Magnetic Compass Orientation in Larval Iberian Green Frogs, *Pelophylax Perezi*

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Abstract

Experiments were carried out to investigate whether Iberian green frog tadpoles *Pelophylax perezi* (formerly *Rana perezi*) are able of using the geomagnetic field for y-axis orientation (i.e. orientation toward and away from shore). Tadpoles were trained outdoor for 5 d, in two different training configurations: (i) a training tank aligned along the magnetic north–south axis, with shore facing south, and (ii) a training tank aligned along the magnetic east–west axis, with shore located east, and similar to the shore–deep water axis ('y-axis') found in their home stream, which flows from south to north. After training, tadpoles were individually tested for magnetic orientation in a water-filled circular outdoor arena surrounded by a pair of orthogonally aligned cube-surface-coils used to alter the alignment of the earth's magnetic field. Tadpoles held in the east–west training tank oriented towards shore, indicating that they were able to distinguish between the shoreward and waterward direction along the y-axis. Tadpoles trained in the tank that was aligned along the north–south axis showed bimodal magnetic compass orientation along the shore–deep water magnetic axis. These findings provide evidence for the use of magnetic compass cues for y-axis orientation by *P. perezi* tadpoles.

Introduction

Magnetic compass orientation has been demonstrated in a wide variety of animals (Wiltschko & Wiltschko 1995; Diego-Rasilla 2004; Wiltschko & Wiltschko 2005). Among urodele amphibians, use of directional information derived from the earth's magnetic field has been demonstrated in the cave salamander, *Eurycea lucifuga* (Phillips 1977), Eastern red-spotted newts, *Notophthalmus viridescens* (Phillips 1986a,b, 1987; Phillips & Borland 1994; Phillips et al. 1995; Deutschlander et al. 2000) and, more recently, in alpine newts, *Mesotriton alpestris* (Diego-Rasilla 2003; Diego-Rasilla et al. 2005) and Bosca's newts, *Lissotriton helveticus* (Rodríguez-García &

Diego-Rasilla 2006). The Eastern newt's magnetic compass is light dependent (Phillips & Borland 1992a,b; Deutschlander et al. 1999; Phillips et al. 2001) and experimental evidence is consistent with a mechanism of magnetoreception involving a photo-induced biochemical reaction that forms radical pair intermediates (i.e. 'radical pair mechanism'; Schulten & Windemuth 1986; Ritz et al. 2000).

In contrast to urodele amphibians, relatively little is known about the use of magnetic compass by anuran amphibians. Studies by Sinsch (1987, 1990, 1992) have shown that the geomagnetic field plays a role in the homing behaviour of *Bufo bufo*, *B. calamita* and *B. spinulosus*; however, these studies did not determine whether the magnetic field was involved

in the compass or map component of homing. Magnetic compass orientation by anurans has only been shown in the bullfrog *Rana catesbeiana* (Freake et al. 2002; Freake & Phillips 2005). Bullfrog tadpoles exhibit bimodal magnetic compass orientation along the shore–deep water magnetic axis (Freake et al. 2002), also referred to as 'y-axis' orientation (Ferguson & Landreth 1966).

In this study, we investigate whether Iberian green frog tadpoles are sensitive to the geomagnetic field and can use it for orienting along a learned y-axis. The experiments presented here constitute the first step in understanding the orientation behaviour of *Pelophylax perezi* (formerly *Rana perezi*).

Methods

Iberian green frog tadpoles (stages 41–45, Gosner 1960) used in this study were collected in mid summer (6 August 2005) from a stream situated in Barros (Cantabria, northern Spain; 43°16′58″N, 4°4′52″W; elevation 82 m). Frogs were captured along an 85 m transect in which the stream flows from south towards north (194°–14°); hence, with the shores towards east and west. The stream is approx. 2 m wide, and the tadpoles were collected along the east shore of the stream.

Training and testing took place in an open area completely shaded by a group of hazels, *Corylus avellana*, approx. 400 m away from the stream (43°17′07″N, 4°4′44″W; elevation 59 m). Tadpoles were trained under natural conditions and natural light-dark cycle in water-filled outdoor tanks. Testing protocols were based on those of Rodríguez-García & Diego-Rasilla (2006). Tadpoles used in the experiment were returned to their home stream after testing.

The y-axis training tanks consisted of two 96 l (80 × 40 × 30 cm) all glass aquaria, each one with a sloping bottom (over 25°slope) in order to provide a deep end, and a shallow end (Fig. 1). In each aquarium the deep end was shaded by means of black cardboard attached to the exterior walls of the aquarium around the deep end, and over the top of the last 30 cm of the tank. The rest of the aquarium (top and sides) was uncovered, so tadpoles had an unobstructed view of the sky and surroundings during training. The shaded deep end was intended to reinforce the y-axis orientation of larvae by providing a refuge or hiding place at the deep (i.e. waterward) end.

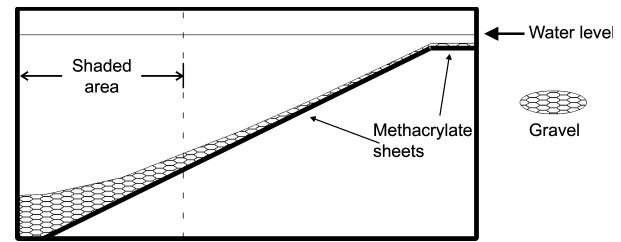Training tanks were filled with tap water treated with aquarium water conditioner ('TetraAcqua

Fig. 1: Training tank design (side view). The sloping bottom was constructed from a sheet of methacrylate sheet, fastened into the aquarium walls with silicone rubber ('Quilosa Orbasil K95'). A small horizontal shore (8 cm length) was added to the shallow end with an additional methacrylate sheet. The bottom of the tank, including the horizontal shore, was completely covered with gravel that sloped up towards the shore

AquaSafe®, Tetra GmbH, Germany') so that water depth at the shore end was 1 cm.

Each day the aquarium water was partly replaced with fresh treated water. This was done at the time of maximum temperature (i.e. 14:00–16:00 GMT) to maintain the water temperature of the training tanks between 17 and 23°C. Such daily fluctuation of water temperature approximately resembled natural conditions of the home stream, where water temperature varied between 18 and 21°C.

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.

Tadpoles were introduced into the tanks on 6 August, at 11:00 GMT. Two groups of 20 and 22 tadpoles were introduced into the shallow end of each training tank (N–S and E–W training tanks, respectively) and allowed five days (i.e. from 6 to 11 August) to learn the y-axis direction before testing. Tadpoles were fed boiled lettuce, which was placed in the shallow end of the tank daily.

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 (11 August 2005) during daylight hours (09:30–12:30 GMT).

In our experimental design, four different magnetic field conditions were used (see Phillips 1986b): 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, visually symmetrical, test arena (circular plastic container, 43 cm

diameter and 24 cm high) enclosed within a 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 treated tap water to a depth of 1 cm. The arena configuration afforded a view of the overhead sky but not the horizon. Temperature of arena water was maintained between 20 and 23°C, by replacing some of the arena water with fresh water as soon as temperature reached 23°C.

Prior to testing, each larva was removed from the training tank with a small dip net and placed in a rectangular plastic container (34 × 24 × 16 cm) with 1 cm water depth and its length along the same alignment as the training tank (N–S or W–E). After 5 min in this individual isolation tank, the tadpole was carried to the testing arena, and placed in the arena centre beneath an opaque, cylindrical plastic container (9 cm diameter, 14.5 cm high) that served as a release device. Tadpoles were held in the container for 1 min to overcome effects of handling before the release device was lifted, allowing them to move freely about the arena. Larvae 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 to 10° accuracy by means of symmetrical radial marks drawn on the exterior edge of arena walls.

Bearings of larvae that contacted arena wall in less than 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 larva failed to score within 10 min.

Each tadpole was tested only once in one of the four magnetic field alignments. The first larva was taken from North to South training tank and tested in the ambient field [i.e. magnetic north (magN) = geographic north (gN)]. Then, another N–S larva was tested, now in a condition with magnetic north rotated to the east (magN = gE), followed by two more N–S larvae in magN = gW and magN = gS conditions respectively. Once this first group of four alignments was completed, the next four larvae were taken from the East to West training tank, and tested following the same sequence of magnetic field

alignments. Then, four more larvae from North to South tank were tested, and then four more from East to West tank, and so on.

Pooling the magnetic bearings from an approximately equal number of tadpoles tested in each of the four magnetic field alignments made it possible to factor out any consistent non-magnetic bias and retain only that component of the tadpoles' orientation that was consistent response to the magnetic field (see Phillips 1986b).

The distributions of bearings were analysed using standard circular statistics (Batschelet 1981). Mean vectors were calculated by vector addition, and tested for significance using the Rayleigh test. Statistics for bimodal distributions were calculated by doubling each data value and reducing any greater than 360 using modulo arithmetic. 95% confidence intervals were used to test for orientation along the y-axis.

Data from the two different training directions were analyzed separately and the Watson U^2-test was used to test for differences between the two distributions (Batschelet 1981; Mardia & Jupp 2000). Finally, the absolute or 'topographic' bearings (i.e. the distribution of bearings with respect to topographic directions ignoring the alignment of the four test magnetic fields) were also examined for any evidence of an effect of nonmagnetic directional cues on the tadpoles' orientation using the Rayleigh test.

Results

The distribution of magnetic bearings from tadpoles held in the training tank aligned east–west with the deep end towards the west showed significant unimodal orientation ($100 \pm 42°$, r = 0.39, n = 22, p = 0.036, Rayleigh test; Fig. 2a). In addition, the 95% confidence limits for the mean vector bearing include the trained y-axis. The distribution of this sample appeared somewhat bimodal, but a bimodal Rayleigh test was not significant ($102-282 \pm 24°$, r = 0.35, n = 22, p = 0.069, Rayleigh test).

Tadpoles trained in the tank that was aligned along the north–south axis, with the deep end towards the north, exhibited a significant bimodal orientation relative to the direction of the magnetic field ($7-187 \pm 20°$, r = 0.45, n = 17, p = 0.028, Rayleigh test; Fig. 2b). Again, the 95% confidence limits include the trained y-axis.

The two distributions of magnetic bearings (north–south and east–west training configurations) were significantly different ($U^2 = 0.193$, p < 0.05, Watson U^2-test) and their mean axes of orientation differed by approx. 90°.
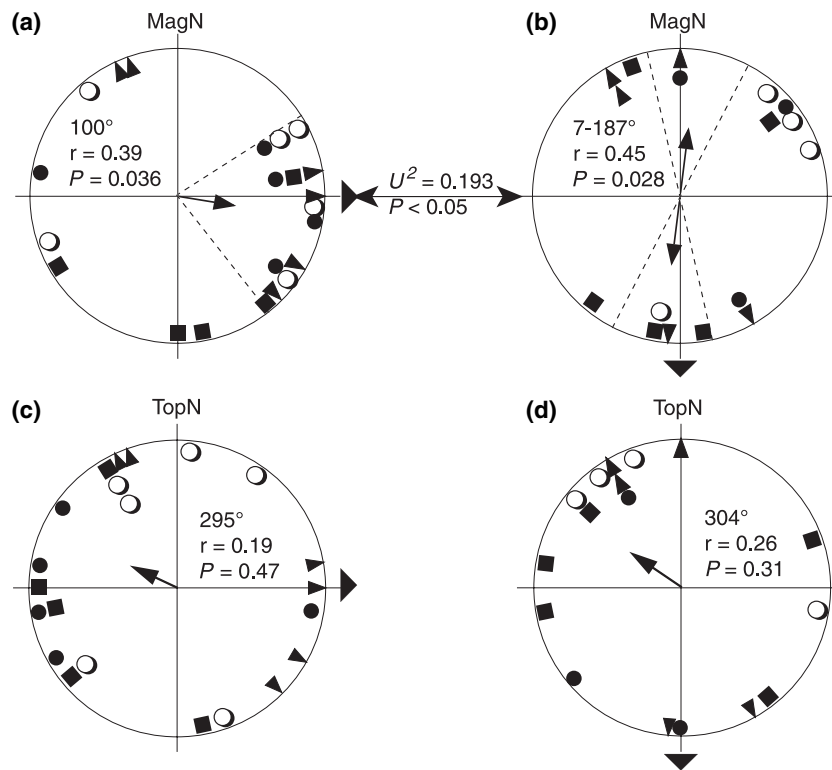
Fig. 2: Magnetic orientation of Iberian green frog tadpoles along a trained y-axis. Each data point represents the magnetic bearing of an individual tested only once in one of the four magnetic field alignments. Symbols inside the diagrams: (triangles) tadpoles tested in magnetic north (magN) = geographic north (gN) condition, (squares) tadpoles tested in magnetic north rotated to the east (magN = gE) condition, (open circles) tadpoles tested in magN = gW condition, and (closed circles) tadpoles tested in magN = gS condition. Single/double-headed arrows at the center of each circle indicate the mean unimodal/bimodal axis of each distribution. The length of each arrow is proportional to the mean vector length (r), with the radius (unimodal distributions) or diameter (bimodal distributions) of the circle corresponding to r = 1. Dashed lines represent the 95% confidence intervals for the mean vector or axis. Black triangles outside the plots indicate the magnetic direction of the shore (shallow end of the y-axis). (a) Magnetic bearings of tadpoles from the east–west tank (shore towards east). (b) Magnetic bearings of tadpoles trained in the tank with the y-axis aligned north–south (shore towards south). (c) Topographic bearings of tadpoles from the east–west tank (shore towards east). (d) Topographic bearings of tadpoles trained in the tank with the y-axis aligned north–south (shore towards south)

When the two distributions of magnetic bearings were normalized with respect to the magnetic direction of shore (i.e. pooling all bearings so that shore direction is rotated to 180°), we did not find a significant difference ($U^2 = 0.077$, $p > 0.05$, Watson U^2-test), indicating that the distribution of bearings relative to the trained y-axis was not different for tadpoles trained along the north–south and east–west axes.

The topographic bearings from both training configurations were randomly distributed (east–west tank: 295°, r = 0.19, p = 0.47, Rayleigh test, Fig. 2c; north–south tank: 304°, r = 0.26, p = 0.31, Rayleigh test, Fig. 2d).

We also examined orientation relative to solar azimuth; when the topographic bearings were plotted as deviations from the azimuth of the sun, the distribution was uniform (north–south tank: 162°, r = 0.31, p = 0.189; east–west tank: 156°, r = 0.16, p = 0.581).

Discussion

Our results indicate that *P. perezi* tadpoles use a magnetic compass to orient along the y-axis. Because the orientation of the two training groups was significantly different, and the mean vectors coincided with the shore-deep water axes of their respective training tanks, these findings indicate the tadpoles's magnetic compass response was learned. Hence, *P. perezi* is the second species of anuran amphibian in which the use of a magnetic compass has been demonstrated. Although both groups of tadpoles showed magnetic y-axis orientation, those held in

the training tank aligned east–west were oriented towards shore, whereas tadpoles trained in the tank that was aligned along the north–south axis showed bimodal magnetic compass orientation along the shore–deep water magnetic axis. However, the distribution of bearings relative to the trained y-axis does not differ between the groups (p > 0.05).

Several studies have shown a direction reversal in y-axis orientation of amphibians at the moment of metamorphosis (Goodyear & Altig 1971; Tomson 1972; Adler & Taylor 1980). For instance, larval salamanders of the genus *Ambystoma* have been shown to orient initially towards deep water but when metamorphosis takes place they reverse their directional response and orient in the shoreward direction (Taylor 1972; Tomson 1972; Adler & Taylor 1980). Presumably, both groups of Iberian green frog tadpoles used in this study should have shown unimodal orientation towards the shore because of their stage of development; tadpoles were in the last stages of metamorphosis [i.e. metamorphosis begins in stage 41 and is completed in stage 46 (Gosner 1960)]. However, only tadpoles trained in the E–W training tank oriented unimodally towards shore. Bimodal y-axis magnetic compass orientation has also been observed in amphibian urodeles (eastern red-spotted newts, *N. viridescens*: Deutschlander et al. 2000; Bosca's newts, *L. boscai*: Rodríguez-García & Diego-Rasilla 2006) and anurans (bullfrogs, *R. catesbeiana*: Freake et al. 2002; Freake & Phillips 2005), and it has been suggested that variation in training/testing protocol, and in individual animal motivation, may produce either unimodal or bimodal orientation relative to the y-axis (Freake et al. 2002).

The electric vector, or e-vector, of polarised light provides an axial cue; consequently, the use of a celestial compass based on sky polarisation patterns could result in bimodal orientation (Freake 1999). Extraocular photoreception of polarized light and its use in compass orientation have been demonstrated in amphibians (Adler 1976; Taylor & Adler 1978; Taylor & Auburn 1978). However, although the tadpole Iberian green frogs were trained and tested under natural sky light, they did not use this sort of compass information because topographic bearings (i.e. the distribution of bearings with respect to topographic directions ignoring the alignment of the four test magnetic fields) were oriented randomly. Also, there was no evidence of orientation relative to solar azimuth. Furthermore, any consistent nonmagnetic bias, including any response to celestial compass cues, was factored out of the resulting distributions by pooling the magnetic bearings from an approximately equal number of tadpoles tested in each of the four magnetic field alignments (Phillips 1986b).

In summary, the findings reported here provide the first direct experimental support for the use of a magnetic compass by *P. perezi*. These findings, in conjunction with the results of previous studies (Phillips 1986a,b; Deutschlander et al. 2000; Freake et al. 2002; Diego-Rasilla et al. 2005; Freake & Phillips 2005; Rodríguez-García & Diego-Rasilla 2006) indicate that sensitivity to the alignment of the magnetic field is a widespread sensory capability not only among urodele, but also among anuran, amphibians.

Acknowledgements

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