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# Light-dependent magnetic compass orientation in amphibians and insects: candidate receptors and candidate molecular mechanisms

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Magnetic compass orientation by amphibians, and some insects, is mediated by a light-dependent magnetoreception mechanism. Cryptochrome photopigments, best known for their role in circadian rhythms, are proposed to mediate such responses. In this paper, we explore light-dependent properties of magnetic sensing at three levels: (i) behavioural (wavelength-dependent effects of light on magnetic compass orientation), (ii) physiological (photoreceptors/photopigment systems with properties suggesting a role in magnetoreception), and (iii) molecular (cryptochrome-based and non-cryptochrome-based signalling pathways that are compatible with behavioural responses). Our goal is to identify photoreceptors and signalling pathways that are likely to play a specialized role in magnetoreception in order to definitively answer the question of whether the effects of light on magnetic compass orientation are mediated by a light-dependent magnetoreception mechanism, or instead are due to input from a non-light-dependent (e.g. magnetite-based) magnetoreception mechanism that secondarily interacts with other light-dependent processes.

**Keywords:** magnetoreception; photoreception; cryptochrome; pineal; compound eye; magnetic compass

## 1. INTRODUCTION

The last 20 years have seen an explosion of new information about the functional properties and potential mechanisms underlying the magnetic sense in animals. Evidence points to magnetoreception mechanisms using at least two different biophysical mechanisms: (i) particles of biogenic magnetite or other iron-containing materials (magnetite-based mechanisms or MBMs) and (ii) light-induced biochemical reactions involving a specialized class of photopigments (cryptochromes) that form long-lived, spin-correlated radical pairs (RPs) (radical-pair mechanisms or RPMs).

Magnetic information is involved in at least two distinct aspects of spatial behaviour. (i) Some birds, reptiles, amphibians and crustaceans derive geographical positioning ('map') information from spatial variation in the intensity and/or inclination of the geomagnetic field (reviewed by Freake *et al.* 2006). Only MBMs are likely to exhibit the high level of sensitivity necessary

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One contribution to a Theme Supplement 'Magnetoreception'.

to detect spatial variation in the Earth's magnetic field (e.g. magnetic intensity varies only about 0.01% km<sup>-1</sup>, and inclination only about 0.01° km<sup>-1</sup>; Kobayashi & Kirschvink 1995; Freake *et al.* 2006). (ii) A far greater and more diverse range of animals has been shown to obtain directional ('compass') information from the geomagnetic field (Wiltschko & Wiltschko 1995). The functional properties of magnetic compass orientation in most animals studied to date fall into one of two groupings, consistent with either a light-dependent RPM or a non-light-dependent MBM (Schulten 1982; Ritz *et al.* 2000, 2002; Johnsen & Lohmann 2008; Hogben *et al.* 2009; Rodgers & Hore 2009; Winklhofer 2009; but see Jensen 2009). Effects of light on magnetic compass orientation have been observed in birds, amphibians and some insects (Phillips & Borland 1992*a,b*; Phillips & Sayeed 1993; Wiltschko *et al.* 1993; Deutschlander *et al.* 1999*a,b*; Vácha *et al.* 2008; Wiltschko *et al.* 2010). Amphibians and insects are the only two groups for which there is evidence of a direct effect of light on the underlying magnetoreception mechanism, and are the focus of this paper.

Cryptochromes, best known for their role in circadian rhythms (Emery *et al.* 1998; Partch & Sancar 2005), are the only animal photopigments shown to

produce persistent, spin-correlated RPs (Liedvogel *et al.* 2007; Biskup *et al.* 2009; Schleicher *et al.* 2009). Moreover, recent experiments with adult flies (*Drosophila melanogaster*) suggest that cryptochromes may be involved in UV/blue-light-dependent responses to magnetic stimuli (i.e. fields 6–10 times the intensity of the Earth's magnetic field; Gegeer *et al.* 2008; Yoshii *et al.* 2009). In this paper, we explore possible links between light-dependent properties of magnetic compass sensing at behavioural, physiological and molecular levels, with the goal of identifying photoreceptors and signalling pathways that play a specialized role in magnetoreception. Targeting specific receptors is necessary to definitively answer the question of whether the effects of light on magnetic compass orientation are mediated by a light-dependent magnetoreception mechanism (Ritz *et al.* 2000, 2009; Timmel *et al.* 2001; Cintolesi *et al.* 2003; Maeda *et al.* 2008; Rodgers & Hore 2009), or instead reflect input from a non-light-dependent (e.g. magnetite-based) magnetoreception mechanism that secondarily interacts with other light-dependent processes (Jensen 2009).

Magnetite and related ferromagnetic materials appear to be involved in non-compass responses in some vertebrates (e.g. Beason & Semm 1996; Walker *et al.* 1997; Fleissner *et al.* 2003, 2007; Mora *et al.* 2004; Zapka *et al.* 2009; Wiltshcko *et al.* 2010; see also Burda *et al.* 1990; Marhold *et al.* 1997). In contrast, candidate receptors that could mediate RPM-based magnetic compass responses have not been identified, although interest has focused on cryptochromes present in ganglion cells in the avian retina that could modulate primary visual responses originating from retinal photoreceptors (Möller *et al.* 2004; Mouritsen *et al.* 2004; Liedvogel *et al.* 2007). Rather than considering systems where magnetic field effects on higher order neurons might modulate visual responses originating from primary photoreceptors, our goal is (i) to locate/identify specific photoreceptors that are likely to have magnetoreception as their primary function and (ii) to suggest specific photopigment systems and/or photochemical reactions that are involved in sensing the magnetic field, or in providing a non-magnetically sensitive reference to facilitate detection of the magnetic field's effect on another (i.e. cryptochrome-based) photosystem.

## 2. BEHAVIOURAL PROPERTIES OF LIGHT-DEPENDENT MAGNETIC COMPASS ORIENTATION

Light-dependent magnetic compass orientation has been demonstrated in flies and beetles, anuran and urodele amphibians and in passerine and non-passerine birds. Of these, the most detailed information comes from nocturnally migrating birds, which show dramatic changes in orientation behaviour depending on both the wavelength and intensity of light (Muheim *et al.* 2002; Wiltshcko & Wiltshcko 2005; Wiltshcko *et al.* 2010). Although there is a strong case for the involvement of an RPM in the avian magnetic compass (Ritz *et al.* 2004; Henbest *et al.* 2008; Hogben *et al.* 2009; Rodgers &

Hore 2009), the nature of the light effects on magnetic behaviour of birds and which, if any, of these effects reflect properties of the magnetic compass remain to be determined (Johnsen *et al.* 2007; Johnsen & Lohmann 2008; Jensen 2009). In contrast, although amphibians have been tested under a more limited range of lighting conditions, the directional response of the magnetic compass has been shown to be light-dependent (Phillips & Borland 1992*a,b*; Deutschlander *et al.* 1999*a,b*; Phillips *et al.* 2001).

### 2.1. Light-dependent magnetic compass orientation of amphibians

In newts, the magnetic compass provides directional information under both diurnal and dim nocturnal lighting conditions, but not in total darkness (Phillips & Borland 1992*a*; Diego-Rasilla *et al.* 2005, 2008). Laboratory studies of the Eastern red-spotted newt (ERSN) have provided evidence for a light-dependent magnetoreception mechanism that receives antagonistic short-wavelength ( $\lambda \leq 450$  nm) and long-wavelength ( $\lambda \geq 500$  nm) inputs, mediated by extraocular photoreceptor(s) in the pineal organ (table 1 and figure 1; Phillips & Borland 1992*b*; Deutschlander *et al.* 1999*a,b*; Phillips *et al.* 2001). ERSNs are able to orient under both short- and long-wavelength light. However, the directional response of the magnetic compass under these two lighting conditions differs by 90° (figure 1*a–d*; Phillips & Borland 1992*a*), suggesting that both spectral inputs are sensitive to the magnetic field (figure 1*e–g*). The orientation of ERSNs tested under white (UV and visible) light is indistinguishable from that of newts tested under short-wavelength light, indicating that the short-wavelength ('blue') input is preferentially excited under full-spectrum light and, therefore, is appreciably more sensitive than the long-wavelength input. A similar wavelength-dependent 90° shift in magnetic compass orientation has been shown in larval frogs (Freake & Phillips 2005). Consistent with antagonistic magnetic inputs causing the 90° shift, the magnetic compass response of ERSNs is eliminated under intermediate wavelengths of light (approx. 475 nm) that should excite the two spectral mechanisms equally and cause the antagonistic inputs to cancel out (Phillips & Borland 1992*b*).

### 2.2. Light-dependent magnetic compass orientation of insects

Adult flies and beetles also exhibit a light-dependent 90° shift in magnetic compass orientation (table 2). Recent experiments with larval *Drosophila* provide a detailed picture of the response of the light-dependent magnetic compass, confirming the presence of antagonistic short-wavelength and long-wavelength inputs that produce complementary patterns of response (Dommer *et al.* 2008; D. H. Dommer 2008, unpublished data). Owing to the limited number of light stimuli used in the fly and beetle experiments, the findings provide relatively little information about the spectral properties of the underlying magnetoreception system. The two wavelengths used in the fly experiments were

Table 1. Light-dependent magnetic compass orientation in ERSN, *Nothophthalmus viridescens*, tested for shoreward orientation under monochromatic light. Included are studies where newts were trained for 1 or 5 days under natural light conditions to the magnetic compass orientation of an artificial shore and then tested indoors under a discrete wavelength of light. Given are the peak wavelength, half bandwidth and irradiance of the testing light, and the orientation response of the animals relative to the trained magnetic direction.

peak wavelength (nm)	half bandwidth (nm)	total photon irradiance (photons s <sup>-1</sup> m <sup>-2</sup> )	orientation relative to trained magnetic direction	references
5 day training				
400	40	2.2E + 16	179°/359 <sup>oa</sup>	Phillips & Borland (1992a)
450	40	4.5E + 16	7°	Phillips & Borland (1992a)
475	40	4.5E + 16	n.s.	Phillips & Borland (1992a)
500	40	4.5E + 16	253°	Phillips & Borland (1992a)
550	40	4.5E + 16	268°	Phillips & Borland (1992a)
600	40	4.5E + 16	241°	Phillips & Borland (1992a)
1 day training				
450	40	3.2E + 16	12°/192 <sup>oa</sup>	Deutschlander <i>et al.</i> (1999a)
550	40	3.2E + 16	97°/277 <sup>oa</sup>	Deutschlander <i>et al.</i> (1999a)

<sup>a</sup>Controls tested under full-spectrum (UV and visible) light also oriented bimodally along the trained axis in these experiments.

selected because they coincide with the peak sensitivities of the antenna pigments ( $\lambda_{\max}$  360–370 nm) and associated photopigments ( $\lambda_{\max}$  430–520 nm) found in several classes of photoreceptors in the compound eye (Kirschfeld *et al.* 1978, 1983, 1988; Hardie & Kirschfeld 1983), two of which exhibit unusual properties, suggesting a possible role in magnetoreception (§3). The effects of light on magnetic compass orientation in both *Drosophila* and *Tenebrio* are also compatible with a cryptochrome-based mechanism (§4).

### 3. CANDIDATE PHOTORECEPTION MECHANISMS

The RPM proposes that an Earth-strength magnetic field can influence photoreduction of the fully oxidized flavin chromophore of cryptochrome to the partially reduced, radical form (Ritz *et al.* 2000; Cintolesi *et al.* 2003; Rodgers & Hore 2009; and see §4). However, behavioural studies of light-dependent magnetic compass orientation in insects and amphibians point to the involvement of not one, but two, magnetically sensitive processes (see earlier discussion). One possibility is that both of these light-dependent processes are mediated by a single cryptochrome-based photosystem in which the magnetic field affects both (i) short-wavelength light-dependent photoreduction of the fully oxidized flavin chromophore to the partially reduced, radical form ( $\text{FAD}_{\text{ox}} \rightarrow \text{FAD}\cdot^-$  or  $\text{FADH}\cdot$ ) and (ii) long-wavelength light-dependent photo-oxidation of the flavin radical back to the fully oxidized form ( $\text{FAD}\cdot^-$  or  $\text{FADH}\cdot \rightarrow \text{FAD}_{\text{ox}}$ ). Under constant illumination, these opposing reactions, as well as the further photoreduction of the radical to the fully reduced form ( $\text{FADH}^-$ ) and dark reoxidation back to the fully oxidized form ( $\text{FAD}_{\text{ox}}$ ), should produce a photo-equilibrium among the three redox forms of the flavin chromophore (Hoang *et al.* 2008; Shirdel *et al.* 2008; Zikihara *et al.* 2008; Balland *et al.* 2009) that determines the ratio of the signalling (either radical or fully reduced; see below) and non-signalling forms of

the cryptochrome molecule. In an ordered array of cryptochrome molecules, the effect of the magnetic field on the persistence of the signalling state and, thus, on the response to light could provide a source of compass information, as long as the magnetic field's effect on the RP system can be distinguished from changes caused by variation in the intensity and spectral properties of incident light. Ideally, this would require a non-magnetically sensitive reference exhibiting the same spectral/intensity dependence as the cryptochrome photosystem. Such a reference may or may not involve another cryptochrome-based photosystem, but in either case should mirror the light-dependent properties of the magnetically sensitive photosystem and, therefore, the light-dependent magnetic compass response.

Unlike photoreceptors involved in vision, which rapidly adapt to changing lighting conditions, the proposed reference system is likely to involve (i) a chromatic response mediated by photo-interconverting forms of the same photopigment molecule (avoiding nonlinearities likely to result from synaptic interactions between different classes of photoreceptors) and (ii) little or no membrane-level adaptation. Photoreceptors exhibiting these properties are found in the pineal complex of fish, amphibians and some reptiles (Eldred & Nolte 1978; Solessio & Engbretson 1993; Koyanagi *et al.* 2004). It is worth noting, however, that the same properties would make these photoreceptors well suited to mediate a spectral gradient compass like that proposed by Jensen (2009).

#### 3.1. Candidate photo(magneto)receptors in the pineal complex

Extraocular photoreceptors in the pineal complex of frogs, salamanders and lizards have been shown to mediate celestial compass orientation (i.e. Sun and polarized light orientation; Adler & Taylor 1973; Taylor & Auburn 1978; Adler & Phillips 1985; Freake 1999; Foa *et al.* 2009), suggesting that the involvement

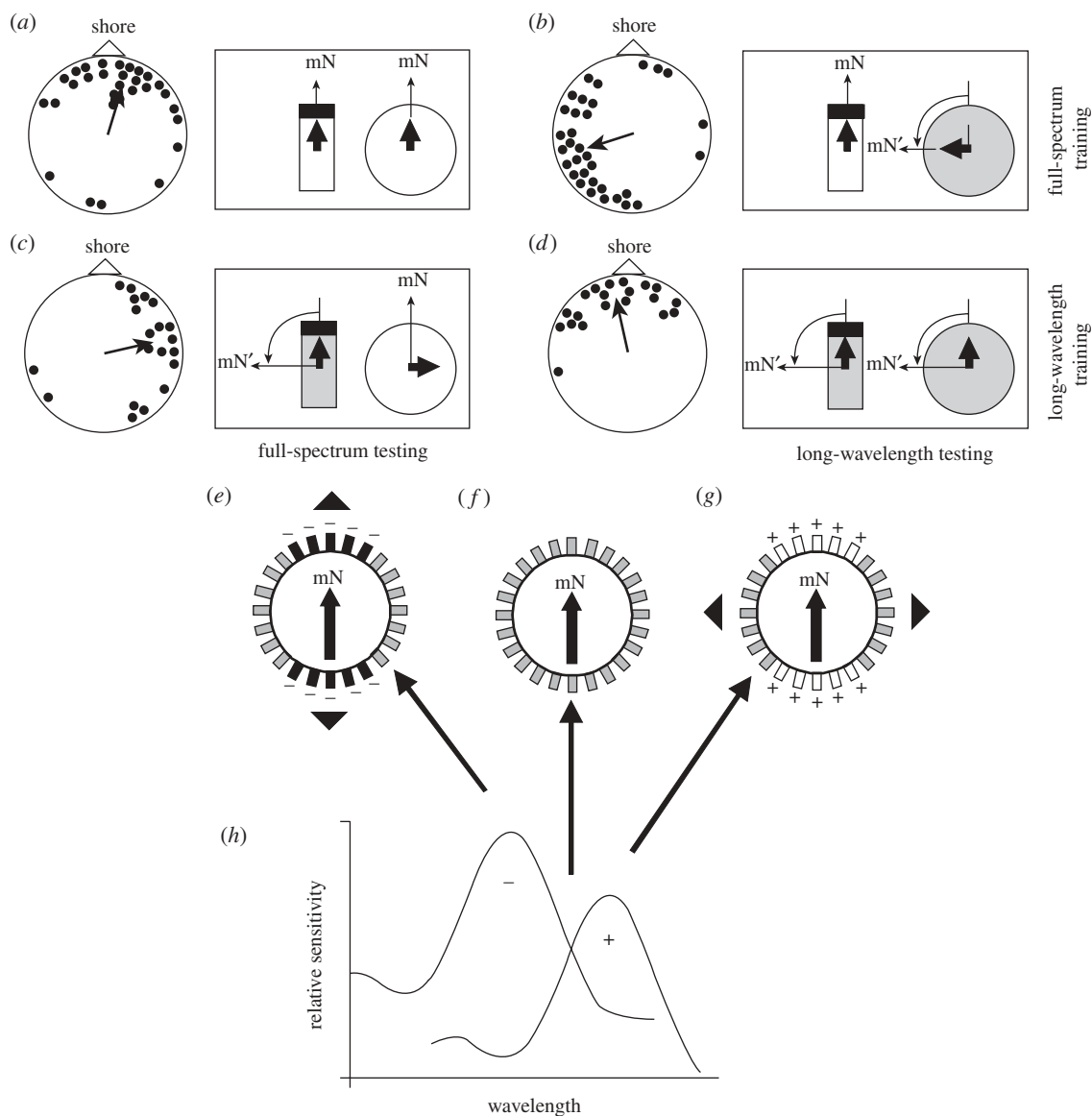


Figure 1. (*a–d*) Evidence for a light-dependent magnetic compass (Phillips & Borland 1992*a*). Orientation of newts (*a*) trained and tested under full-spectrum light, (*b*) trained under full-spectrum light and tested under wavelengths greater than 500 nm, (*c*) trained under wavelengths greater than 500 nm and tested under full-spectrum light, and (*d*) trained and tested under wavelengths greater than 500 nm. Data are the magnetic bearings of individual newts tested in one of four magnetic field alignments (magnetic north at geographical north, east, south or west). Bearings are plotted relative to the magnetic direction of shore in the outdoor training tanks ( $=360^\circ$ ); data from two different training tank alignments are combined. Arrows at centres of the circular distributions indicate the mean vector bearings, with the length of the arrows proportional to the vector lengths ' $r$ ' (radius of the circle corresponds to  $r = 1$ ). For each condition, a schematic of a training tank (rectangle with artificial shore indicated in black) and test arena shows predicted effects of exposure to full-spectrum or long-wavelength (grey shading) light. Thin arrows indicate the true (mN) or  $90^\circ$ -rotated (mN') alignments of magnetic north. Wide arrows indicate expected (i.e. shoreward) direction if newts perceive magnetic north to be rotated  $90^\circ$  counterclockwise under long-wavelength light. (*e–h*) Antagonistic spectral inputs and patterns of response proposed to mediate the light-dependent magnetic compass in a hypothetical magnetoreception system consisting of a circular array of axially sensitive receptors (small rectangles). Under (*e*) short-wavelength and (*g*) long-wavelength light, receptors in specific alignments relative to the magnetic axis show an increase (white rectangles labelled 'plus') or decrease (dark rectangles labelled 'minus') in response to light, relative to receptors in alignments unaffected by the magnetic field (grey rectangles). Arrowheads at the edge of the circular array indicate the axes with the lowest level of response, which differ by  $90^\circ$  in (*e*) and (*g*). (*f*) The directional response is eliminated under intermediate wavelengths of light that activate the two mechanisms equally. (*h*) Hypothetical antagonistic inputs to the light-dependent magnetic compass showing higher sensitivity of the short-wavelength ('blue-sensitive') mechanism inferred from the similar responses of newts tested under full-spectrum (white) and short-wavelength ( $\leq 450$  nm) light.

of the pineal organ in light-dependent compass orientation is the ancestral condition in terrestrial vertebrates. Furthermore, evidence that photoreceptors in the pineal complex of both amphibians (Adler &

Taylor 1973; Taylor & Auburn 1978) and lizards (Freake 1999) are sensitive to the plane of polarized light indicates that at least one class of photoreceptors contains an ordered array of light-absorbing molecules

Table 2. Light-dependent magnetic compass orientation of insects trained to the magnetic orientation of a UV light source and tested under monochromatic light. For explanation, see table 1.

peak wavelength (nm)	half bandwidth (nm)	total irradiance (photons s <sup>-1</sup> m <sup>-2</sup> )	orientation relative to trained magnetic direction	species	references
365	12.0	1.3E + 14	7°	<i>Drosophila</i> larvae	Dommer <i>et al.</i> (2008)
365	60.0	2.0E + 15	0°	<i>Drosophila</i> adult	Phillips & Sayeed (1993)
390	14.0	1.5E + 15	2°	mealworm beetle	Vácha <i>et al.</i> (2008 <i>b</i> )
500	31.0	1.8E + 15	86°	mealworm beetle	Vácha <i>et al.</i> (2008 <i>b</i> )
500	40.0	2.0E + 15	92°	<i>Drosophila</i> adult	Phillips & Sayeed (1993)

(Wehner 1989), a requirement of the RPM (Rodgers & Hore 2009). Behavioural studies of ERSNs, using small spectral filters attached to the top of the head, have implicated the pineal organ as the site of the light-dependent magnetic compass (Deutschlander *et al.* 1999*a*; Phillips *et al.* 2001). The avian pineal organ is also magnetically sensitive (Demaine & Semm 1985), although it does appear not to be the site of the magnetic compass (Schneider *et al.* 1994).

**3.1.1. Frog frontal organ.** In frogs, the pineal complex consists of the intracranial pineal organ and the frontal organ, homologous to the parapineal lobe of the salamander pineal organ, that grows up through the skull to a location beneath a small patch of the unpigmented skin on top of the head (Adler 1976). The frontal organ contains at least three classes of photoreceptors (Dodt & Heerd 1962; Eldred & Nolte 1978; Vigh-Teichmann & Vigh 1990). The spectral properties of the magnetic compass response in amphibians (Phillips & Borland 1992*b*; Deutschlander *et al.* 1999*a*; Freake & Phillips 2005) closely resemble those of one class of responses in the frontal organ nerve that exhibits chromatic sensitivity. These chromatic responses receive excitatory input from a green-sensitive mechanism (maximal spectral sensitivity at approx. 515 nm) and inhibitory input from a UV-sensitive mechanism (maximal spectral sensitivity at approx. 370 nm). Inputs to the chromatic response appear to be photo-interconvertible forms of the same photopigment molecule (Eldred & Nolte 1978; Koyanagi *et al.* 2004). UV input to the chromatic mechanism is substantially more sensitive (approx. 2 log units) than the green input, and the two inputs are equally sensitive at around 475 nm (Dodt & Heerd 1962), closely matching the antagonistic spectral inputs proposed to mediate the light-dependent magnetic compass in newts (figure 1*e–h*; Phillips *et al.* 2001). The spectral properties of the chromatic response suggest that the underlying photoreception mechanism(s) could be directly involved in magnetoreception, or could provide a non-magnetically sensitive spectral reference for another magnetically sensitive (e.g. cryptochrome-based) photosystem. Consistent with the involvement of frontal organ photoreceptors in magnetoreception, behavioural experiments with ERSNs have implicated the pineal organ as the site of the light-dependent magnetic compass (Deutschlander *et al.* 1999*a*; Phillips *et al.* 2001), and

neurophysiological recordings from the front organ nerve of bullfrogs have provided evidence for the presence of at least one class of magnetically sensitive photoreceptors (J. B. Phillips & S. C. Borland 1998, unpublished data). Further work is needed to replicate these findings, and to characterize the distribution of cryptochromes in different classes of frontal organ photoreceptors.

Interestingly, photo-interconvertible UV-sensitive and green-sensitive forms of the rhodopsin-based photopigment parapinopsin, with spectral properties matching those of the frog frontal organ chromatic response, have been recently identified in the pineal organ of the lamprey, *Lethenteron japonica* (Koyanagi *et al.* 2004). The photo-interconverting forms of parapinopsin produce a photo-equilibrium that depends on the relative UV versus green content of incident light. Thus, lamprey parapinopsin, and homologues isolated from fish and frog pineal (Koyanagi *et al.* 2004), could provide a ‘dynamic reference’ for a cryptochrome-based magnetoreception system (see below), and/or provide the primary input for a spectral gradient compass (Jensen 2009).

### 3.2. Evidence for magnetic sensitivity in photoreceptors in the compound eye of flies

In the compound eye of flies, most ommatidia contain seven rhabdomeres, made up of tightly packed, photopigment-containing microvilli, each of which functions as a separate optic waveguide. Six rhabdomeres located around the periphery of the ommatidia, the retinula 1–6 cells (R1–6; figure 2*a,c*), exhibit roughly equal peaks of sensitivity in the UV ( $\lambda_{\max} \sim 370$  nm) and blue–green ( $\lambda_{\max} \sim 490$  nm) regions of the spectrum (figure 2*d*; Kirschfeld *et al.* 1983). The blue–green peak is due to absorption by a photopigment (xanthopsin), while the UV peak is due to absorption by a light-harvesting ‘antenna’ pigment (tentatively identified as a carotenoid, 3-hydroxy retinol; Vogt & Kirschfeld 1983, 1984), which transfers excitation energy to the photopigment (Kirschfeld *et al.* 1983). The seventh (centrally located) rhabdomere in each ommatidia is made up of microvilli from the retinula 7 (distal) and retinula 8 (proximal) cells (R7 and R8; figure 2*b,c*). There are two types of R7 and two types of R8, each with distinct spectral sensitivities (figure 2*e* and see below).

In the blowfly *Calliphora vicina*, intracellular recordings have provided evidence for a weak effect of a near-Earth-strength magnetic field on the response

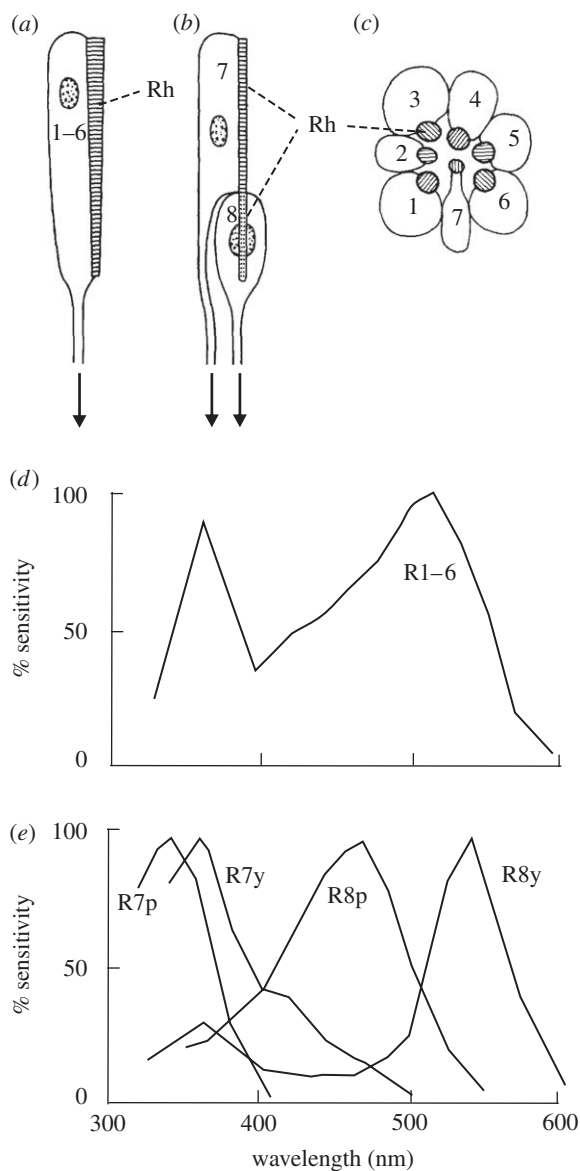


Figure 2. Morphology and spectral sensitivity of photoreceptors in the compound eye of the blowfly *C. vicina*. (a–c) Schematic diagrams showing side views of (a) R1–6 and (b) R7 and R8, and (c) a cross section through an ommatidia showing the relative positions of the rhabdomeres (adapted from Kirschfeld *et al.* 1978). Parallel striping indicates the alignments of microvilli. Microvilli in R7 are perpendicular to those of the underlying R8. Spectral sensitivities of (d) R1–6 and (e) R7p, R7y, R8p and R8y (adapted from Hardie & Kirschfeld 1983).

of R1–6 to light (Phillips 1987; see the electronic supplementary material). In a small number of R1–6 cells that yielded stable long-term recordings, the effects of magnetic field alignment on the response to 20 ms flashes of 370 and 490 nm light (adjusted in intensity to produce similar amplitudes of response) were compared in successive 360° sweeps. Figure 3 shows the data from one such cell. Magnetic field alignments associated with low-amplitude responses to both wavelengths were bimodally distributed (figure 3*a,b*;  $p < 0.05$ , Rayleigh test on doubled angles; Batschelet 1981), but the mean axes differed by approximately 90° and the two distributions were significantly

different ( $p < 0.001$ , Watson  $U^2$  test on doubled angles). The difference in the magnetic field's effect on the responses to 370 and 490 nm light is reminiscent of the wavelength-dependent 90° shift in magnetic compass orientation exhibited by adult *Drosophila* (Phillips & Sayeed 1993) and ERSNs (figure 1; Phillips & Borland 1992*a*), and raises the possibility that the magnetic field may have antagonistic effects on photo-signalling pathways resulting from absorption of light by the photopigment ( $\lambda_{\max} \sim 490$  nm) and antenna pigment ( $\lambda_{\max} \sim 370$  nm) in R1–6, or from absorption by other light-absorbing molecule(s) with similar spectral properties. Most importantly, these findings demonstrate the value of targeting specific classes of photoreceptors with intracellular recording techniques to directly test for magnetic field sensitivity.

Despite preliminary evidence for magnetic field sensitivity in the R1–6 cells (figure 3; electronic supplementary material, figure S1; Phillips 1987), it is unlikely that these cells play a role in magnetoreception. This is because of rhabdomeric twisting and neural superposition of the outputs of six R1–6 cells with different microvillar alignments onto the same second-order cell (Hardie 1986), both of which may reduce, but not necessarily eliminate, magnetic sensitivity, e.g. magnetic field sensitivity could be retained in a population of RPs that are non-randomly aligned along the long axis of the R1–6 rhabdomeres (see the electronic supplementary material from Rodgers & Hore 2009). In addition, under natural broad-band illumination, the similar sensitivities of the antenna pigment and photopigment in R1–6 would cause antagonistic effects of the magnetic field like those shown in figure 3 to at least partially cancel out.

### 3.3. Retinula 7y and 8y: photoreceptors specialized for detection of the geomagnetic field?

Like R1–6, the retinula 7y and 8y cells (R7y and R8y) in flies like *Musca* and *Calliphora* contain a UV-absorbing antenna pigment that transfers energy to a photopigment with peak sensitivity in the visible region of the spectrum (Kirschfeld *et al.* 1983, 1988). In both R7y and R8y, however, wavelengths of light preferentially absorbed by one of the two pigments are selectively screened (i.e. the photopigment in R7y, and the sensitizing pigment in R8y; McIntyre & Kirschfeld 1981; Kirschfeld *et al.* 1988). If either of these photoreceptors exhibit magnetic field sensitivity similar to the R1–6 recording shown in figure 3, reduction of the sensitivity of one of the two pigments would prevent balanced excitation of these two inputs under naturally occurring broad-band light (figure 1*e–h*; Phillips & Borland 1992*b*), making the photoreceptor(s) well suited to function in magnetoreception.

The unusual properties of the R7y and R8y cells are difficult to explain in terms of a traditional role in vision. Although the R7y cells contain both a UV-sensitive antenna pigment ( $\lambda_{\max} \sim 370$  nm) and a blue-sensitive photopigment ( $\lambda_{\max} \sim 3430$  nm), the action spectrum has a single peak of spectral sensitivity

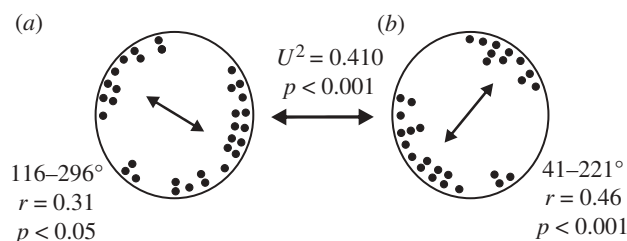


Figure 3. Magnetic field alignments associated with low-amplitude responses to 20 ms flashes of (a) 370 nm and (b) 490 nm light recorded intracellularly from a blowfly R1–6 cell. Individual bearings (black dots) are horizontal alignments of a 100  $\mu$ T magnetic field in which the responses to the flashes of light were at least 2% lower in amplitude than either of the two adjacent control responses (i.e. responses to identical flashes of light measured with the magnetic field cancelled). Intensities of the two wavelengths were adjusted initially to produce similar amplitudes of responses. Responses to five flashes of light were averaged in each of 32 equally spaced alignments of the magnetic field and in each of the control conditions; control responses were obtained after every two field alignments. Data in (a) and (b) are from a total of six horizontal ‘sweeps’ of the magnetic field, alternated between the two wavelengths (three sweeps each). See the electronic supplementary material for details.

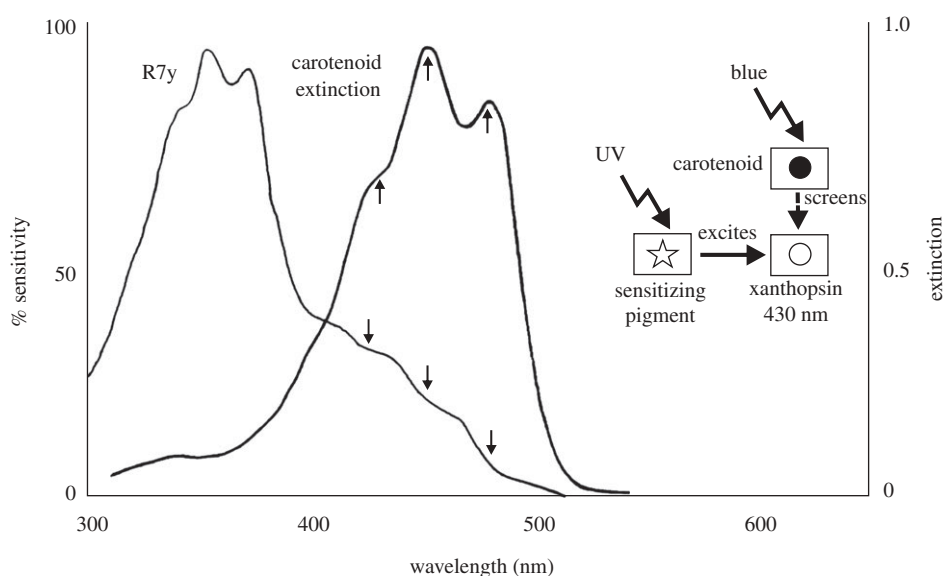


Figure 4. Photopigment system of the retina 7y cell of the blowfly (adapted from Hardie 1986). Curve at the left shows spectral sensitivity of R7y. Curve labelled ‘carotenoid extinction’ is the absorption spectrum of the optically dense, blue-absorbing screening pigment in R7y. Inset shows that energy resulting from the absorption of UV light by the sensitizing (antenna) pigment excites the photopigment xanthopsin, while blue light is absorbed by the carotenoid, which screens the photopigment from direct absorption of light (Hardie & Kirschfeld 1983; Kirschfeld *et al.* 1988).

in the UV corresponding to the absorption maximum of the antenna pigment (figures 2*e* and 4). Wavelengths of light to which the R7y photopigment are maximally sensitive are blocked by an optically dense, blue-absorbing screening pigment (figure 4; Kirschfeld *et al.* 1978; McIntyre & Kirschfeld 1981; Hardie & Kirschfeld 1983). Consequently, activation of the photopigment in R7y is primarily due to energy transfer from the antenna pigment, rather than to direct absorption of light. This unusual pigment system is not required to produce a UV-sensitive photoreceptor, since another class of photoreceptor in the compound eye (R7p) contains a UV-absorbing photopigment (figure 2*e*; Hardie & Kirschfeld 1983; Kirschfeld *et al.* 1983).

Like R1–6 and R7y, R8y also contains a UV-absorbing antenna pigment that transfers light energy to a photopigment that has a peak of sensitivity in the visible range ( $\lambda_{\max} \sim 530$  nm). Owing to the

attenuation of UV and blue light by the overlying R7y cells, however, the spectral sensitivity of the R8y is dominated by a single long-wavelength peak of sensitivity ( $\lambda_{\max} \sim 550$  nm) corresponding to the long-wavelength tail of the photopigment absorption curve (figure 2*e*). R8y has been proposed to provide a long-wavelength (‘green’) input to a tri-chromatic colour-processing system in flies (Briscoe & Chittka 2001). However, this does not explain the presence of the antenna pigment in R8y, since enhanced UV sensitivity (with or without screening by the overlying tissue) would decrease hue discrimination ability.

In contrast to primary roles in vision, if R7y and R8y mediate a light-dependent magnetic compass, selective screening of wavelengths of light that activate one of the two photo-signalling pathways may be necessary for the light-dependent magnetic compass to operate under natural (i.e. broad band) lighting conditions. R8y appears to have the same antenna pigment as

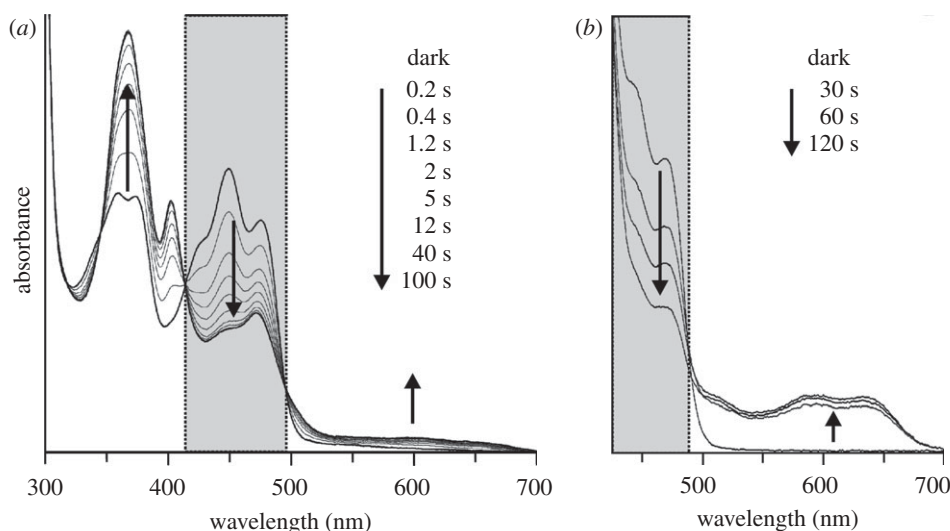


Figure 5. Blue-light-induced absorption changes in (a) insect (*Drosophila* DmCRY1) and (b) vertebrate (zebrafish ZfCRY-DASH) cryptochromes resulting from conversion of the flavin chromophore to the radical form. (a) Change in absorbance of DmCRY1 caused by progressively longer periods of exposure to 445 nm light ( $4.3 \times 10^{16}$  photons  $\text{cm}^{-2} \text{s}^{-1}$ ) showing conversion from the fully reduced form ( $\text{FAD}_{\text{ox}}$ ) to the partially reduced, anionic radical form ( $\text{FAD}\cdot^-$ ); exposure times given in seconds (adapted from Berndt *et al.* 2007). (b) Change in absorbance of ZfCRY-DASH caused by progressively longer exposure to 460 nm light ( $1.5 \times 10^{16}$  photons  $\text{cm}^{-2} \text{s}^{-1}$ ; adapted from Zikihara *et al.* 2008). UV sensitivity in (b) (data not shown) may be enhanced *in vivo* by a light-harvesting chromophore (methenyltetrahydrofolate or MTHF) that transfers energy to the flavin chromophore. (a,b) Regions of the spectrum where there is preferential excitation of the fully reduced form (grey shaded) and partially reduced radical form (unshaded) are indicated. Vertical dotted lines show (a) isosbestic points or (b) equivalent transition point for the fully oxidized and partially reduced forms. Vertical arrows indicate changes in absorbance that occur with increased duration of blue-light exposure.

R1–6 (Kirschfeld *et al.* 1983) in which we have obtained evidence for magnetic field sensitivity (figure 3; Phillips 1987); see the electronic supplementary material. It is also intriguing to note that the absorbance spectra of the R7y screening pigment (which also screens the underlying R8y) is virtually identical to that of the insect type 1 cryptochromes (compare figures 4 and 5a), suggesting that further consideration should be given to the possibility of a magnetic field effect on spectral properties of the R7y screening pigment (see the electronic supplementary material). Unfortunately, there is no information on the distribution of cryptochromes in the compound eyes of *Calliphora* and *Musca*. The presence of cryptochrome has been demonstrated in photoreceptor cells in the *Drosophila* compound eye, including R7 and R8 (Yoshii *et al.* 2008), but direct measurements of the spectral sensitivity of R7 and R8 cells in *Drosophila* are not yet available.

Whether or not a cryptochrome-based photo-signalling is responsible for magnetic field sensitivity in fly photoreceptors (§4), it is noteworthy that, within the central rhabdomere, the microvilli of R7y (dominated by the antenna pigment absorption) and those of the R8y (dominated by the photopigment absorption) are aligned perpendicularly. If excitation pathways associated with the UV-absorbing antenna pigment in R7y and the green-absorbing photopigment in R8y exhibit directional responses to the magnetic field like those shown in figure 3, summation of outputs from R7y and R8y onto a second-order cell would reinforce the magnetic field's effect, while at the same

time reducing its dependence on the spectral properties of incident light.

#### 4. CANDIDATE MOLECULAR MECHANISMS: CRYPTOCHROMES

Plant and animal cryptochromes evolved independently from blue-light-dependent DNA photolyase ancestors (Daiyasu *et al.* 2004; Lin & Todo 2005). Nevertheless, there are important sequence homologies and functional similarities within the photolyase and cryptochrome family, including binding sites for the FAD/FADH chromophore and the pterin or flavin antenna pigment, and a triad of tryptophans thought to be involved in electron transfer (Lin & Todo 2005; Banerjee *et al.* 2007; Bouly *et al.* 2007; Müller & Carell 2009; but see Öztürk *et al.* 2008). The RPM (Ritz *et al.* 2000; Cintolesi *et al.* 2003; Solov'yov *et al.* 2007; Rodgers & Hore 2009) proposes that the light-dependent magnetic compass is mediated by a cryptochrome-based RP system, formed by the transfer of an electron from the triad of tryptophan residues to the fully oxidized form of the flavin chromophore (but see Öztürk *et al.* 2008), and is initially in an overall singlet state. In an ordered array of RPs, the alignment of an Earth-strength magnetic field may influence the rate of inter-system crossing between the singlet and triplet excited states. Owing to conservation of spin, back transfer of an electron to reform the fully oxidized ( $\text{FAD}_{\text{ox}}$ ) form is possible only when the RP is in an overall singlet state. Consequently, alignments of the

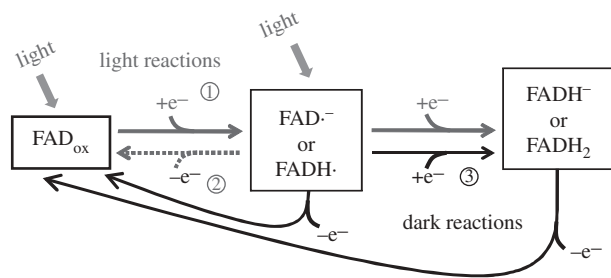


Figure 6. Interconversion of the three redox states of the flavin chromophore in cryptochrome photosystems. Grey lines indicate reactions resulting from absorption of light. Black lines show reactions that occur in the dark. A magnetic field effect on photoreduction of the fully oxidized flavin chromophore ( $\text{FAD}_{\text{ox}}$ ) has been proposed by a number of authors to be involved in the light-dependent magnetic compass (1) (Ritz *et al.* 2000). Photoexcitation of the flavo-semiquinone radical ( $\text{FAD}\cdot^-$  or  $\text{FADH}\cdot$ ) can result in further photoreduction to the fully reduced form ( $\text{FADH}^-$ ), but can also result in photo-oxidation back to the fully oxidized form ( $\text{FAD}_{\text{ox}}$ ). The possibility that this photo-reoxidation reaction plays a significant role in cryptochrome signalling systems, and furthermore is sensitive to the magnetic field, is proposed here to explain the antagonistic effect of the magnetic field on the response of the magnetic compass in amphibians and some insects under long-wavelength light (2). A dark reduction reaction involving flavin-superoxide RP has also been proposed to play a role in magnetoreception (3) (Solov'yov & Schulten 2009; but see Hogben *et al.* 2009).

magnetic field that produce greater singlet  $\leftrightarrow$  triplet mixing and, therefore, decrease the overall singlet character of the RP will enhance the persistence of the radical form. Regardless of which redox form of the flavin chromophore is the signalling state (see below), an effect of the magnetic field that increases the persistence of the radical form is likely to increase the response to light. The magnetic field's effect is predicted to be independent of polarity (north versus south), and so is consistent with birds, amphibians and some insects, using an inclination magnetic compass that is dependent on the slope or dip angle, rather than on the polarity, of the magnetic field (Wiltschko & Wiltschko 1972; Phillips 1986; Vácha *et al.* 2008a; D. H. Dommer 2008, unpublished data).

Theoretical treatments of the RPM have generally assumed that the ground or resting state of the cryptochrome molecule occurs when the flavin chromophore is in the fully oxidized state ( $\text{FAD}_{\text{ox}}$ ), which is photoreduced by blue light to the partially reduced (flavo-semiquinone) radical state ( $\text{FAD}\cdot^-$  or  $\text{FADH}\cdot$ ; figure 6; Ritz *et al.* 2000; Cintolesi *et al.* 2003; Solov'yov *et al.* 2007). According to this view, redistribution of charge during the formation of the cryptochrome RP produces a conformational change in the protein that triggers an interaction with one or more, as yet unidentified, signalling partners (Hoang *et al.* 2008; Müller & Carell 2009). The flavo-semiquinone may be further photoreduced to the fully reduced  $\text{FADH}^-$  form, and both the radical or fully reduced forms reoxidized by a dark reaction back to the fully oxidized form (figure 6). However, recent evidence suggests that the predominance of the fully oxidized ( $\text{FAD}_{\text{ox}}$ ) form

prior to irradiation with light in some cryptochrome systems may be an artefact of the isolation and purification process, e.g. in type 1 insect cryptochromes (Song *et al.* 2007; Öztürk *et al.* 2008). According to this alternative view, the radical form is the resting state (as occurs in the blue-light-dependent photolyases), and is photoreduced to  $\text{FADH}^-$ , which is the signalling form.

While there is as yet no definitive evidence indicating the photo-signalling pathway(s) involved in magnetoreception, it is important to emphasize that this photo-signalling pathway need not be the same as that involved in photo-entrainment of circadian rhythms, or in any other light-dependent process involving cryptochromes, including those coexisting in the same organism (Berndt *et al.* 2007; Hoang *et al.* 2008). Furthermore, an effect of the magnetic field on photoreduction of the fully oxidized form to the partially reduced (flavo-semiquinone) form of the flavin chromophore (Ritz *et al.* 2000; Cintolesi *et al.* 2003; Solov'yov *et al.* 2007) could play a role in magnetoreception even if this is not the primary photo-transduction pathway (Song *et al.* 2007; Öztürk *et al.* 2008). For example, magnetic-field-dependent photo-interconversion of the fully oxidized and radical forms ( $\text{FAD}_{\text{ox}} \leftrightarrow \text{FAD}\cdot^-$  or  $\text{FADH}\cdot$ ; see below) might compete with a photo-transduction pathway in which the semiquinone resting state is photoreduced to the fully reduced signalling state (i.e.  $\text{FAD}\cdot^-$  or  $\text{FADH}\cdot \rightarrow \text{FADH}^-$ ; figure 6).

Three features of cryptochrome photo-signalling systems are important for the current discussion.

- *In vivo*, cryptochrome photo-signalling systems may involve both resting and photo-induced equilibria that involve all three redox forms of the flavin chromophore ( $\text{FAD}_{\text{ox}}$ ,  $\text{FAD}\cdot^-$  or  $\text{FADH}\cdot$  and  $\text{FADH}^-$ ; Song *et al.* 2007; Van Vickle-Chavez & Van Gelder 2007; Öztürk *et al.* 2008; Zikihara *et al.* 2008; Balland *et al.* 2009). The stability of the radical form can be increased by ATP binding to a site adjacent to the flavin chromophore, which is homologous to the DNA-binding pocket in the photolyases (Immeln *et al.* 2007; Hoang *et al.* 2008; Burney *et al.* 2009). ATP binding, with or without autophosphorylation of the cryptochrome protein, provides a mechanism that could alter the relative stability of the different redox states and, thus, the predominant resting state of the flavin chromophore in different photo-signalling systems within and between organisms.
- The availability of an amino acid residue in the vicinity of the flavin chromophore that can serve as a proton donor determines which of the flavin radicals (i.e.  $\text{FAD}\cdot^-$  or  $\text{FADH}\cdot$ ) is formed by photoreduction of  $\text{FAD}_{\text{ox}}$  (Öztürk *et al.* 2008). *Drosophila* cryptochrome (DmCRY1) and other insect CRYs form the anionic radical ( $\text{FAD}\cdot^-$ ; Öztürk *et al.* 2008), while amphibians and fish cryptochromes (including CRY-DASH; Zikihara *et al.* 2008; Biskup *et al.* 2009) form the neutral radical ( $\text{FADH}\cdot$ ), with corresponding differences in the absorption spectra (figure 5).

— The radical (flavo-semiquinone) form of cryptochrome may undergo both photoreduction, in which the flavin chromophore is converted to  $\text{FADH}^-$ , and photo-oxidation, in which the chromophore is converted to  $\text{FAD}_{\text{ox}}$  (Shirdel *et al.* 2008; Usman *et al.* 2009). For example, Usman *et al.* (2009) found that heterologously expressed, purified 6-4 photolyase from the green algae *Ostreococcus tauri* (OtCPF1), belonging to the same gene family as the animal cryptochromes (Lin & Todo 2005), undergoes photo-oxidation of the radical ( $\text{FADH}\cdot$ ) form to the fully oxidized ( $\text{FAD}_{\text{ox}}$ ) form with a quantum yield comparable to that of the photoreduction of the radical to the fully reduced ( $\text{FADH}^-$ ) form. Photo-oxidation of the radical form of OtCPF1 is suggested to involve a two-step process. Both steps in the proposed process involve electron-transfer reactions: (i) oxidation of an external electron donor (R) in the buffer by the flavin radical ( $\text{FADH}\cdot \rightarrow \text{FADH}\cdot^* + \text{R} \rightarrow \text{FADH}^- + \text{R}^+$ ) and (ii) diffusion of the  $\text{R}^+$  in solution and subsequent oxidization by another flavin radical ( $\text{R}^+ + \text{FADH}\cdot \rightarrow \text{R} + \text{FAD}_{\text{ox}} + \text{H}^+$ ). Owing to the GST-fusion system used by Usman *et al.* to purify OtCPF1, the external electron donor (R) is probably reduced L-glutathione present in the elution buffer (Usman *et al.* 2009). Whether or not a comparable reaction can occur in cryptochrome photo-signalling systems remains to be determined. In purified *Drosophila* cryptochrome (DmCRY), Shirdel *et al.* (2008) found that wavelengths of light that excite both the fully oxidized ( $\text{FAD}_{\text{ox}}$ ) and radical ( $\text{FAD}\cdot^-$ ) forms of DmCRY produce a steady-state photo-induced equilibrium between photoreduction ( $\text{FAD}_{\text{ox}} \rightarrow \text{FAD}\cdot^-$ ) and photo-oxidation ( $\text{FAD}\cdot^- \rightarrow \text{FAD}_{\text{ox}}$ ). Shirdel *et al.* (2008) suggest that the photo-cycle dynamics of DmCRY involve a photo-induced forth and back intermolecular electron transfer followed by an intramolecular electron transfer between the flavin chromophore and adjacent tryptophan residue. In either of these proposed photo-oxidation reactions, an effect of the magnetic field that shifts the photo-equilibrium towards or away from the signalling state (i.e. either the radical or fully reduced forms) could alter the response of the system to light (see below).

#### 4.1. Can the properties of cryptochrome-based photo-signalling systems be reconciled with those of light-dependent magnetic compass orientation in amphibians and insects?

Amphibian CRY-DASH has been shown to form spin-correlated RPs, making this molecule (and potentially other amphibian cryptochromes) a suitable candidate for involvement in an RPM (Biskup *et al.* 2009). In CRY-DASHs found in aquatic vertebrates (Zikihara *et al.* 2008; Biskup *et al.* 2009), and other cryptochromes that produce the neutral form of the flavin radical ( $\text{FADH}\cdot$ ), wavelengths of light less than 480 nm convert the fully oxidized form of the flavin chromophore ( $\text{FAD}_{\text{ox}}$ ) into the radical (flavo-semiquinone) form ( $\text{FADH}\cdot$ ), which is preferentially

excited at wavelengths from approximately 480 nm to at least 650 nm (e.g. figure 5*b*). The correspondence between the spectral properties of magnetic compass orientation in newts (§2) and the spectral absorption properties of amphibian CRY-DASH (Biskup *et al.* 2009), and potentially other amphibian cryptochromes (van der Schalie *et al.* 2007), is consistent with the fully oxidized and radical (flavo-semiquinone) forms of cryptochrome providing the short- and long-wavelength inputs to the light-dependent magnetic compass, i.e. providing antagonistic magnetically sensitive inputs. In insects, the effects of UV and visible light on behavioural (table 2) and neurophysiological (figure 3) responses to magnetic stimuli are also consistent with the action spectra of photo-signalling pathways involving different redox forms of insect cryptochrome (figure 5*a*).

Spectral antagonism has been demonstrated in a number of *in vivo* cryptochrome photo-signalling systems. For example, in both plants (Folta & Maruhnich 2007) and flies (Hoang *et al.* 2008), green light has an antagonistic effect on cryptochrome-dependent blue-light signalling. The blue-light-dependent responses in these systems are presumed to reflect a signalling pathway involving photoreduction of the fully oxidized to the radical form, while the antagonistic effects of long-wavelength light are attributed to further photoreduction of the radical form to the fully reduced form, which is subsequently converted back into the fully oxidized form in the dark (figure 6; Berndt *et al.* 2007; Hoang *et al.* 2008; but see Öztürk *et al.* 2008). Despite the superficial similarity of these antagonistic spectral effects on cryptochrome-mediated responses to those proposed to explain the effects of light on magnetic compass orientation (§2), it is by no means clear that the magnetic field could have an effect on the long-wavelength-dependent photoreduction of the cryptochrome radical to the fully reduced form ( $\text{FADH}\cdot \rightarrow \text{FADH}^-$ ) that would be antagonistic to its effect on the initial photoreduction reaction leading to radical formation, i.e. that the magnetic field would produce inverse or complementary patterns of response when exposed to short- and long-wavelength light (figure 1*e-h*).

The magnetic field reduction has also been proposed to affect dark reduction of the flavin radical to the fully reduced form ( $\text{FADH}\cdot \rightarrow \text{FADH}^-$  or  $\text{FADH}_2$ ), involving the formation of a flavin-superoxide RP (Ritz *et al.* 2009; Solov'yov & Schulten 2009). However, this mechanism has been criticized on theoretical grounds (Hogben *et al.* 2009) and, whether or not such an effect of the magnetic field occurs, is unlikely to account for the light-dependent properties of magnetic compass orientation in amphibians and insects.

To account for the light-dependent properties of the amphibian and insect magnetic compass responses, we propose that specific alignments of the magnetic field affect both UV/blue-light-dependent photoreduction of the fully oxidized form of the flavin chromophore to the semiquinone radical form ( $\text{FAD}_{\text{ox}} \rightarrow \text{FAD}\cdot^-$  or  $\text{FADH}\cdot$ ), and long-wavelength-dependent photo-oxidation reaction of the radical form back to the fully

oxidized form ( $\text{FAD}\cdot^-$  or  $\text{FADH}\cdot \rightarrow \text{FAD}_{\text{ox}}$ ). The effect of the magnetic field will be to shift the photo-equilibrium of the three redox forms either towards or away from the signalling state, which may be either the radical (Hoang *et al.* 2008; Müller & Carell 2009) or the fully reduced form (Öztürk *et al.* 2008). Theoretical treatments of the RPM have generally assumed that the initial photoreduction of the fully oxidized form of the cryptochrome chromophore ( $\text{FAD}_{\text{ox}}$ ) to the radical form ( $\text{FAD}\cdot^-$  or  $\text{FADH}\cdot$ ) produces a flavin-Trp RP in an overall singlet state (Ritz *et al.* 2000; Cintolesi *et al.* 2003; Solov'yov *et al.* 2007). Magnetic field alignments that increase mixing of the single and triplet states decrease the singlet character of the RP, resulting in a decrease in the efficiency of the back-transfer reaction (Ritz *et al.* 2000; Cintolesi *et al.* 2003; Hogben *et al.* 2009; Rodgers & Hore 2009), which would shift in the photo-equilibrium towards the radical form of the flavin chromophore.

How then could the same alignments of the magnetic field that affect the photoreduction reaction have an opposite effect on photo-reoxidation of the radical ( $\text{FAD}\cdot^-$  or  $\text{FADH}\cdot$ ) back to the fully oxidized form ( $\text{FAD}_{\text{ox}}$ ), i.e. shift the photo-equilibrium away from the radical form? One possibility is that an opposite effect of the magnetic field might occur if the first step of the photo-reoxidation reaction results in the initial formation of a RP in an overall triplet state, since the consequences of increased singlet  $\leftrightarrow$  triplet mixing for the photo-signalling system may depend on the initial state of the RP system (K. Schulten 1997, personal communication). For example, chance encounters between an external electron donor and the flavin radical in the second step of a two-step photo-reoxidation reaction like that suggested by Usman *et al.* (2009) would result in 25 per cent singlet and 75 per cent triplet states, owing to the random population of the one singlet and three triplet states. As singlet RPs are depleted by the electron-transfer reaction, an effect of the magnetic field that increases  $\text{S} \leftrightarrow \text{T}$  mixing might help to replenish singlets and, thus, could increase the efficiency of the photo-oxidation reaction, shifting the photo-equilibrium away from the radical state. In other words, alignments of the magnetic field that increase  $\text{S} \leftrightarrow \text{T}$  mixing could increase the rate of formation of the radical state by means of the UV/blue-light-dependent photoreduction reaction (shifting the photo-equilibrium towards the radical form), while a similar effect of the magnetic field might increase the conversion of the radical form back into the fully oxidized form by means of the long-wavelength-dependent photo-reoxidation reaction (shifting the photo-equilibrium away from the radical form). The result would be an antagonistic effect of the magnetic field on the response light, i.e. the effect of magnetic field alignment on the photo-signalling system would produce inverse or complementary patterns of response under short- and long-wavelength light (figure 1e–h). Whether or not the particular mechanism proposed here is plausible, we suggest that an explanation for the spectrally antagonistic effects of light on magnetic compass orientation in amphibians and insects holds the key to understanding the photo-

excitation pathway(s) responsible for sensitivity to the geomagnetic field.

It is interesting to note that the proposed antagonistic effects of the magnetic field on the response of a cryptochrome-based photosystem to short- and long-wavelength light would tend to decrease the overall sensitivity to the magnetic field under natural (i.e. broad band) lighting conditions. Indeed, we have argued that the selective screening of long- and short-wavelength light in the R7y and R8y cells of flies like *Calliphora* and *Musca* are specific adaptations that enable these photoreceptors to function as magnetoreceptors under natural lighting conditions (§3). It seems likely, therefore, that an antagonistic effect of the magnetic field on the responses to short- and long-wavelength light is the ancestral state of the cryptochrome photo-signalling system that has evolved to function in magnetoreception and/or, for reasons yet to be identified, is a by-product of component(s) of the photo-signalling system that otherwise enhance magnetic field sensitivity.

The hypothesis that the wavelength-dependent effects of light on magnetic compass orientation are caused by an antagonistic effect of the magnetic field on photo-interconversion of the fully oxidized and radical ( $\text{FAD}_{\text{ox}} \leftrightarrow \text{FAD}\cdot^-$ ) forms of cryptochrome makes several predictions about the wavelength dependence of these responses. In cryptochromes that produce the neutral form of the flavin radical (e.g. Biskup *et al.* 2009), photo-interconversion of the fully oxidized ( $\text{FAD}_{\text{ox}}$ ) and neutral radical ( $\text{FADH}\cdot$ ) forms have a single isosbestic point or equivalent transition around 480 nm that agrees quite well with the transition between normal ( $\lambda \leq 450$  nm) and 90°-shifted ( $\lambda \geq 500$  nm) orientation observed in newts (Phillips & Borland 1992a). Unlike the insect type 1 cryptochromes (e.g. DmCRY; figure 5a), vertebrate CRY-DASHs do not exhibit a second isosbestic point at short wavelengths (figure 5b; Zikihara *et al.* 2008; Biskup *et al.* 2009), apparently owing to the presence of a UV-sensitive light-harvesting pigment (methenyltetrahydrofolate (MTHF)) that transfers energy to the fully oxidized form of the flavin chromophore ( $\text{FAD}_{\text{ox}}$ ) more efficiently than to the radical form ( $\text{FADH}\cdot$ ). As a consequence, newts trained under natural lighting conditions and tested under UV light (less than 400 nm) should exhibit unshifted magnetic compass orientation, while newts tested under long-wavelength light (wavelengths up to approx. 675 nm) should exhibit 90°-shifted orientation, i.e. there should be only one transition between normal and 90°-shifted orientation.

In flies, photo-interconversion of the fully oxidized ( $\text{FAD}_{\text{ox}}$ ) and anionic radical ( $\text{FAD}\cdot^-$ ) forms of DmCRY exhibit two isosbestic points at 410–415 nm and at 490–495 nm (figure 5a). Wavelengths of light below approximately 400 nm and above approximately 510 nm preferentially excite the radical form, while intermediate wavelengths between approximately 420 and 490 nm preferentially excite the fully oxidized form (Berndt *et al.* 2007; Song *et al.* 2007; Öztürk *et al.* 2008; Hoang *et al.* 2008). In the absence of a UV-absorbing MTHF chromophore that transfers energy to the flavin chromophore (see below), therefore,

there should be transitions in the flies' magnetic compass orientation at both approximately 410 and approximately 495 nm (figure 5a). As a consequence, the axis of magnetic compass orientation of flies tested under long-wavelength light (greater than 510 nm) and under short-wavelength light (less than 400 nm) should be identical, and differ by 90° from that of flies tested at intermediate wavelengths (between 420 and 490 nm).

The predicted wavelength dependence is consistent with experiments by Gegear *et al.* (2008) in which adult *Drosophila* were conditioned under full-spectrum light to associate a food reward with a  $\times 10$  magnetic stimuli. Wild-type flies (but not flies lacking a functional cryptochrome gene) exhibited conditioned responses under full-spectrum light. However, wild-type flies failed to exhibit conditioned responses when wavelengths less than 420 nm were eliminated. The absence of conditioned responses under light greater than 420 nm is difficult to reconcile with a simple cryptochrome-based photo-signalling pathway involving photoreduction of the fully oxidized flavin chromophore ( $\text{FAD}_{\text{ox}} \rightarrow \text{FAD}\cdot^-$ ) or of the partially reduced flavin chromophore ( $\text{FAD}\cdot^- \rightarrow \text{FADH}^-$ ), both of which exhibit appreciable blue-light sensitivity (420–490 nm; figure 5a). However, the wavelength dependence of the conditioned responses is compatible with the proposed antagonistic effects of the magnetic field on photo-interconversion of the fully oxidized and radical forms of the flavin chromophore (figure 5a; and see Van Vickle-Chavez & Van Gelder 2007). Specifically, the failure of flies to exhibit conditioned responses after elimination of wavelengths less than 420 nm may not have been caused by an inability to detect the magnetic stimuli, but rather by a change in the nature of the response (figure 1; Phillips & Borland 1992a; Phillips & Sayeed 1993; Deutschlander *et al.* 1999a). As discussed earlier (section 2), adult male *Drosophila* exhibit magnetic compass orientation under both 365 and 500 nm light (40 nm bandwidth) at an intensity comparable to that used in the experiments by Gegear *et al.* (2008), although the direction of orientation under 500 nm light is shifted 90° clockwise from that of flies tested under 365 nm light (Phillips & Sayeed 1993). Broad-band light with and without wavelengths less than 420 nm may produce inverse (i.e. complementary) patterns, as proposed to explain the wavelength-dependent 90° shift in magnetic compass orientation in amphibians and some insects (section 2). Consistent with this possibility, Gegear *et al.* found that untrained wild-type flies avoided the stronger field under full-spectrum light, but preferred the stronger field under wavelengths greater than 420 nm, i.e. flies could discriminate the magnetic stimuli under both lighting conditions, but exhibited opposite responses.

If this explanation of Gegear *et al.*'s findings is correct, flies that failed to exhibit conditioned response under broad-band light consisting of wavelengths greater than 420 nm would be expected to again exhibit conditioned responses if trained and tested under wavelengths  $\gtrsim 510$  nm of sufficiently high intensity. (Note that the absence of conditioned responses

by flies tested in the 'greater than 500 nm' condition by Gegear *et al.* may have occurred because this light stimulus had sufficient energy below 495 nm to produce 'balanced' excitation of the more-sensitive  $\text{FAD}_{\text{ox}}$  absorption (preferentially excited by wavelengths less than 495 nm) and the less-sensitive  $\text{FAD}\cdot^-$  absorption (preferentially excited by wavelengths greater than 495; figure 5a.) It should also be possible to condition flies under monochromatic light consisting of either short ( $\lambda < 400$  nm) and long ( $\lambda > 510$  nm), but not intermediate (420–490 nm), wavelengths.

Interestingly, conditioned responses to spectral stimuli in the blowfly, *Lucilia* sp. (Troje 1993), thought to be mediated by R7 and R8 (Briscoe & Chittka 2001), show properties that are also consistent with the earlier predictions. In contrast to other animals with well-developed colour vision in which colour discrimination improves smoothly with increasing colour difference between two spectral stimuli (Briscoe & Chittka 2001), Troje (1993) found that blowflies associate colours in three broad 'categories', with transitions between spectral categories occurring at approximately 410 and approximately 500 nm (figure 7). Moreover, experiments carried out by Fukushi (1994) showed that blowflies treat certain mixtures of monochromatic 'blue' and 'yellow' light not as a distinct hue, but instead as neutral stimuli possibly resulting from 'cancellation between inputs elicited by the two monochromatic lights'. While these responses may be indicative of a novel form of colour vision, or of some other more rudimentary wavelength-dependent response, an alternative explanation is that the flies conditioned by Troje (1993) and Fukushi (1994) were responding to the ambient magnetic field using a light-dependent magnetoreception mechanism that produces two alternative categories of response depending on the wavelength of light (figure 1), consistent with the findings of recent studies of magnetic compass orientation by larval *Drosophila* (D. H. Dommer 2008, unpublished data).

The 'magnetic sensing' hypothesis makes three predictions. Discrimination of spectral stimuli in experiments like those carried out by Troje (figure 7) should be compromised (i) if the magnetic field is cancelled or (ii) if the magnetic detector is 'jammed' by exposure to low-level radio-frequency fields, which have been shown to disrupt the light-dependent magnetic compass response of migratory birds (Ritz *et al.* 2004). (iii) In the presence of the ambient magnetic field, flies should treat short-wavelength (less than 400 nm) and long-wavelength (greater than 500 nm) stimuli as belonging to the same spectral category, e.g. flies should fail to distinguish short-wavelength UV stimuli (less than approx. 400 nm) from the constant 580 nm stimulus used in Troje's experiments (dashed line in figure 7).

## 5. CONCLUSIONS

Clearly, the next step in understanding the sensory mechanism(s) that mediate light-dependent magnetic compass orientation is to sample the effects of magnetic

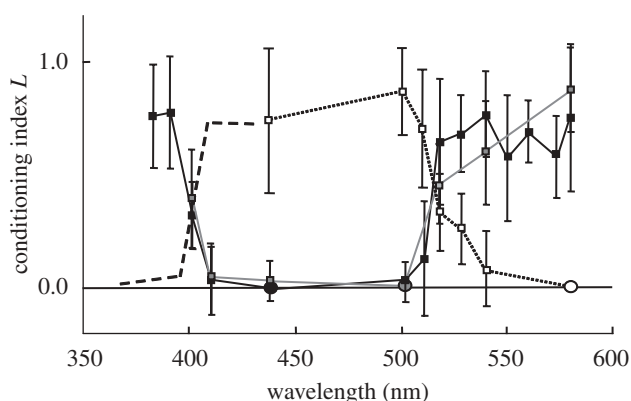


Figure 7. Results of colour discrimination experiments in blowflies, *Lucilia* sp. (Adapted from Troje 1993). Data show the ability of blowflies to discriminate between two spectral stimuli. The wavelength of one stimulus was held constant (reference wavelength, round symbol), while the wavelength of the second stimulus was varied. Solid black line and black symbols—experiments with 438 nm as reference wavelength. Solid grey line and grey-filled symbols—experiments with 501 nm as reference wavelength. Dotted black line and white-filled symbols—experiments with 580 nm as reference wavelength. Dashed black line—predicted pattern if responses are mediated by a light-dependent magnetoreception mechanism (see text). Note that transitions between ‘no discrimination’ ( $L \cong 0.0$ ) and ‘high discrimination’ occurred at approximately 400 and 515 nm regardless of the reference wavelength. In each experiment, the intensity of the variable wavelength stimulus was initially adjusted so that the flies showed little or no spontaneous preference when presented with the two stimuli. Vertical axis in each graph is the conditioning index ( $L$ ), defined as the difference between the average choice frequencies of the two paired spectral stimuli obtained from 10–20 flies each tested two or more times (40 trials per test). Vertical bars indicate the 95% confidence intervals for the  $L$  values. See Troje (1993) for a description of the experimental methods.

field alignment on the response of specific photoreceptor cells to light. Photoreceptors in the compound eye of flies and pineal complex of amphibians are especially good candidates to play a primary role in magnetoreception. In large flies, the unusual properties of R7y and R8y, coupled with the evidence for rudimentary responses to magnetic stimuli in the functionally similar R1–6 cells (figure 3 and electronic supplementary material, figure S1), suggest that one or both of these photoreceptors function as specialized magnetoreceptors. Among vertebrates, behavioural evidence that the pineal organ of newts is the site of the light-dependent magnetic compass (Deutschlander *et al.* 1999b; Phillips *et al.* 2001), along with neurophysiological evidence for magnetic field sensitivity in the isolated pineal organ of birds (Demaine & Semm 1985) and parapineal (‘frontal organ’) of frogs (J. B. Phillips & S. C. Borland 1998, unpublished data), indicate that these sensory organs also contain photoreceptors/photo-signalling pathways specialized for magnetoreception. Indeed, it would not be surprising to find that photoreceptors and photo-signalling pathways specialized for sensing the geomagnetic field are present in a wide variety of animals. The possibility that birds (Wiltschko & Wiltschko 2005; but see Johnsen *et al.* 2007; Jensen

2009) and some mammals (Olcese *et al.* 1985) have photoreceptor-based magnetoreceptors in the retina is especially intriguing, and suggests that integration of magnetic and visual information could play an important role in the spatial perception of these vertebrates.

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