

Light-dependent magnetic compass in Iberian green frog tadpoles

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Abstract Here, we provide evidence for a wavelength-dependent effect of light on magnetic compass orientation in *Pelophylax perezi* (order Anura), similar to that observed in *Rana catesbeiana* (order Anura) and *Notophthalmus viridescens* (order Urodela), and confirm for the first time in an anuran amphibian that a 90° shift in the direction of magnetic compass orientation under long-wavelength light (≥ 500 nm) is due to a direct effect of light on the underlying magnetoreception mechanism. Although magnetic compass orientation in other animals (e.g., birds and some insects) has been shown to be influenced by the wavelength and/or intensity of light, these two amphibian orders are the only taxa for which there is direct evidence that the magnetic compass is light-dependent. The remarkable similarities in the light-dependent magnetic compasses of anurans and urodeles, which have evolved as separate clades for at least 250 million years, suggest that the light-dependent magnetoreception mechanism is likely to have evolved in the common ancestor of the Lissamphibia (Early Permian, ~294 million years) and, possibly, much earlier. Also, we discuss a number of similarities between the functional properties of the light-dependent magnetic

compass in amphibians and blue light-dependent responses to magnetic stimuli in *Drosophila melanogaster*, which suggest that the wavelength-dependent 90° shift in amphibians may be due to light activation of different redox forms of a cryptochrome photopigment. Finally, we relate these findings to earlier studies showing that the pineal organ of newts is the site of the light-dependent magnetic compass and recent neurophysiological evidence showing magnetic field sensitivity in the frog frontal organ (an outgrowth of the pineal).

Keywords Anuran · Magnetic compass · Light-dependent magnetoreception · *Pelophylax perezi*

Introduction

Since Ferguson and Landreth (1966) coined the term “ γ -axis orientation” to describe orientation by shore-dwelling amphibians that intersects the home shoreline at a right angle, this kind of orientation behavior has been shown in a wide variety of amphibian species (Wells 2007). Y -axis orientation allows amphibians to orient along the most direct route to and from shore, which is of great importance in the ecology of amphibians (Russell et al. 2005; Wells 2007). Whether they move toward or away from the shore depends on the species, its physiological state, risk of predation, and/or stage of development (Stebbins and Cohen 1997). In the case of tadpoles, orientation along the γ -axis can take them from deeper cooler water to shallower warmer water or facilitate rapid movement to places of refuge when escaping danger (Stebbins and Cohen 1997).

Amphibians can use different types of directional information for γ -axis orientation, including the sun

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(Ferguson and Landreth 1966; Ferguson et al. 1967; Landreth and Ferguson 1967, 1968; Adler and Taylor 1973), sky polarization patterns (Taylor and Ferguson 1970; Taylor 1972; Adler and Taylor 1973; Taylor and Adler 1973; Taylor and Auburn 1978), and the earth's magnetic field (Phillips 1986; Deutschlander et al. 2000; Freake et al. 2002; Rodríguez-García and Diego-Rasilla 2006; Diego-Rasilla and Phillips 2007). Although most studies of magnetic compass orientation in amphibians have been carried out with urodeles, among anurans, y -axis magnetic compass orientation has been shown in tadpoles of the bullfrog *Rana catesbeiana* (Freake et al. 2002; Freake and Phillips 2005) and tadpoles of the Iberian green frog *Pelophylax perezi* (Diego-Rasilla and Phillips 2007). Although the behavior of tadpoles clearly differs from that of adult frogs, with corresponding differences in their orientation behavior, the ease with which tadpoles can be trained and tested makes them well suited for studies of the biophysical mechanism underlying the magnetic compass.

There is evidence that the magnetic compass is mediated by a light-dependent magnetoreception mechanism in both newts (Phillips and Borland 1992a, b, c; Deutschlander et al. 1999a, b; Phillips et al. 2001) and frogs (Freake and Phillips 2005). In particular, exposure to different wavelengths of light alters the magnetic compass orientation of newts (Phillips and Borland 1992c). Furthermore, both the homing orientation of alpine newts (*Mesotriton alpestris*; Diego-Rasilla 2003) and the shoreward magnetic compass orientation of Eastern red-spotted newts (*Nothophthalmus viridescens*; Phillips and Borland 1992b) are eliminated in total darkness.

At moderate intensities (12.3–12.6 log quanta/cm²/s) of monochromatic (~400-nm bandwidth) light, Eastern red-spotted newts and bullfrog tadpoles trained outdoors to orient in the direction of an artificial shore under natural (i.e., full spectrum) light, oriented in the shoreward magnetic direction when tested in a visually symmetrical indoor arena under full-spectrum and short-wavelength (400 and 450 nm) light, but oriented 90° counterclockwise from the shoreward direction under long wavelength (500, 550, and 600 nm; Phillips and Borland 1992a; Freake and Phillips 2005). Subsequent experiments showed that newts trained outdoors under long-wavelength (>500 nm) light and tested under full spectrum oriented 90° clockwise from the shore direction, while newts trained and tested under long wavelength oriented in the shore direction. These findings indicated that the wavelength-dependent shift resulted from a direct effect of light on the underlying magnetoreception mechanism.

The cause of the 90° shift in newts was shown to be an antagonistic interaction between short-wavelength (≤ 450 nm) and less sensitive long-wavelength (≥ 500 nm) inputs that produce complimentary (inverse) patterns of response and are mediated by extra-ocular photoreceptors

located in the pineal organ (Phillips and Borland 1992a, b; Phillips et al. 2001; Ritz et al. 2002). Consistent with the antagonistic input model, intermediate wavelengths of light around 475 nm that should excite the two inputs equally and cause the complementary patterns to cancel out produce disorientation (Phillips and Borland 1992a).

To date, comparable data showing that the light-dependent 90° shift in the magnetic compass orientation of anuran amphibians is due to a direct effect on the underlying magnetoreception mechanism have not been available. In this study, we investigated whether magnetic compass orientation of Iberian green frog tadpoles (Diego-Rasilla and Phillips 2007) is mediated by a light-dependent magnetoreception similar to that shown in the earlier experiments with newts.

Methods

Iberian green frog tadpoles (stages 41–45; Gosner 1960) used in this study were collected from a stream situated in Barros (Cantabria, northern Spain; 43°16'58" N, 4°4'52" W; elevation 82 m). Tadpoles were captured along an 85-m transect in which the stream flows from south toward north (194°–14°), hence with the shores toward east and west. The stream is about 2 m wide, and the tadpoles were collected along the east shore of the stream.

Two groups of animals were collected in 2006 and 2007. In 2006, 63 tadpoles were collected in midsummer (5 August), allowed to learn the direction of an artificial shore for 5 days under natural (i.e., full spectrum) skylight conditions, and subsequently tested under short- or long-wavelength lighting conditions ($\lambda \leq 450$ nm/ $\lambda \geq 500$ nm). In 2007, 59 tadpoles were captured in the same season (4 August), allowed 5 days to learn an artificial shore direction under long wavelength, and then tested under full-spectrum or long-wavelength lighting conditions (see below for details).

Training and testing apparatus have been described elsewhere (Diego-Rasilla and Phillips 2007), as well as general testing protocols. Training and testing took place in an open area completely shaded by a group of hazels, *Corylus avellana*, about 400 m away from the stream (43°17'07" N, 4°4'44" W; elevation 59 m). Tadpoles were trained under natural light–dark cycle in water-filled outdoor tanks and exposed to natural variation in light intensity. Tadpoles used in the experiments were returned to their home stream after testing.

The y -axis training tanks consisted of two 96-L all-glass aquaria (length, 0.81 m; width, 0.31 m; height, 0.41 m), each one with a sloping bottom (over 25° slope) in order to provide a gradual gradient from the deep end to the shallow end. Training tank design has been presented in detail elsewhere (Diego-Rasilla and Phillips 2007). In each

aquarium, sides were covered by means of 1.5-cm-thick wooden boards to prevent light penetration from the sides of the tanks, whereas the top was uncovered. Training tanks were filled with water from the tadpoles' home stream and water depth at the shore end was 1 cm. Each day, the aquarium water was partly replaced with freshwater from the stream; this was done at the time of maximum temperature (i.e., 1400–1600 GMT) to maintain the water temperature of the training tanks between 17°C and 23°C. Such daily fluctuation of water temperature approximately resembled natural conditions of the home stream where water temperature varied between 19°C and 23°C. Tadpoles were fed boiled lettuce, which was placed in the shallow end of the tank daily.

Two different training configurations, with perpendicular shore directions, were used in these experiments. One training tank was aligned along the magnetic north–south axis, with shore facing south, whereas the other training tank was aligned along the east–west axis, with shore to the east.

In 2006, tadpoles were introduced into the tanks on 5 August, at 1130 GMT. In both of them, the aquaria top glass remained transparent. Two groups of 31 and 32 tadpoles were introduced into the shallow end of each training tank (N–S and E–W training tanks, respectively) and allowed 5 days (i.e., from 5 to 10 August) to learn the *y*-axis direction before testing. In 2007, animals were introduced into the tanks on 4 August, at 1145 GMT. This time, the top side of each training tank was covered with a ≥ 500 -nm long-wavelength light pass filter comprising one layer of a spectral gel filter (Lee no. 101; Lee Filters©, Andover, Hants, UK) placed between two transparent methacrylate sheets (82×42 cm, 0.4 cm thick). Two groups of 28 and 31 tadpoles were introduced into the shallow end of each training tank (N–S and E–W training tanks, respectively) and allowed 5 days (i.e., from 4 to 9 August) to learn the *y*-axis direction. After training, Iberian green frog tadpoles were tested individually in a water-filled outdoor arena for magnetic orientation. All tests were conducted on a single day during daylight hours.

In our experimental design, four different magnetic field conditions were used (see Phillips 1986): the ambient magnetic field and three altered fields (magnetic north rotated to east, west, and south) produced by means of a doubly wrapped cube-surface coil (Rubens 1945). The testing apparatus was a circular, featureless test arena open only to the sky (circular plastic container, 43-cm diameter and 24-cm height) enclosed within a doubly wrapped cube-surface coil powered by a dual-DC power supply (Protek 30158) used to alter the alignment of the Earth's magnetic field. The sides of the apparatus were covered with a black cotton curtain. The testing apparatus was located under dense foliage that blocked a direct view of the overhead sky.

Prior to testing, the arena was filled with water from the tadpoles' home stream to a depth of 1 cm. Temperature of arena water was maintained between 19°C and 23°C by replacing some of the arena water with freshwater as soon as temperature reached 23°C.

Each tadpole was removed from the training tank with a small dip net and placed in the arena center beneath an opaque, cylindrical plastic container (9-cm diameter, 14.5-cm height) that served as a release device. Tadpoles remained in the cylindrical container for 1 min to overcome the effects of handling before the release device was lifted and were allowed to move freely within the testing arena. Tadpoles' movements were observed through tiny holes in the black curtain that covered the test apparatus. A tadpole's directional response was recorded at the first point where it made contact with the wall. Directional bearings were recorded by means of symmetrical radial marks drawn on the exterior edge of arena walls.

Bearings of tadpoles that contacted the arena wall in <10 s were not recorded. These individuals, which moved immediately as soon as the release device was lifted, were considered to exhibit a randomly oriented escape response and were excluded from the analyses. In addition, trials were abandoned if the tadpole failed to score within 10 min (in those cases, the next tadpole was tested under the same magnetic and lighting conditions; Diego-Rasilla and Phillips 2007).

Iberian green frog tadpoles trained under full-spectrum natural skylight (2006 experiment) were tested for their directional preferences under short-wavelength light (≤ 450 nm) and long-wavelength light (≥ 500 nm), whereas tadpoles trained under long-wavelength light (2007 experiment) were tested under full-spectrum and long-wavelength light (≥ 500 nm).

Short-wavelength and long-wavelength training and testing conditions were produced by covering the top of the training or testing apparatus with filters consisting of a layer of a specific spectral gel filter (Lee no. 101 for obtaining ≥ 500 -nm-long wavelength light and Lee HT-120 $\lambda \leq 450$ -nm transmitting gel filter for creating short-wavelength lighting, Lee Filters©) between two transparent methacrylate sheets (105×105-cm side). Since the sides of the training and the testing apparatus were opaque, only light passing through the spectral filters reached the animal in the testing arena. For full-spectrum tests, the top of the apparatus was uncovered, exposing the animals to full-spectrum natural lighting. Light intensity in all lighting conditions varied over the course of the day due to normal changes in sun position and cloud cover.

Each tadpole was tested only once in one of the four magnetic field alignments and under one of the lighting conditions. In both years, the order of the four magnetic field alignments (magnetic *N*=N, S, E, or W) was

determined using a random number sequence. In 2006, the first individual was tested in magN=S, the next individual in magN=W, followed by one in magN=N and one in magN=E, whereas in 2007, the first individual was tested in magN=N, the next individual in magN=E, followed by one in magN=W and one in magN=S. These sequences were repeated until the tests were completed.

One experimenter set the horizontal alignment of the field using remote switches, whereas the second experimenter carried each tadpole from the tanks and recorded its directional response without knowing the alignment of the magnetic field. Thus, the same sequence of four magnetic field alignments was used for successive groups of four tadpoles without the observer being aware of the magnetic field alignment. The sequence of fields was not revealed to the observer until after the experiment was completed.

Also, in the 2006 experiment, the first four tadpoles were taken individually from N–S training tank and tested following the explained sequences of magnetic field alignments. Once this first group of four alignments was completed, the next four larvae were taken from the E–W training tank and tested following the same sequence of magnetic field alignments. In 2007, the first four tadpoles were taken individually from the E–W training tank, and the next four tadpoles were taken from the N–S training tank. Lighting conditions were the same for these two groups of four tadpoles. Then, after modifying lighting conditions, four more larvae from the N–S tank (2006 experiment) or E–W tank (2007 experiment) were tested, and then four more from the E–W tank (2006 experiment) or N–S tank (2007 experiment), and so on.

Absolute or “topographic” bearings (i.e., actual directions of movement ignoring the alignment of the magnetic field in testing) were recorded and then normalized with respect to the direction of magnetic north during testing by rotating the distributions of bearings from each of the four magnetic alignments (magN=N, magN=E, magN=W, magN=S) so that the direction of magnetic north coincides at 0°. By pooling bearings from an equal number of tadpoles tested in each of the four field alignments, we were able to factor out any non-magnetic bias from the resulting distribution and retain only that component of the tadpoles’ orientation relative to the magnetic field (Phillips 1986; Diego-Rasilla and Phillips 2007).

The distributions of bearings were analyzed using standard circular statistics (Batschelet 1981). Mean vectors were calculated by vector addition and tested for significance using the Rayleigh test for a non-random distribution; statistics for bimodal distributions were calculated by doubling the magnetic bearings. Ninety-five percent confidence intervals were used to determine whether the mean for the distribution included the shoreward direction. If the mean vector was longer before doubling the angles, than

after doubling the angles, the distribution of bearings was considered to be unimodal rather than bimodal.

Data from the two different training directions and training/testing lighting conditions were analyzed separately, and the Watson U^2 test was used to test for differences between the two distributions of magnetic bearings (Batschelet 1981; Mardia and Jupp 2000).

We pooled all magnetic bearings from the same wavelength activation in training and testing (i.e., full-spectrum light in training and short wavelength in testing, and long-wavelength light in both training and testing) with respect to the magnetic direction of the shore in training (combining the magnetic distributions from the two training tanks). All magnetic bearings from different wavelength activations in training and testing (i.e., full-spectrum light in training and long-wavelength light in testing, and long-wavelength light in training and full-spectrum light in testing) were also pooled with respect to the magnetic direction of the shore in training. Similar analyses to those explained previously were performed with these data.

Finally, the absolute or “topographic” bearings were also examined for any evidence of an effect of nonmagnetic directional cues on the tadpoles’ orientation using the Rayleigh test.

Results

Experiments performed in 2006 (full-spectrum training)

After training under natural skylight, tadpoles tested under short-wavelength light (≤ 450 nm, magnetic bearings pooled from four magnetic field alignments) exhibited bimodal orientation that coincided with the magnetic direction of the trained y -axis (Tables 1 and 2 and Fig. 1a). In contrast, tadpoles tested under long-wavelength light (≥ 500 nm) exhibited bimodal orientation that was perpendicular to the magnetic direction of the y -axis, although their orientation was significant only in one trained group and approached significance when both groups were combined (Tables 1 and 2 and Fig. 1b).

The pooled distributions of magnetic bearings from the two shore directions (i.e., bearings pooled with respect to the magnetic direction of shore) were significantly different under ≤ 450 - and ≥ 500 -nm light (Watson U^2 test: $U^2=0.374$, $p<0.001$; Fig. 1a, b).

The topographic bearings (i.e., absolute bearings, ignoring the alignment of the magnetic field in testing; see “Methods”) were non-randomly distributed in only one training/treatment condition (i.e., animals from the 0–180° alignment tank tested under short-wavelength light; Table 2), but the 95% confidence interval did not include the tank y -axis direction.

Table 1 Directional responses of tadpoles

Training light conditions	Tank alignment	Testing light conditions								
		Test field (mN)	TB	MB	MBRS	Test field (mN)	TB	MB	MBRS	
Full spectrum	0–180°	Short-wavelength (≤ 450 nm)				Long-wavelength (≥ 500 nm)				
		MagN=S	180	0	180	MagN=S	90	270	90	
		MagN=W	220	310	130	MagN=W	120	210	30	
		MagN=N	330	330	150	MagN=N	280	280	100	
		MagN=E	300	210	30	MagN=E	200	110	290	
		MagN=S	320	140	320	MagN=S	240	60	240	
		MagN=W	50	140	320	MagN=W	330	60	240	
		MagN=N	10	10	190	MagN=N	20	20	200	
		MagN=E	230	140	320	MagN=E	240	150	330	
		MagN=S	340	160	340	MagN=S	110	290	110	
		MagN=W	320	50	230	MagN=W	260	350	170	
		MagN=N	340	340	160	MagN=N	150	150	330	
		MagN=E	260	170	350	MagN=E	90	0	180	
		MagN=S	0	180	0	MagN=S	250	70	250	
						MagN=W	40	130	310	
					MagN=N	0	0	180		
					MagN=E	150	60	240		
		90–270°	MagN=S	130	310	220	MagN=S	160	340	250
			MagN=W	40	130	40	MagN=W	250	340	250
			MagN=N	290	290	200	MagN=N	350	350	260
			MagN=E	40	310	220	MagN=E	60	330	240
			MagN=S	110	290	200	MagN=S	160	340	250
			MagN=W	350	80	350	MagN=W	70	160	70
			MagN=N	70	70	340	MagN=N	250	250	160
			MagN=E	180	90	0	MagN=E	110	20	290
			MagN=S	100	280	190	MagN=S	190	10	280
			MagN=W	280	10	280	MagN=W	110	200	110
							MagN=N	230	230	140
							MagN=E	250	160	70
							MagN=S	350	170	80
						MagN=W	300	30	300	
						MagN=N	240	240	150	
					MagN=E	90	0	270		
Long wavelength (≥ 500 nm)	0–180°	Full spectrum				Long wavelength (≥ 500 nm)				
		MagN=N	250	250	70	MagN=N	200	200	20	
		MagN=E	240	150	330	MagN=E	120	30	210	
		MagN=W	185	275	95	MagN=W	165	255	75	
		MagN=S	305	125	305	MagN=S	345	165	345	
		MagN=N	265	265	85	MagN=N	220	220	40	
		MagN=E	35	305	125	MagN=E	335	245	65	
		MagN=W	160	250	70	MagN=W	130	220	40	
		MagN=S	300	120	300	MagN=S	15	195	15	
		MagN=N	70	70	250	MagN=N	205	205	25	
		MagN=E	90	0	180	MagN=E	285	195	15	
		MagN=W	0	90	270	MagN=W	80	170	350	
		MagN=S	70	250	70	MagN=S	325	145	325	
		MagN=N	100	100	280					

Table 1 (continued)

Training light conditions	Tank alignment	Testing light conditions							
		Test field (mN)	TB	MB	MBRS	Test field (mN)	TB	MB	MBRS
90–270°		MagN=E	175	85	265				
		MagN=N	310	310	220	MagN=N	20	20	290
		MagN=E	145	55	325	MagN=E	30	300	210
		MagN=W	65	155	65	MagN=W	310	40	310
		MagN=S	30	210	120	MagN=S	0	180	90
		MagN=N	135	135	45	MagN=N	270	270	180
		MagN=E	180	90	0	MagN=E	230	140	50
		MagN=W	120	210	120	MagN=W	0	90	0
		MagN=S	175	355	265	MagN=S	290	110	20
		MagN=N	25	25	295	MagN=N	80	80	350
		MagN=E	135	45	315	MagN=E	165	75	345
		MagN=W	75	165	75	MagN=W	230	320	230
		MagN=S	45	225	135	MagN=S	110	290	200
		MagN=N	135	135	45	MagN=N	105	105	15
		MagN=E	120	30	300	MagN=E	200	110	20
		MagN=W	135	225	135	MagN=W	330	60	330
		MagN=S	70	250	160				

TB absolute (topographic) bearings, MB magnetic bearings, MBRS magnetic bearings relative to the magnetic direction of shore (0°)

Experiments performed in 2007 (long-wavelength training)

To determine whether light has a direct effect on the magnetic compass, tadpoles were trained in tanks covered with sheets of spectral gel filter that only transmitted wavelengths ≥ 500 nm. Tadpoles were then tested under either natural full-spectrum or long-wavelength (≥ 500 nm) light.

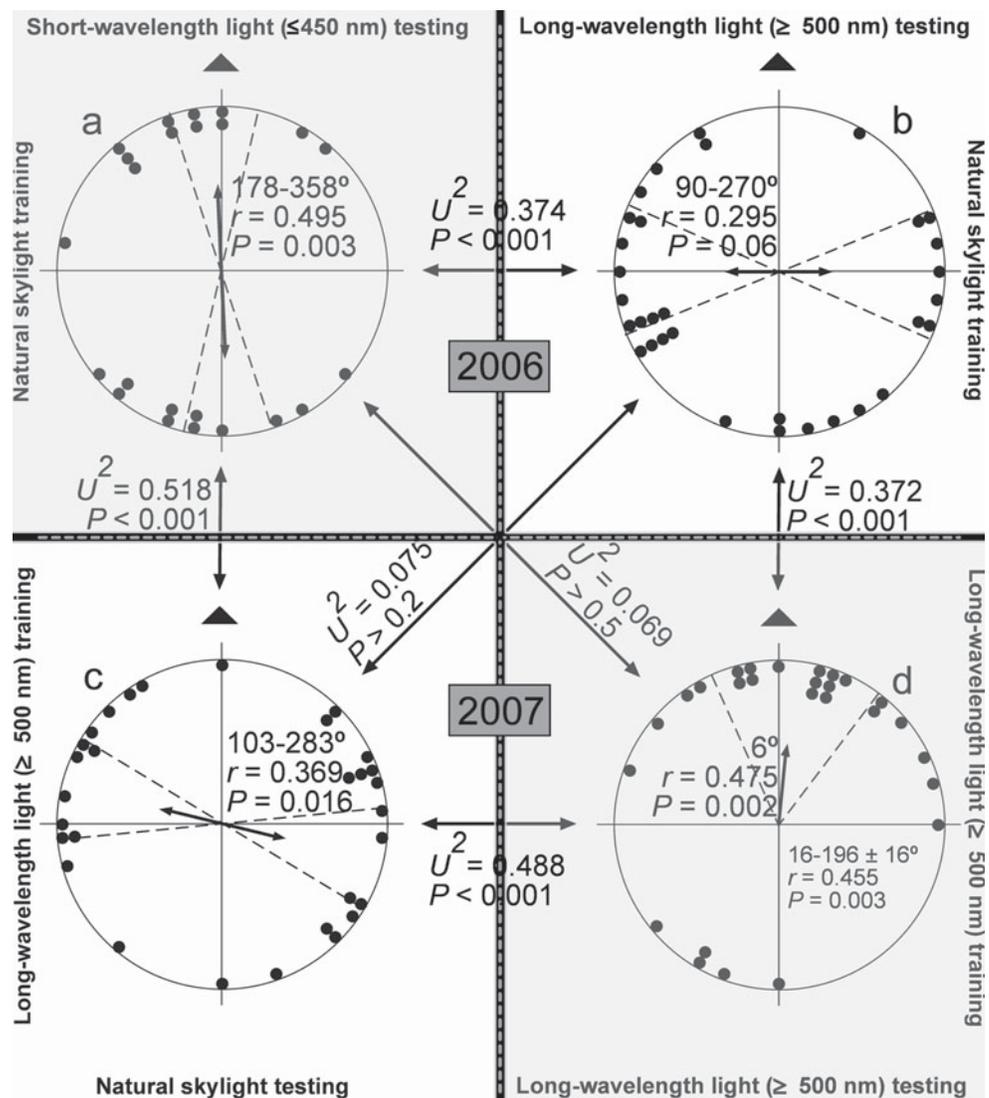
After training under ≥ 500 -nm light, Iberian green frog tadpoles tested under full-spectrum light exhibited bimodal orientation perpendicular to the magnetic direction of the y -axis (Fig. 1c), although their orientation was significant only in the 0–180° trained group (Tables 1 and 2). In contrast, the combined distribution of bearing from tadpoles tested under long-wavelength light was oriented unimodally

Table 2 Statistical analysis of data

Training light conditions	Bearings	Tank alignment	Testing light conditions								
			$\alpha \pm 95\%$ IC	r	P	N	$\alpha \pm 95\%$ IC	r	P	N	
Full spectrum	TB	0–180°	315±41°	0.523	0.025	13	167°	0.083	0.9	16	
		90–270°	67°	0.307	0.4	10	191°	0.153	0.694	16	
	MB	0–180°	164–344±17°	0.602	0.007	13	87–267°	0.044	0.97	16	
		90–270°	107–287±21°	0.577	0.032	10	1–181±17°	0.547	0.007	16	
Bearings pooled with respect to the magnetic direction of shore (0°)			178–358±16°	0.495	0.003	23	90–270±23°	0.295	0.06	32	
Long wavelength (>500 nm)	TB	0–180°	147°	0.039	0.98	14	218°	0.067	0.95	12	
		90–270°	106±31°	0.578	0.003	16	332°	0.154	0.707	15	
	MB	0–180°	94–274±18°	0.553	0.011	14	200±31°	0.702	0.001	12	
		90–270°	28–208°	0.265	0.331	16	99–279°	0.4	0.089	15	
	Bearings pooled with respect to the magnetic direction of shore (0°)			103–283±19°	0.369	0.016	30	6±30°	0.475	0.002	27

α mean direction (deg), r mean vector length, P probability, N sample size, TB absolute (topographic) bearings, MB magnetic bearings

Fig. 1 Magnetic compass orientation of tadpoles trained under natural skylight and tested under ≤ 450 -nm short-wavelength light (a) or ≥ 500 -nm long-wavelength light (b) or trained under ≥ 500 -nm long-wavelength light and tested under natural skylight (c) or ≥ 500 -nm long-wavelength light (d). In each circular diagram, bearings of tadpoles trained in a tank with the y-axis aligned east–west (shore= 90°) and bearings of tadpoles trained in a tank with the y-axis aligned north–south (shore= 180°) are pooled with respect to the magnetic direction of shore. Each dot represents the bearing of one tadpole, tested only once. *Single-headed* and *double-headed arrows* at the center of each plot indicate the mean vector or mean bimodal axis, respectively, for each distribution. The length of each arrow is proportional to the mean vector length (r), with the radius of the circle corresponding to $r=1$ (*single-headed arrows*) or with the diameter of the circle corresponding to $r=1$ (*doubled-headed arrow*). *Dashed lines* represent the 95% confidence intervals for the mean vector. *Triangles outside the plots* indicate the magnetic direction of the shore end of the y-axis



along the y-axis in the shore direction (Fig. 1d); tadpoles from the $0-180^\circ$ trained group oriented toward shore and from the $90-270^\circ$ trained group oriented bimodally along the y-axis (Tables 1 and 2). The combined distributions of tadpoles tested under ≥ 500 -nm and full-spectrum light were significantly different (Watson U^2 test: $U^2=0.488$, $p < 0.001$; Fig. 1c, d).

The topographic bearings were non-randomly distributed in only one training/treatment condition (i.e., animals from $90-270^\circ$ alignment tank tested under full-spectrum light; Table 2), and the 95% confidence interval included the tank y-axis direction.

Pooled data from 2006 and 2007 experiments: same vs. different spectral activation in training and testing

When data from the two training tank alignments were combined, there was no difference in the axis of orientation

between Iberian green frog tadpoles trained under full-spectrum and tested under short-wavelength light when compared to those trained and tested under long-wavelength light (Watson U^2 test: $U^2=0.069$, $p > 0.5$; Fig. 1a, d), suggesting that the short-wavelength mechanism is preferentially excited under natural, full-spectrum light and therefore exhibits higher overall sensitivity than the long-wavelength mechanism, as shown previously in newts (Phillips and Borland 1992a). Similarly, there were no differences in orientation between tadpoles trained under full-spectrum and tested under long-wavelength light and those trained under long wavelength and tested under full spectrum (Watson U^2 test: $U^2=0.075$, $p > 0.2$), i.e., tadpoles experiencing activation of different spectral inputs in training and testing (Fig. 1b, c).

When data were pooled from Iberian green frog tadpoles experiencing the same wavelength activation in training and testing (either full-spectrum light in training and short-

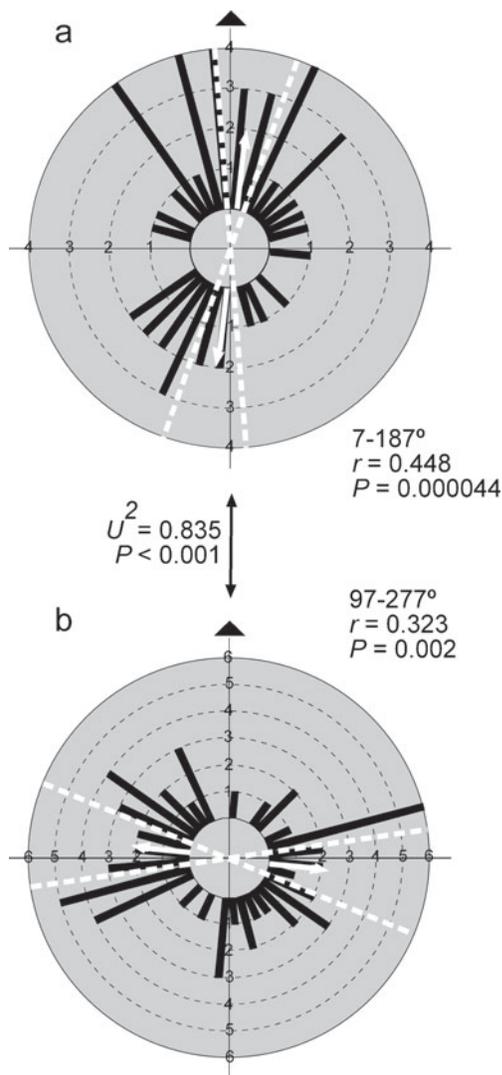


Fig. 2 Same (a) vs. different (b) spectral activation in training and testing (see text). Circular histograms of the data where the *concentric circles* represent frequency increments of 1. **a** Data from Fig. 1a, d combined. **b** Data from Fig. 1b, c combined. *Double-headed arrows* at the center of each plot indicate the mean bimodal axis for each distribution. The length of each arrow is proportional to the mean vector length (r), with the diameter of the circle corresponding to $r=1$. *Dashed lines* represent the 95% confidence intervals for the mean vector. *Triangles outside the plots* indicate the magnetic direction of the shore end of the y-axis

wavelength in testing, or long-wavelength light in both training and testing, i.e., data in Fig. 1a, d combined), the tadpoles showed bimodal magnetic orientation along the shore–deep water magnetic axis (Fig. 2a). In contrast, when data were pooled from tadpoles experiencing different wavelength activation in training and testing (full-spectrum light in training and long-wavelength light in testing, or long-wavelength light in training and full-spectrum light in testing, i.e., Fig. 1b, c combined), the tadpoles showed bimodal magnetic orientation perpendicular to the shore–

deep water magnetic axis (Fig. 2b). The two distributions (Fig. 2a, b) were significantly different (Watson U^2 test: $U^2 = 0.835$, $p < 0.001$; Fig. 2) and their mean axes of orientation differed by 90° .

Discussion

The responses of *P. perezii* tadpoles in the experiments reported here provide support for a light-dependent magnetic compass mechanism in anuran amphibians and confirm that the 90° shift in the direction of magnetic compass orientation under long-wavelength light (≥ 500 nm) reported earlier in bullfrog tadpoles *R. catesbeiana* (Freake and Phillips 2005) is due to a direct effect of light on the underlying magnetoreception mechanism.

A similar light-dependent mechanism has been found in urodele amphibians where wavelength-dependent 90° shift in magnetic compass orientation results from a light-dependent magnetoreception mechanism (Phillips and Borland 1992a). Likewise, our results provide further evidence that this response is caused by antagonistic short-wavelength and long-wavelength inputs and that full-spectrum light preferentially activates the short-wavelength spectral mechanism, which has higher sensitivity than the long-wavelength spectral mechanism.

Overall, we found that tadpoles exhibited the strongest magnetic compass orientation when the same spectral mechanism (i.e., short-wavelength spectral mechanism or long-wavelength spectral mechanism) was activated in training and testing; tadpoles showed increased scatter in magnetic compass responses when lighting conditions in training and testing activated different spectral inputs (Table 2 and Figs. 1 and 2).

The finding that similar light-dependent magnetoreception mechanisms are present in anuran and urodele amphibians has implications for the evolution of this sensory ability in amphibians. Although there is some controversy concerning the origin of Lissamphibia (the smallest clade that includes Apoda, Anura, and Urodela, but not Amniota; Schoch and Milner 2004; Lee and Anderson 2006; Marjanović and Laurin 2007), most investigators suggest that Lissamphibia is monophyletic and derived from the temnospondyls (Milner 1990; Trueb and Cloutier 1991; Ruta et al. 2003). Batrachia is the smallest clade that contains both Salientia (including Anura, but not Urodela or Apoda) and Caudata (including Urodela, but not Anura or Apoda). The oldest batrachians are *Triadobatrachus massinoti* (Rage and Roček 1989) and *Czatkobatrachus polonicus* (Borsuk-Białynicka and Evans 2002), both from the Early Triassic. Since their precise age within the Early Triassic is unknown, the minimal age of Batrachia should be around 250 Mya, in the Induan-

Olenekian boundary (249.7 ± 0.7 Mya; Gradstein et al. 2004), which lies very close to the Permian–Triassic (Chanxingian–Induan) boundary itself (251.0 ± 0.4 Mya; Marjanović and Laurin 2007). Therefore, anurans and urodeles have been separate clades for at least 250 Mya. Consequently, this light-dependent magnetoreception mechanism could have been present in the common ancestor of the Lissamphibia whose origin took place in Early Permian (~ 294 Mya; Zhang and Wake 2009). Although, previous available time tree estimates for the origin of Lissamphibia are controversial, with about 87 Mya difference between the youngest (282 Mya) and oldest (368,8 Mya) estimates (Cannatella et al. 2009).

The light-dependent magnetic compass is proposed to involve a light-absorbing molecule that forms radical pair intermediates (radical pair mechanism; Schulten et al. 1978; Schulten 1982; Schulten and Windemuth 1986; Ritz et al. 2000; Cintolesi et al. 2003; Solov'yov et al. 2007; Rodgers and Hore 2009). Cryptochromes, which contain a flavin chromophore, are the only animal photopigments that are known to form radical pair intermediates and, as a consequence, have been implicated in the light-dependent magnetic compass (Ritz et al. 2000; Mouritsen et al. 2004; Rodgers and Hore 2009; Liedvogel and Mouritsen 2010; Phillips et al. 2010). Cryptochromes are found in many organisms, including amphibians (Cashmore et al. 1999; Zhu and Green 2001; Bailey et al. 2002; Lin 2004; Möller et al. 2004; Kyriacou 2009), where they are involved in diverse non-visual physiological responses, such as light-driven pupillary responses in mammals (van Gelder et al. 2003) and birds (Tu et al. 2004), as well as developmental and circadian (both light-dependent and light-independent) responses (Stanewsky et al. 1998; van der Horst et al. 1999; Rosato et al. 2001; Klarsfeld et al. 2004; Sancar 2004; Ahmad et al. 2007; Hoang et al. 2008; Robinson 2008; Zhu et al. 2008).

Adult male *Drosophila melanogaster* have been shown to exhibit a light-dependent 90° shift in the direction of magnetic compass orientation (Phillips and Sayeed 1993) consistent with the involvement of a light-dependent magnetoreception similar to that in amphibians. A recent study of *D. melanogaster* (Gegear et al. 2008) showed that various fly strains exhibited magnetic field sensitivity only when illuminated with broadband light that includes short wavelengths below 420 nm. These wavelengths fit the action spectrum of *Drosophila* cryptochrome (CRY), which show the highest sensitivity at wavelengths < 420 nm and very little activity above 500 nm, and may result from the activation of multiple redox forms of the flavin chromophore of cryptochrome (Busza et al. 2004; Van Vickle-Chavez and Van Gelder 2007; Phillips et al. 2010). Furthermore, magnetosensitivity was severely compromised in loss-of-function cryptochrome mutants of *D.*

melanogaster (Gegear et al. 2008). Yoshii et al. (2009) found that cryptochrome mediates light-dependent magnetosensitivity of *Drosophila*'s circadian clock, concluding that *Drosophila*'s circadian clock is sensitive to magnetic fields and that this sensitivity depends on both the light activation of CRY and on the applied field strength, providing further support for the cryptochrome-based magnetoreception mechanism (Ritz et al. 2000; Rodgers and Hore 2009). Evidence consistent with the involvement of cryptochromes in a light-dependent magnetic compass has also been obtained in migratory birds (Möller et al. 2004; Mouritsen et al. 2004; Ritz et al. 2004; Mouritsen and Ritz 2005; Wiltschko and Wiltschko 2006; Liedvogel et al. 2007; Liedvogel and Mouritsen 2010).

In amphibians, the spectral sensitivity of the light-dependent magnetic compass (Phillips and Borland 1992a; Deutschlander et al. 1999a, b; Freake and Phillips 2005; this study) is consistent with the involvement of a cryptochrome photopigment in which the flavin chromophore is photo-interconverted among three redox forms (FAD, $\text{FADH}^{\cdot-}$, and FADH_2 ; Zikihara et al. 2008; Biskup et al. 2009). Phillips et al. (2010) proposed that the antagonistic effects of short- and long-wavelength light on the magnetic compass orientation in amphibians arise from an antagonistic effect of the magnetic field on the short-wavelength-dependent photoreduction of the fully oxidized form of the flavin chromophore to the radical semiquinone ($\text{FAD} \rightarrow \text{FADH}^{\cdot-}$) and the long-wavelength-dependent photooxidation of the radical form back into the fully oxidized form ($\text{FADH}^{\cdot-} \rightarrow \text{FAD}$). This could produce inverse or complimentary patterns of response to light either by having opposing effects on the concentration of the radical signaling state (Ritz et al. 2000; Cintolesi et al. 2003; Giovani et al. 2003; Banerjee et al. 2007; Bouly et al. 2007; Solov'yov et al. 2007; Rodgers and Hore 2009; Solov'yov and Schulten 2009; Phillips et al. 2010) or by competing with the formation of the fully reduced form of the flavin chromophore (FADH_2) that serves as the signaling state (Song et al. 2007; Öztürk et al. 2008).

The pineal complex has been implicated as the site of the light-dependent magnetic compass in newts (Deutschlander et al. 1999a, b), and a recent neurophysiological study has provided evidence for sensitivity to the alignment of an Earth-strength magnetic field in the frontal organ (an outgrowth of the pineal organ) of adult bullfrogs *R. catesbeiana* (Phillips and Borland, in preparation). A chromatic mechanism has been identified in the frontal organ of frogs with spectral properties that closely match the wavelength dependence of the light-dependent magnetic compass in newts and tadpoles, i.e., antagonistic high-sensitivity, short-wavelength (peak sensitivity ~ 370 nm) and low-sensitivity, long-wavelength (peak sensitivity ~ 515 nm) inputs that are equally sensitive at 470–480 nm

(Dodt and Heerd 1962; Eldred and Nolte 1978; Koyanagi et al. 2004). The antagonistic inputs appear to arise from photo-interconverting forms of the same light-absorbing molecule and show little or no membrane-level adaptation (Eldred and Nolte 1978; Koyanagi et al. 2004; Phillips et al. 2010). At least one such mechanism has been attributed to the rhodopsin-based photopigment, parapinopsin (Koyanagi et al. 2004). Whether or not this photopigment is directly involved in magnetoreception or provides a non-magnetically sensitive reference for another (possibly cryptochrome-based) magnetosensitive pathway (Phillips et al. 2010) remains a topic for future research.

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References

- Adler K, Taylor DH (1973) Extraocular perception of polarized light by orienting salamanders. *J Comp Physiol* 87:203–212
- Ahmad M, Galland P, Ritz T, Wiltschko R, Wiltschko W (2007) Magnetic intensity affects cryptochrome-dependent responses in *Arabidopsis thaliana*. *Planta* 225:615–624. doi:10.1007/s00425-006-0383-0
- Bailey MJ, Chong NW, Xiong J, Cassone VM (2002) Chickens' Cry2: molecular analysis of an avian cryptochrome in retinal and pineal photoreceptors. *FEBS Lett* 513:169–174
- Banerjee R, Schleicher E, Meier S, Viana RM, Pokorny R, Ahmad M, Bittl R, Batschauer A (2007) The signaling state of *Arabidopsis* cryptochrome 2 contains flavin semiquinone. *J Biol Chem* 282:14916–14922. doi:10.1074/jbc.M700616200
- Batschelet E (1981) Circular statistics in biology. Academic, New York
- Biskup T, Schleicher E, Okafuji A, Link G, Hitomi K, Getzoff Elizabeth D, Weber S (2009) Direct observation of a photoinduced radical pair in a cryptochrome blue-light photoreceptor. *Angew Chem Int Ed* 48:404–407. doi:10.1002/anie.200803102
- Borsuk-Białynicka M, Evans SE (2002) The scapulocoracoid of an Early Triassic stem-frog from Poland. *Acta Palaeontol Pol* 47:79–96
- Bouly JP, Schleicher E, Dionisio-Sese M, Vandenbussche F, Van Der Straeten D, Bakrim N, Meier S, Batschauer A, Galland P, Bittl R, Ahmad M (2007) Cryptochrome blue light photoreceptors are activated through interconversion of flavin redox states. *J Biol Chem* 282:9383–9391. doi:10.1074/jbc.M609842200
- Busza A, Emery-Le M, Rosbash M, Emery P (2004) Roles of the two *Drosophila* cryptochrome structural domains in circadian photoreception. *Science* 304:1503–1506
- Cannatella DC, Vieites DR, Zhang P, Wake MH, Wake DB (2009) Amphibians (Lissamphibia). In: Hedges SB, Kumar S (eds) The timetree of life. Oxford University Press, New York, pp 353–356
- Cashmore A, Jarillo JA, Wu Y, Liu D (1999) Cryptochromes: blue light receptors for plants and animals. *Science* 284:760–765
- Cintolesi F, Ritz T, Kay CWM, Timmel CR, Hore PJ (2003) Anisotropic recombination of an immobilized photoinduced radical pair in a 50- μ T magnetic field: a model avian photomagneto-receptor. *Chem Phys* 294:385–399. doi:10.1016/S0301-0104(03)00320-3
- Deutschlander ME, Borland SC, Phillips JB (1999a) Extraocular magnetic compass in newts. *Nature* 400:324–325. doi:10.1038/22450
- Deutschlander ME, Phillips JB, Borland SC (1999b) The case for light-dependent magnetic orientation in animals. *J Exp Biol* 202:891–908
- Deutschlander ME, Phillips JB, Borland SC (2000) Magnetic compass orientation in the eastern red-spotted newt, *Notophthalmus viridescens*: rapid acquisition of the shoreward axis. *Copeia* 2000:413–419
- Diego-Rasilla FJ (2003) Homing ability and sensitivity to the geomagnetic field in the alpine newt, *Triturus alpestris*. *Ethol Ecol Evol* 15:251–259
- Diego-Rasilla FJ, Phillips JB (2007) Magnetic compass orientation in larval Iberian green frogs, *Pelophylax perezi*. *Ethology* 113:1–6. doi:10.1111/j.1439-0310.2007.01334.x
- Dodt E, Heerd E (1962) Mode of action of pineal nerve fibers in frogs. *J Neurophysiol* 25:405–429
- Eldred WD, Nolte J (1978) Pineal photoreceptors: evidence for a vertebrate visual pigment with two physiologically active states. *Vis Res* 18:29–32. doi:10.1016/0042-6989(78)90073-1
- Ferguson DE, Landreth HF (1966) Celestial orientation of Fowler's toad (*Bufo fowleri*). *Behaviour* 26:105–123
- Ferguson DE, Landreth HF, McKeown JP (1967) Sun compass orientation of the northern cricket frog, *Acris crepitans*. *Anim Behav* 15:45–53
- Freaker MJ, Phillips JB (2005) Light-dependent shift in bullfrog tadpole magnetic compass orientation: evidence for a common magnetoreception mechanism in anuran and urodele amphibians. *Ethology* 111:241–254. doi:10.1111/j.1439-0310.2004.01067.x
- Freaker MJ, Borland SC, Phillips JB (2002) Use of a magnetic compass for Y-axis orientation in larval bullfrogs (*Rana catesbeiana*). *Copeia* 2002:466–471
- Gegear RJ, Casselman A, Waddell S, Reppert SM (2008) Cryptochrome mediates light-dependent magnetosensitivity in *Drosophila*. *Nature* 454:1014–1018. doi:10.1038/nature07183
- Giovani B, Byrdin M, Ahmad M, Brettel K (2003) Light-induced electron transfer in a cryptochrome blue-light photoreceptor. *Nat Struct Biol* 10:489–490
- Gosner KL (1960) A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* 16:183–190
- Gradstein FM, Ogg JG, Smith AG, Agterberg FP, Bleeker W, Cooper RA, Davydov V, Gibbard P, Hinnov LA, House MR, Lourens L, Luterbacher HP, McArthur J, Melchin MJ, Robb LJ, Shergold J, Villeneuve M, Wardlaw BR, Ali J, Brinkhuis H, Hilgen FJ, Hooker J, Howarth RJ, Knoll AH, Laskar J, Monechi S, Plumb KA, Powell J, Raffi I, Röhl U, Sadler P, Sanfilippo A, Schmitz B, Shackleton NJ, Shields GA, Strauss H, Dam JV, Tv K, Veizer J, Wilson DM (2004) A geologic time scale. Cambridge University Press, Cambridge
- Hoang N, Schleicher E, Kacprzak S, Bouly J-P, Picot M, Wu W, Berndt A, Wolf E, Bittl R, Ahmad M (2008) Human and *Drosophila* cryptochromes are light activated by flavin photoreduction in living cells. *PLoS Biol* 6:e160. doi:10.1371/journal.pbio.0060160
- Klarsfeld A, Malpel S, Michard-Vanhee C, Picot M, Chelot E, Rouyer F (2004) Novel features of cryptochrome-mediated photoreception in the brain of the circadian clock of *Drosophila*. *J Neurosci* 24:1468–1477
- Koyanagi M, Kawano E, Kinugawa Y, Oishi T, Shichida Y, Tamotsu S, Terakita A (2004) Bistable UV pigment in the lamprey pineal.

- Proc Natl Acad Sci USA 101:6687–6691. doi:10.1073/pnas.0400819101
- Kyriacou CP (2009) Clocks, cryptochromes and Monarch migrations. *J Biol* 8:55.3–55.4. doi:10.1186/jbiol1153
- Landreth HF, Ferguson DE (1967) Newts: sun-compass orientation. *Science* 158:1459–1461
- Landreth HF, Ferguson DE (1968) The sun compass of Fowler's toad, *Bufo woodhousei fowleri*. *Behaviour* 30:27–43
- Lee MSY, Anderson JS (2006) Molecular clocks and the origin(s) of modern amphibians. *Mol Phylogenet Evol* 40:635–639
- Liedvogel M, Mouritsen H (2010) Cryptochromes—a potential magnetoreceptor: what do we know and what do we want to know? *J R Soc Interface* 7:S147–S162. doi:10.1098/rsif.2009.0411.focus
- Liedvogel M, Maeda K, Henbest K, Schleicher E, Simon T, Timmel CR, Hore PJ, Mouritsen H (2007) Chemical magnetoreception: bird cryptochrome 1a is excited by blue light and forms long-lived radical-pairs. *PLoS ONE* 2:e1106. doi:10.1371/journal.pone.0001106
- Lin C (2004) Photoreceptors and associated signaling II: cryptochromes. In: *Encyclopedia of plant and crop science*. University of California, Los Angeles, California, USA
- Mardia KV, Jupp PE (2000) *Directional statistics*. Wiley, New York
- Marjanović D, Laurin M (2007) Fossils, molecules, divergence times, and the origin of Lissamphibians. *Syst Biol* 56:369–388
- Milner AR (1990) The radiations of temnospondyl amphibians. In: Taylor PD, Larwood GP (eds) *Major evolutionary radiations*. Clarendon, Oxford, pp 321–349
- Möller A, Sagasser S, Wiltschko W, Schierwater B (2004) Retinal cryptochrome in a migratory passerine bird: a possible transducer for the avian magnetic compass. *Naturwissenschaften* 91:585–588. doi:10.1007/s00114-004-0578-9
- Mouritsen H, Ritz T (2005) Magnetoreception and its use in bird navigation. *Curr Opin Neurobiol* 15:406–414
- Mouritsen H, Janssen-Bienhold U, Liedvogel M, Feenders G, Stalleicken J, Dirks P, Weiler R (2004) Cryptochromes and neuronal-activity markers colocalize in the retina of migratory birds during magnetic orientation. *Proc Natl Acad Sci USA* 101:14294–14299. doi:10.1073/pnas.0405968101
- Öztürk N, Song S-H, Selby CP, Sancar A (2008) Animal type 1 cryptochromes: analysis of the redox state of the flavin cofactor by site-directed mutagenesis. *J Biol Chem* 283:3256–3263. doi:10.1074/jbc.M708612200
- Phillips JB (1986) Magnetic compass orientation in the Eastern red-spotted newt (*Notophthalmus viridescens*). *J Comp Physiol A* 158:103–109
- Phillips JB, Borland SC (1992a) Behavioral evidence for the use of a light-dependent magnetoreception mechanism by a vertebrate. *Nature* 359:142–144. doi:10.1038/359142a0
- Phillips JB, Borland SC (1992b) Magnetic compass orientation is eliminated under near-infrared light in the Eastern red-spotted newt (*Notophthalmus viridescens*). *Anim Behav* 44:796–797. doi:10.1016/S0003-3472(05)80311-2
- Phillips JB, Borland SC (1992c) Wavelength-specific effects of light on magnetic compass orientation of the Eastern red-spotted newt (*Notophthalmus viridescens*). *Ethol Ecol Evol* 4:33–42
- Phillips JB, Sayeed O (1993) Wavelength-dependent effects of light on magnetic compass orientation in *Drosophila melanogaster*. *J Comp Physiol A* 172:303–308. doi:10.1007/BF00216612
- Phillips JB, Deutschlander ME, Freake MJ, Borland SC (2001) The role of extraocular photoreceptors in newt magnetic compass orientation: evidence for parallels between light-dependent magnetoreception and polarized light detection in vertebrates. *J Exp Biol* 204:2543–2552
- Phillips JB, Jorge PE, Muheim R (2010) Light-dependent magnetic compass orientation in amphibians and insects: candidate receptors and candidate molecular mechanisms. *J R Soc Interface* 7:S241–S256. doi:10.1098/rsif.2009.0459.focus
- Rage JC, Roček Z (1989) Redescription of *Triadobatrachus massinoti* (Piveteau, 1936) an anuran amphibian from the Early Triassic. *Palaeontogr A* 206:1–16
- Ritz T, Adem S, Schulten K (2000) A model for photoreceptor-based magnetoreception in birds. *Biophys J* 78:707–718. doi:10.1016/S0006-3495(00)76629-X
- Ritz T, Phillips JB, Dommer DH (2002) Shedding light on vertebrate magnetoreception. *Neuron* 34:503–506. doi:10.1016/S0896-6273(02)00707-9
- Ritz T, Thalau P, Phillips JB, Wiltschko R, Wiltschko W (2004) Resonance effects indicate a radical pair mechanism for avian magnetic compass. *Nature* 429:177–180. doi:10.1038/nature 02534
- Robinson R (2008) Monarchs, Cry2 is king of the clock. *PLoS Biol* 6:e12
- Rodgers CT, Hore PJ (2009) Chemical magnetoreception in birds: the radical pair mechanism. *Proc Natl Acad Sci USA* 106:353–360. doi:10.1073/pnas.0711968106
- Rodríguez-García L, Diego-Rasilla FJ (2006) Use of a magnetic compass for Y-axis orientation in premetamorphic newts (*Triturus boscai*). *J Ethol* 24:111–116. doi:10.1007/s10164-005-0169-z
- Rosato E, Codd V, Mazzotta G, Piccin A, Zordan M, Costa R, Kyriacou CP (2001) Light-dependent interaction between *Drosophila* CRY and the clock protein PER mediated by the carboxy terminus of CRY. *Curr Biol* 11:909–917
- Rubens SM (1945) Cube-surface coil for producing a uniform magnetic field. *Rev Sci Instrum* 16:243–245
- Russell AP, Bauer AM, Johnson MK (2005) Migration in amphibians and reptiles: an overview of patterns and orientation mechanisms in relation to life history strategies. In: Elewa AMT (ed) *Migration of organisms*. Climate. Geography. Ecology. Springer, Berlin, pp 151–203
- Ruta M, Coates MI, Quicke DDL (2003) Early tetrapod relationships revisited. *Biol Rev* 78:251–345
- Sancar A (2004) Regulation of the mammalian circadian clock by cryptochrome. *J Biol Chem* 279:34079–34082. doi:10.1074/jbc.R400016200
- Schoch RR, Milner AR (2004) Structure and implications of theories on the origin of lissamphibians. In: Arratia G, Wilson MVH, Cloutier R (eds) *Recent advances in the origin and early radiations of vertebrates*. Dr. Friedrich Pfeil, Munich, pp 345–377
- Schulten K (1982) Magnetic field effects in chemistry and biology. In: Treusch J (ed) *Festkörperprobleme [Advances in solid state physics]*, vol 22. Vieweg, Braunschweig, pp 61–83
- Schulten K, Windemuth A (1986) Model for a physiological magnetic compass. In: Maret G, Boccara N, Kiepenheuer J (eds) *Biophysical effects of steady magnetic fields*. Springer, Berlin, pp 99–106
- Schulten K, Swenberg CE, Weller A (1978) A biomagnetic sensory mechanism based on magnetic field modulated coherent electron spin motion. *Z Phys Chem NF* 111:1–5
- Solov'yov IA, Schulten K (2009) Magnetoreception through cryptochrome may involve superoxide. *Biophys J* 96:4804–4813. doi:10.1016/j.bpj.2009.03.048
- Solov'yov IA, Chandler D, Schulten K (2007) Magnetic field effects in *Arabidopsis thaliana* cryptochrome-1. *Biophys J* 92:2711–2726
- Song S-H, Öztürk N, Denaro TR, NÄz A, Kao Y-T, Zhu H, Zhong D, Reppert SM, Sancar A (2007) Formation and function of flavin anion radical in cryptochrome 1 blue-light photoreceptor of monarch butterfly. *J Biol Chem* 282:17608–17612. doi:10.1074/jbc.M702874200
- Stanewsky R, Kaneko M, Emery P, Beretta B, Wager-Smith K, Kay SA, Rosbash M, Hall JC (1998) The cryb mutation identifies cryptochrome as a circadian photoreceptor in *Drosophila*. *Cell* 95:681–692. doi:10.1016/S0092-8674(00)81638-4

- Stebbins RC, Cohen NW (1997) A natural history of amphibians. Princeton University Press, Princeton
- Taylor DH (1972) Extra-optic photoreception and compass orientation in larval and adult salamanders (*Ambystoma tigrinum*). *Anim Behav* 20:233–236
- Taylor DH, Adler K (1973) Spatial orientation by salamanders using plane-polarized light. *Science* 181:285–287
- Taylor DH, Auburn J (1978) Orientation of amphibians by linearly polarized light. In: Schmidt-Koenig K, Keeton W (eds) Animal migration, navigation and homing. Springer, Berlin, pp 334–346
- Taylor DH, Ferguson DE (1970) Extraoptic celestial orientation in the southern cricket frog *Acris gryllus*. *Science* 168:390–392
- Trueb L, Cloutier R (1991) A phylogenetic investigation of the inter- and intrarelationships of the Lissamphibia (Amphibia: Temnospondyli). In: Schultze H-P, Trueb L (eds) Origins of the higher groups of Tetrapods—controversy and consensus. Cornell University Press, Ithaca, pp 223–313
- Tu DC, Batten ML, Palczewski K, Van Gelder RN (2004) Nonvisual photoreception in the chick iris. *Science* 306:129–131. doi:10.1126/science.1101484
- van der Horst GTJ, Muijtjens M, Kobayashi K, Takano R, Kanno S-I, Takao M, Jd W, Verkerk A, Eker APM, Dv L, Buijs R, Bootsma D, Hoeijmakers JHJ, Yasui A (1999) Mammalian Cry1 and Cry2 are essential for maintenance of circadian rhythms. *Nature* 398:627–630. doi:10.1038/19323
- van Gelder RN, Wee R, Lee JA, Tu DC (2003) Reduced pupillary light responses in mice lacking cryptochromes. *Science* 299:222
- Van Vickle-Chavez SJ, Van Gelder RN (2007) Action spectrum of *Drosophila* cryptochrome. *J Biol Chem* 282:10561–10566. doi:10.1074/jbc.M609314200
- Wells DK (2007) The ecology and behavior of amphibians. The University of Chicago Press, Chicago
- Wiltshko R, Wiltshko W (2006) Magnetoreception. *BioEssays* 28:157–168
- Yoshii T, Ahmad M, Helfrich-Förster C (2009) Cryptochrome mediates light-dependent magnetosensitivity of *Drosophila*'s circadian clock. *PLoS Biol* 7:e1000086. doi:10.1371/journal.pbio.1000086
- Zhang P, Wake DB (2009) Higher-level salamander relationships and divergence dates inferred from complete mitochondrial genomes. *Mol Phylogenet Evol* 53:492–508
- Zhu H, Green CB (2001) Three cryptochromes are rhythmically expressed in *Xenopus laevis* retinal photoreceptors. *Mol Vis* 7:210
- Zhu H, Sauman I, Yuan Q, Casselman A, Emery-Le M, Emery P, Reppert SM (2008) Cryptochromes define a novel circadian clock mechanism in monarch butterflies that may underlie sun compass navigation. *PLoS Biol* 6:e4. doi:10.1371/journal.pbio.0060004
- Zikihara K, Ishikawa T, Todo T, Tokutomi S (2008) Involvement of electron transfer in the photoreaction of zebrafish cryptochrome-DASH. *Photochem Photobiol* 84:1016–1023. doi:10.1111/j.1751-1097.2007.00364.x