal phyla. This thesis represents a survey of the basic performance attributes of visual systems which are relatively neglected but this, nonetheless, only touches the surface. A renewed interest within sensory biology for simple and ancient visual systems, combined with a wealth of new techniques (molecular and imaging) promise to make the coming years an exciting time for the field.

The investigations of spatial vision discussed here provide some fundamental estimates of the visual performance of eyes, which are little explored. Further research on a range of systems could greatly improve our understanding and enable a detailed comparative understanding of spatial vision. Additionally, this would allow us to marry methods from sensory biology, including behavioural assays and morphological and optical investigation of visual systems, to studies of development and the underlying molecular networks of vision more completely. This depends on the continued advancement of methods and adoption of new study systems. This could include the usage of new tools, such as the wavelet stimuli used in this thesis, as well as advanced imaging and ray tracing methods.

The analysis of experimental data using statistical models which express uncertainty, and in particular Bayesian inference, is increasingly becoming a feature of experimental analysis in biological research. It presents challenges, in that models must be carefully thought out and crafted for each analysis. However, it is facilitated by a growing armoury of tools, including computing packages which make it more convenient and applicable. In addition, the process of building models obliges the investigator to meditate on experimental design and how the data arise, which is not time wasted. There is, of course, no substitute for well-designed experiments and careful measurement and statistical analysis can only maximize the use of informative data.

Vision science is, at its core, a pluralistic field and, ultimately, it is this synthesis of many methods and different kinds of knowledge, which enriches our understanding of how visual systems function and how they emerged.

Our imagination is struck only by what is great;
but the lover of natural philosophy should reflect equally on little things

Alexander von Humboldt

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There is a crack, a crack in everything
That's how the light gets in

Leonard Cohen