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RESEARCH ARTICLE

Magnetic orientation of marsh warblers (*Acrocephalus palustris*) and spotted flycatchers (*Muscicapa striata*) after simulated crossing of the magnetic equator

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ABSTRACT

Every year, billions of birds migrate to optimize their foraging, shelter and breeding. They use an inclination compass, which, unlike the technical compass, distinguishes between the directions towards the magnetic equator from the magnetic pole based on magnetic inclination angles, which range from ±90 deg at the poles to 0 deg at the equator. During autumn migration, some species cross the magnetic equator, where field lines are horizontal, i.e. the inclination angle is 0 deg. At this point, the avian magnetic compass becomes ambiguous, because the birds can no longer distinguish 'to the pole' from 'to the equator'. Experiments with bobolinks and garden warblers have shown that these birds adaptively change their orientation when exposed to a horizontal magnetic field. We tested this in marsh warblers and spotted flycatchers, but they showed no such response, suggesting they may use other cues. This indicates that different species may rely on varying stimuli, and the current experimental models may not be universally applicable.

KEY WORDS: Behavioral ecology, Animal navigation, Orientation, Bird migration, Long-distance migration, Magnetoreception, Magnetic compass, Trans-equatorial migration, Cross-equatorial migration, Cross-equatorial reorientation, Reproducibility of magnetoreception research

INTRODUCTION

The Earth's magnetic field is a source of information about the cardinal directions and position in space for many classes of vertebrates: fishes (Quinn, 1980; Souza et al., 1988), amphibians (Leucht, 1990), reptiles (Rodda, 1984; Merrill and Salmon, 2011), birds (Wiltschko and Wiltschko, 1972) and mammals (Holland et al., 2006). Many living creatures possess magnetoreception as another sensory system, in addition to vision, smell, mechanoreception and electroreception, which are much better studied. Thanks to magnetoreception, some animals can perceive magnetic fields in the surrounding environment and changes in its properties such as field direction, intensity and gradient, where the effect on organisms can manifest as an array of reactions. Migratory birds have been best studied in this regard (Wynn and Liedvogel, 2023), as they can perceive the Earth's magnetic field and use it as a cue for orientation and navigation (Mouritsen, 2018; Wiltschko and Wiltschko, 2019).

The ability of birds to use magnetic field information for orientation was first discovered and described in the 1960s in experiments in European robins, *Erithacus rubecula* (Merkel and Fromme, 1958; Wiltschko and Wiltschko, 1972). However, the sensory, physiological and biophysical mechanisms of this compass system are still incompletely understood (Mouritsen, 2018). Birds are known to possess a magnetic compass based on inclination, which is sensitive to the direction of the axis dip of the magnetic field, rather than to its polarity (Fig. 1; Wiltschko and Wiltschko, 1972). They cannot determine the direction to the South or North Pole because, unlike the technical compass used by humans, the magnetic compass of birds does not sense the polarity of the magnetic field. Instead, birds can determine the axial course of the field lines (Wiltschko et al., 2021). Thus, migratory birds can only determine the directions 'towards the pole' (poleward), where the field lines are tilted downward (Fig. 1D), and 'towards the equator' (equatorward), where they are pointing upward (Fig. 1B). Of particular interest are avian species that migrate across the magnetic equator, where the magnetic field lines run parallel to the Earth's surface at an angle of 0 deg (horizontal magnetic field; Fig. 1C). In such a situation, it becomes impossible for birds to distinguish between the equatorward and poleward directions. Songbirds can use inclination angles of down to at least 5 deg for orientation (Schwarze et al., 2016), i.e. there is a rather wide corridor around the magnetic equator unsuitable for using a magnetic compass.

It is unclear how the birds that have never migrated and have not yet learnt the magnetic map cross the magnetic equator. How do they determine where to go next to reach their wintering grounds in the southern magnetic hemisphere? The most obvious answer is that being in a horizontal magnetic field triggers a change in direction of flight from equatorward to poleward (in the first migration of young birds, towards the South Pole). It is an equivalent of the innate knowledge of magnetic beacons, where certain magnetic conditions along a migratory route induce hyperphagia in birds, allowing them to store enough fat to overcome a major environmental barrier. These magnetic beacons may serve as natural cues that allow birds to at least roughly realise where they are on their migration route (Fransson et al., 2001).

This solution is inconsistent with the concept that first-time migrants completely rely on an innate spatio-temporal programme during their journey (Gwinner and Wiltschko, 1978). In a simplified form, this programme can be formulated as follows: 'start migration at such-and-such an age, fly in the direction α for n days, then change course to β and maintain it for m days, etc., finally arrive at

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Received 17 June 2024; Accepted 16 December 2024

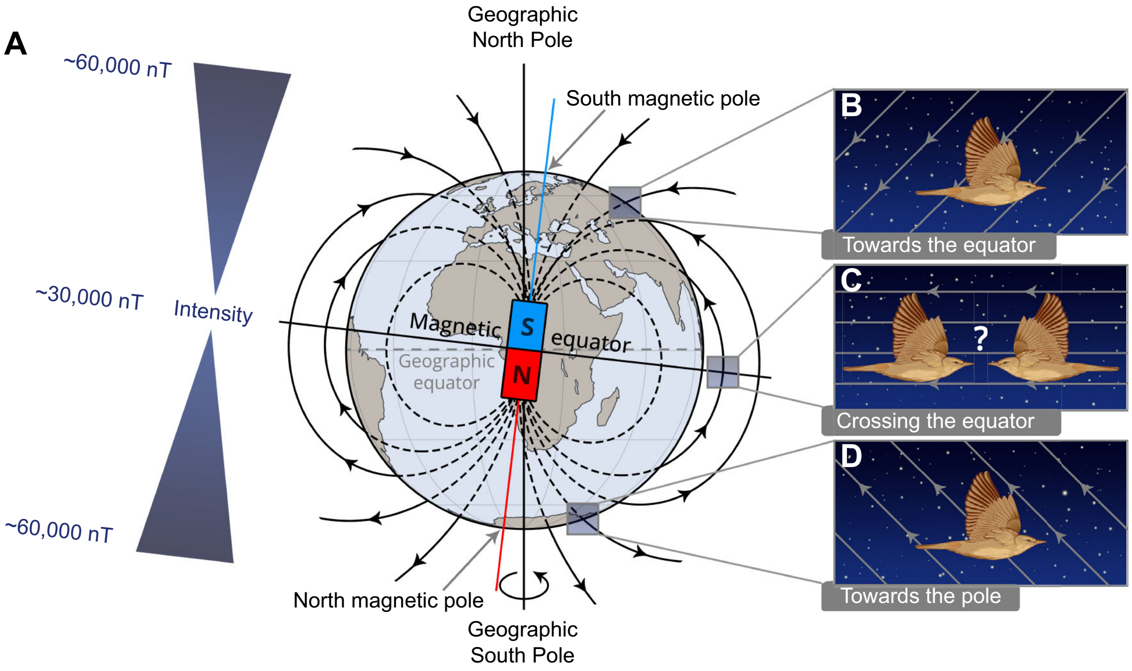

Fig. 1. Autumn transequatorial migration of birds. (A) An image of the Earth's magnetic field showing how the field lines (indicated by black arrows) cross the Earth's surface and how the angle of inclination varies with latitude. At the magnetic equator (the black line crossing the Earth), the field lines are parallel to the surface. The field lines become steeper as they move towards the magnetic poles, where they are perpendicular to the Earth's surface (inclination is 90 deg). The total magnetic field intensity, which diminishes from the magnetic pole to the equator, is displayed on the left. (B–D) Scenario of autumn migration relative to inclination lines. (B) The direction of the magnetic field lines in the Northern Hemisphere. During autumn migration, the bird flies 'towards the equator' (equatorward). (C) The direction of the magnetic field lines at the magnetic equator (horizontal magnetic field, 'blind spot'). (D) The direction of the magnetic field lines in the Southern Hemisphere. During the autumn migration, the bird flies 'towards the pole' (poleward).

the wintering site'. It assumes that naive migrants do not know where they are during their first migration. This 'clock-and-compass' concept has been formulated on the basis of experimental data, and the distribution of long-distance recoveries of ringed birds does not contradict it (Mouritsen, 1998a; Mouritsen and Mouritsen, 2000). Nevertheless, continuation of southward flight after crossing the magnetic equator using a magnetic compass is impossible within the framework of this concept.

At present, there is only a handful of studies that shed light on the mechanisms that allow migratory birds to cross the magnetic equator. Bobolinks, *Dolichonyx oryzivorus*, which breed in the central part of North America and spend their winter in central and far western South America, were placed in a planetarium, where a static drawing of the starry sky was shown to them. During the planetarium tests, they were exposed to a series of successively alternating artificial magnetic fields, which correspond to the natural magnetic fields on the migration path through the equator to the Southern Hemisphere. Bobolinks continued to orient southwards throughout the experiment, which suggests that experiencing the horizontal magnetic field of the equator, possibly in combination with star cues, allowed them to reorient from equatorward to poleward during migration (Beason, 1987, 1989, 1992). However, with this experimental design, it is impossible to separate the operation of the magnetic and star compasses, so we cannot say with certainty that bobolinks used the magnetic field for orientation. Perhaps they ignored the magnetic field and used the stars in the planetarium for orientation.

In another study on garden warblers, *Sylvia borin*, which are also transequatorial migrants, birds were captured as migrants in Frankfurt, central Germany. They were kept in individual cages under conditions mimicking the local geomagnetic field and natural photoperiod. Bird orientation before simulated crossing of the magnetic equator and afterwards was determined in an octagonal cage with a diameter of 1 m and a height of 35 cm, equipped with eight radially arranged twin perches. The test rooms were wooden huts in the garden, lit by dim, diffuse light. For simulating the crossing of the magnetic equator during autumn migration, birds were placed in a horizontal magnetic field created with Helmholtz coils for 2 days and 2 nights in laboratory conditions. Exposure to the horizontal magnetic field caused birds to change their direction from equatorward to poleward (Wiltschko and Wiltschko, 1992), i.e. it triggered significant changes in migration behaviour.

Despite a large body of literature on the ability of songbirds to perceive magnetic field, we still do not have a complete understanding of when, where and how birds use magnetic signals in making migration decisions in natural habitats. This is particularly notable given the low reproducibility of the results in experimental studies involving magnetic signalling (Karwinkel et al., 2024). We tested the hypothesis and results of Wiltschko and Wiltschko (1992), which were obtained on a single species, the garden warbler, and have never been replicated by other researchers on other species. Re-testing such hypotheses is essential to increase their validity and guide future research.

However, we could not exactly replicate that work, as in recent years, garden warblers have not shown a reliable orientation direction when tested in a natural magnetic field on the Curonian Spit (Julia Bojarinova, personal communication). Thus, for this study, we selected marsh warblers, *Acrocephalus palustris*, and spotted flycatchers, *Muscicapa striata*, which are frequently captured at the Biological Station Rybachy, allowing us to gather the number of birds required for the experiment.

Both marsh warblers and spotted flycatchers are transequatorial migrants. Marsh warblers breed in Europe and winter in Central and Southern Africa (Dowsett-Lemaire and Dowsett, 1987; Fig. 2A). Their migration occurs in two stages: from August to September, they reach the Red Sea coast of Sudan and Eritrea, where they stop for several months before continuing southward through eastern Kenya, typically arriving at their wintering grounds by December to January (Åkesson, 1993). Spotted flycatchers are also widely distributed during the breeding season across Europe, western Asia and parts of northwestern Africa. They winter in sub-Saharan Africa, extending to the southernmost parts of the continent (Cramp and Perrins, 1993; Fig. 2B).

MATERIALS AND METHODS

Study site, model species and bird keeping

Trapping of marsh warblers and spotted flycatchers was conducted near the Biological Station Rybachy (BSR) on the Curonian Spit (Kaliningrad Region, Russia). The birds were kept at the BSR, and orientation tests took place in a clearing of reeds on the coast of the Curonian Lagoon (dates for each stage can be found in Table S1). Immediately after capture, we determined age, mass, body fat, moulting stage and general body condition of the birds. For the study, we selected only naive (first-year) birds that had not yet migrated even once and never crossed the equator. All selected birds had a body fat score of 3–4 or higher according to a scale of 0–8 (Kaiser, 1993). Birds with a fat score <3 were released. In total, 21 marsh warblers were captured between 20 July and 6 August 2023, and 25 spotted flycatchers were captured between 26 July and 21 August 2024.

Before starting orientation tests, the birds were housed in a rain-protected outdoor aviary in individual mesh cages (60×40×40 cm). They had access to all celestial cues, such as the sun and polarised light, stars and undisturbed geomagnetic field, and were kept under the natural photoperiod. Throughout the experiment, each bird was placed in a separate compartment of the cage (30×40×40 cm). They were fed a diet consisting of mealworms, mixed food (eggs, carrots,

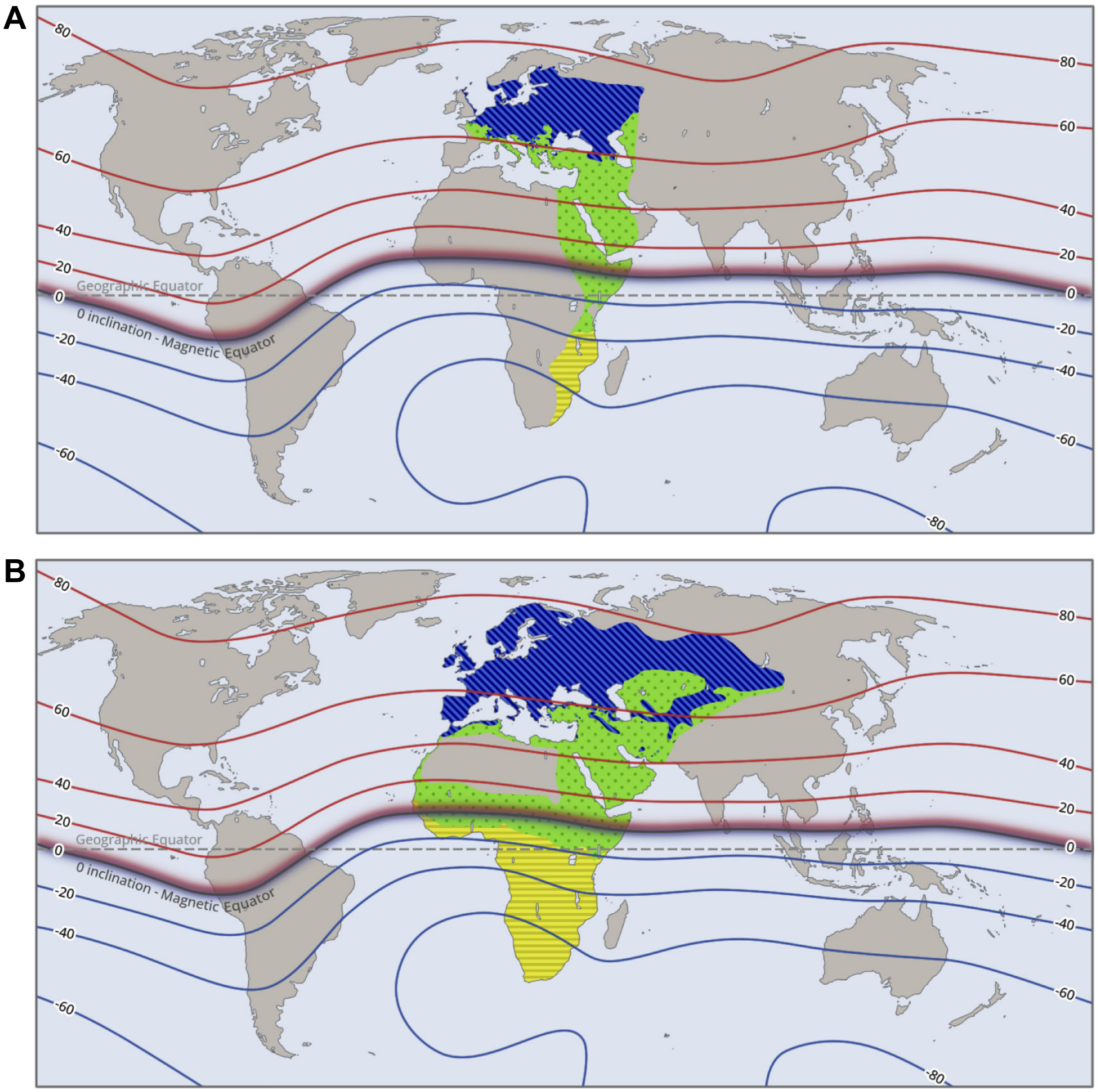

Fig. 2. Map of breeding and wintering areas. (A) Marsh warblers and (B) spotted flycatchers (according to data from BirdLife International, 2024). The map indicates the following: breeding area (blue, diagonal hatching); final non-breeding area (yellow, horizontal hatching); and migration area (green, dotted hatching). Blue and red lines are the inclination lines of the Earth's magnetic field. The grey dashed line is the geographic equator. Bold translucent blue–red line in the middle of the map is the magnetic equator. The line thickness corresponds to the area of the 'blind spot', where the avian magnetic compass cannot be used.

breadcrumbs) and feed for insectivorous birds (Padovan: cereals, breadcrumbs, oils and fats, derivatives of vegetable origin, vegetable protein extracts, fruit, various sugars, minerals, molluscs and crustaceans, sorbitol, insects; VALMAN SRL, Italy). Additionally, the birds received pure water *ad libitum*, in which the vitamin supplement Radostin Vitasil (31500 IU of vitamin A, 6750 IU of vitamin D3, 25 mg of vitamin E, which are in physiologically justified ratios; company producing 'NVT Agrovetzaschita' LLC, Russia) was dissolved.

The fat score and mass of each bird were estimated every day for 3–4 days after trapping. If these parameters constantly decreased for 2 days (which indicates an excessively high level of stress), we released these birds into the wild. The outdoor aviary was equipped with Internet Protocol cameras with infrared LEDs so that we could monitor the activity and behaviour of birds in their cages in real time at night. For experiments, we selected only the birds that exhibited migratory restlessness (known as Zugunruhe; Kramer, 1949) on the night of the tests. Each day before the tests, we monitored the birds' activity in the aviary and recorded our observations on the experiment protocol sheet. Once we had collected the required number of birds, we entered the aviary to catch the necessary individuals and transferred them from the cages to the experimental site inside individual opaque textile bags.

Ethics

The current research was carried out in compliance with the ARRIVE guidelines (https://arriveguidelines.org) and all animal procedures (bird trapping and simple non-invasive behavioural experiments) were approved by the Ethics Committee of the Zoological Institute of the Russian Academy of Sciences (permit nos 3-15/30-08-2023 and 4-15/08-05-2024).

Experimental set-up and test conditions

The experiment consisted of three stages (Fig. 3): (stage I) control tests before exposure to the horizontal magnetic field; (stage II) simulated crossing of the magnetic equator, birds were exposed to the horizontal magnetic field for 2 days and 3 nights; and (stage III) tests after simulated crossing of the magnetic equator.

Stages I and III were conducted on an open experimental site on the shore of the Curonian Lagoon away from buildings, which minimised the possible impact of electromagnetic noise and artificial light sources on the orientation of birds in the experiments. Additionally, we aimed to conduct all orientation tests on moonless nights to avoid phototactic responses in the birds. The trials began with the onset of astronomical twilight. Each test lasted 40–45 min, with a maximum of two tests per bird per night. After 2–3 overnight tests, we tried to give the birds a 1-day rest.

Stage II took place at the same site. We exposed marsh warblers and spotted flycatchers to a horizontal magnetic field that was artificially created using a three-dimensional four-coil Merritt magnetic coil system ('magnetic coils' hereafter; Kirschvink, 1992). This magnetic coil system produces a field with more than 99% uniformity in a space of approximately 110×110×110 cm.

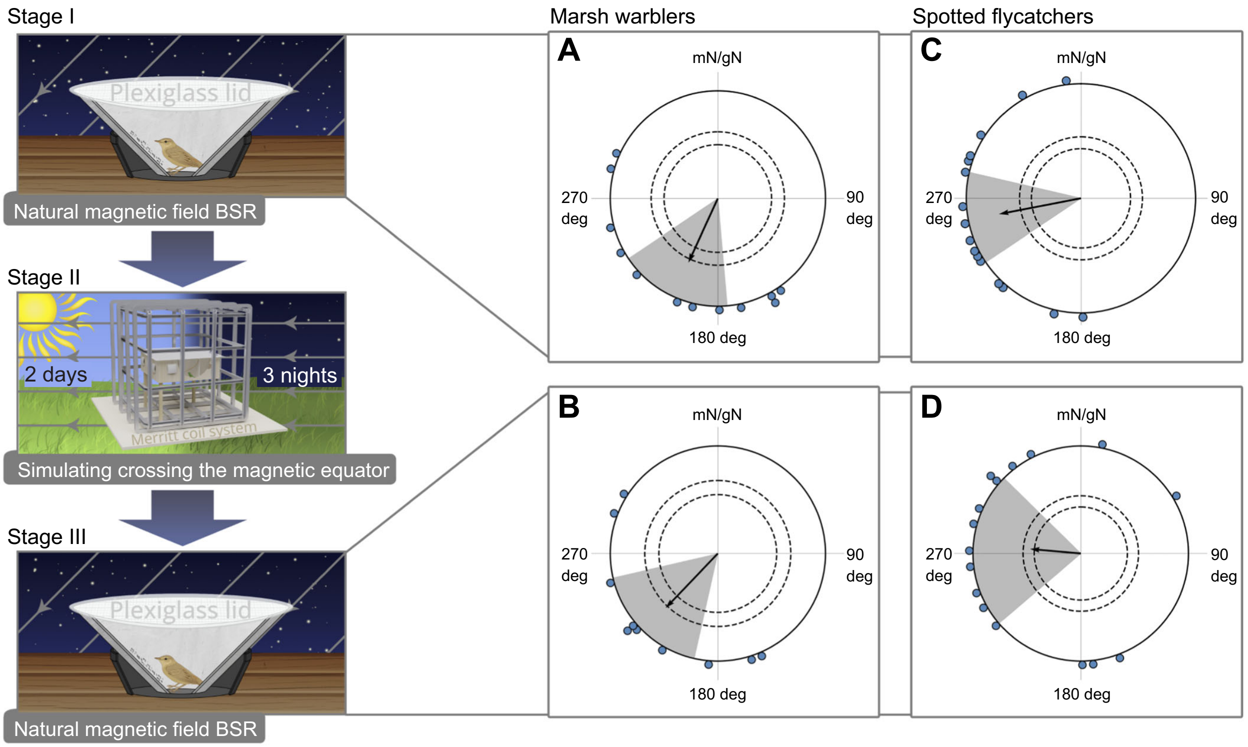

Fig. 3. Design and results of the orientation experiments of marsh warblers and spotted flycatchers. (A,C) The orientation of birds in the Emlen funnel without access to celestial cues in the natural magnetic field of Rybachy (stage I of the study, before exposure in a horizontal magnetic field): (A) the mean direction of marsh warblers was 205 deg; (C) the mean direction of spotted flycatchers was 259 deg. (B,D) The orientation of birds in the Emlen funnel without access to celestial cues in the natural magnetic field of Rybachy [stage III, after exposure for 2 days and 3 nights in a horizontal magnetic field (stage II)]: (B) the mean direction of marsh warblers was 224 deg; (D) the mean direction of spotted flycatchers was 275 deg. In all circular charts, the dots at the periphery of the circle represent the mean heading of a single bird, the arrow represents the group orientation mean vector (circle radius represents a vector length r=1); the grey sector indicates the 95% CI; the inner and outer dashed circles indicate the required length of r for significance levels of 5% and 1% according to the Rayleigh test, respectively; mN, magnetic north; gN, geographical north. The parameters of the magnetic field generated by the Merritt coil system in stage II are intensity 50,500 nT, inclination 0 deg; BSR, Biological Station Rybachy.

Each of the three axes of the coils was powered by a separate BOP 50-4M DC power source (Kepco Inc., USA), placed together with the coil control unit in a shielded and grounded aluminium box located approximately 2 m from the magnetic coil system. The box significantly reduced the noise of the operating power supplies, so that it was much lower than the level of natural ambient noise near the experimental setup. During the exposure, the birds were housed in individual wooden cages (30×30×30 cm; Fig. S2A–D) within the Merritt magnetic coil system (maximum of 9 birds). For the first 2 days of exposure, the birds were placed inside the magnetic coils, in which the magnetic field remained natural. These conditions can be simulated using a double-wrapped copper wire system, allowing current to flow in two directions. When the current flows in the parallel direction, an artificial magnetic field is created, and when it flows in the antiparallel direction, the artificial magnetic field is cancelled out, so that the resulting field does not differ from the natural one. During this short period, the birds had access to all celestial cues as before and could adapt to the new conditions and the slight noise produced by the power units of the magnetic coils. After that, the horizontal magnetic field was simulated for 2 days and 3 nights (intensity 50,500 nT; inclination 0 deg). During this period of exposure, the cages with birds were covered with plexiglass lids (plastic thickness 3 mm, transmission 70%), which did not allow them to observe any celestial cues. In stage III, after the exposure in the horizontal magnetic field, we tested the birds again in the natural magnetic field of the Curonian Spit, without access to any celestial cues.

Orientation tests and data analysis

On 9 August 2023, we began stage I of orientation test on marsh warblers. The same phase of testing for the spotted flycatchers started on 26 August 2024. For both bird species, we used Emlen funnels to determine species-specific orientation in the natural magnetic field of BSR on the Curonian Spit, which we had previously measured using a vector magnetometer FVM400 (Meda Inc., USA) with intensity 50,507±48 nT (mean±s.d.) and inclination 69.9±0.61 deg. All orientation experiments started at least 1 h after local sunset. The tests were conducted under favourable weather conditions, on moonless nights. Usually it is clear skies, calm or light winds, and no rain, storms or other adverse weather events that may interfere with bird behaviour or accuracy of test results. In 2023, during this phase, 12 out of 21 marsh warblers demonstrated significant orientation direction (others were inactive or showed only one value during the set tests). These 12 birds continued to participate in further stages of the study. In 2024, 16 out of 25 spotted flycatchers showed significant orientation direction, and these 16 birds proceeded to the next stage of the study.

Stage II involved exposing the birds to a horizontal magnetic field (simulating the crossing of the magnetic equator), created using the Merritt coil system. Because the magnetic coils can only hold 9 birds, we divided all birds into two groups: 8 and 4 each for marsh warblers (the first group was exposed from 5 September and the second from 11 September); and 8 and 8 each for spotted flycatchers (the first group was exposed from 5 September and the second from 12 September).

In stage III of the experiment, when the birds passed a virtual crossing of the magnetic equator (based only on the magnetic field parameters), they had to assume that they were in the Southern Hemisphere. To avoid conflict between magnetic and astronomical compasses after the exposure, cages with the birds after stage II were covered with polyethylene film that scattered light and made visual landmarks specific to the Northern Hemisphere invisible. We also transferred birds from the outdoor aviary to the experimental site on the shore of the Curonian Lagoon in dark, opaque cloth bags. These measures prevented the birds from observing celestial landmarks characteristic of the Northern Hemisphere until the end of the experiment.

The Emlen funnel for the orientation tests were made of aluminium (diameter of upper part 34 cm, lower part 10 cm, height 14 cm, angle of the wall 45 deg). Inside the funnels, we placed polyethylene films cut to size and covered with a dried mixture of whitewash and glue, in order to fix the scratches of the birds in the direction in which they wanted to move (Emlen and Emlen, 1966; Mouritsen, 1998b; Mouritsen and Larsen, 1998). During the tests (stages I and III), the Emlen funnel was covered with a plexiglass lid (plastic thickness 3 mm, transmission 70%), which completely hid the stars and any other celestial cues, but allowed sufficient light to operate the light-dependent magnetic compass (for details, see Romanova et al., 2023). Thus, the only cue available to the experimental birds during the tests was the local geomagnetic field of BSR.

During the experiments, Emlen funnels were placed on a wooden table according to a pre-prepared random scheme, ensuring a double-blind method and preventing bias in the results owing to researcher expectations. Each evening, one of the researchers created a random placement scheme for the funnels, in which each funnel could be rotated clockwise at 90, 180 or 270 deg, or left at its original position of 0 deg, pointing to the magnetic north. All funnels were numbered and positioned according to this scheme, but only one researcher was aware of their exact orientation angles. When the birds were placed in the funnels, no one knew which specific bird was in each funnel, as the distribution was random. After completing a series of tests, the researchers retrieved the birds, and recorded the ring number on the bird's tarsus and the number of the funnel in which the test took place. The researchers evaluating the birds' orientation direction considered the funnel's seam as 0 deg. After assessing all the funnels, the researcher who placed them used a placement scheme to correct for the actual angle of rotation of each funnel relative to geographic north. For example, if a funnel had been rotated by 270 deg, 270 deg was added to the calculated direction. Thus, if the bird's orientation was determined to be 285 deg, the actual direction, considering the funnel's rotation, would be 195 deg. Also, at the time of counting, the researchers did not know which birds the data were obtained from.

The orientation direction of the birds was determined by the scratches on the polyethylene film left by their claws when they jump in the funnels. The data (the average direction of each bird according to the distribution of scratches) were analysed by a group of three independent researchers (the group was formed from G.U., P.G., A.G., A.P., Dmitry Sannikov and Vladimir Belyakov). In most cases, the average direction can be determined very accurately using a simple visual assessment method (Mouritsen and Larsen, 1998). If two out of three observers thought that the distribution of scratches was random, or if there was more than 30 deg between the two average directions, the bird was considered disoriented, and these results were not included in the final analysis. Also, if the number of scratches was <40, the bird was considered inactive. In cases where the scratch pattern was indistinct (i.e. marks or scratches were unclear, faint or not well defined, possibly owing to their thinness, which made them difficult to detect, or to their irregular or inconsistent arrangement, which made it impossible to visually determine the direction), we counted the scratches in each of the thirty-six 10 deg sectors and used a specially written script to estimate the directionality based on the number of scratches (code is available at https://github.com/MagBBBGroup/BirdScratches_analysis).

Statistical tests were carried out in ORIANA 4.0 (Kovach Computing Services, UK), and only those birds that showed at least two directional non-random orientation values in each experimental stage and had a length of the average vector *r* for all tests of an individual bird greater than 0.2 were included in the final analysis (as in Kobylkov et al., 2019). The differences in orientation between the two test stages (before and after exposure) were checked using the non-parametric Mardia–Watson–Wheeler test (MWW; Batschelet, 1981). Among other things, we used a Monte Carlo method to determine the probability of a type II error to find out at what angle of rotation and with what probability we could detect a change in the direction of movement of the birds after exposure to a horizontal magnetic field (a link to R code can be found in the Fig. S1D,E legend). Such an error could, for example, have occurred because of a small sample size. To this end, we combined data from stages I (n=12) and III (n=10) of the experiment and estimated the parameters of the parent Von Mises distribution. Then, we generated pairs of random samples from this distribution and a similar one, with the mean shifted by a certain value (15, 30, 45 deg and further up to 180 deg with a step of 15 deg). For each shift value, we derived 10,000 such pairs and estimated the probability of obtaining the P-value for the MWW test as high or higher than for testing the original two samples, thus obtaining the type II error probability. The data used for the final analysis are available in Table S1.

After the orientation experiments were completed, we ensured that all birds were returned to their natural habitat. Before release, we assessed their muscle condition and fat levels to ensure they were well prepared to survive in the wild.

RESULTS

Results of experiments with marsh warblers in 2023

At stage I of the study, marsh warblers were oriented to the southwest in the natural magnetic field (Fig. 3A). The mean direction of the group was 205 deg [r=0.65, n=12, P=0.004, 95% confidence interval (CI)=174–236 deg]. At stage III, after exposure to a horizontal magnetic field, the marsh warblers did not change their orientation direction (Fig. 3B), and the mean direction was 224 deg (r=0.70, n=10, P=0.004, 95% CI=189–259 deg). Two birds, after exposure, did not show a mean orientation value with r>0.2; therefore, they were not included in the final analysis at stage III. This indicates that the two birds failed to exhibit a consistent or significant directional preference in their orientation behaviour, as measured by the mean vector length (r). The value of r is a statistical measure of concentration or consistency in circular data, with r>0.2 indicating a meaningful level of directional orientation. As these birds had r-values less than or equal to 0.2, their orientation was deemed too inconsistent to be included in the final analysis at stage III. However, even if we use the average values of these birds in the analysis, the orientation of the group did not change at this stage (225 deg, r=0.60, n=12, P=0.009, 95% CI=190–256 deg; Fig. S1A) and remained significant. We checked for differences in the group orientation at stages I and III of the experiment using the non-parametric MWW test. The test results indicated that the orientation of the birds before and after exposure was statistically indistinguishable (W=0.73, P=0.69 not including two birds after exposure, n=10; W=0.2, P=0.90 including two birds after exposure, n=12), and their 95% CI overlapped widely.

Moreover, the analysis of angular difference within the same individuals in the two conditions (before and after exposure to an artificially generated horizontal magnetic field) showed that most marsh warblers exhibited a consistent directional preference across the two trials. Values exhibited a unimodal distribution with a mean direction of 359 deg (r=0.36, n=12, P=0.21; see Fig. S1B); the 95% CI=287–71 deg includes 0 deg. However, it is important to note that these results were not statistically significant. When looking at individual subject data, the two marsh warblers showed a change in orientation direction similar to that observed for garden warblers (Wiltschko and Wiltschko, 1992). One of these two birds (ring RR03943, Table S1) did not show a significantly oriented result in the post-exposure tests. Nevertheless, if we analyse intra-individual angular differences without considering these two birds, the result does not change: α=0 deg (r=0.43, n=10, P=0.15, 95% CI=298–63 deg). The prolonged exposure to laboratory housing conditions may have caused stress in the birds, which may have affected their ability to orient. In addition, the change in orientation could have been random and unrelated to the magnetic field.

We conducted Monte Carlo simulations to estimate the probability of a type II error and found that, with the concentration parameter of the pooled sample maintained, a 60 deg difference in the mean values of the two general populations would result in the type II error probability below 0.05 (Fig. S1D).

Results of experiments with spotted flycatchers in 2024

At stage I of the study, spotted flycatchers were oriented to the southwest in the natural magnetic field (Fig. 3C). The mean direction of the group was 259 deg (r=0.73, n=16, P<0.001, 95% CI=237–281 deg). At stage III, after exposure to a horizontal magnetic field, spotted flycatchers did not change their orientation direction relative to the magnetic compass (Fig. 3D); the mean direction was 275 deg (r=0.47, n=16, P=0.026, 95% CI=236–315 deg). The differences in the group orientation between stages I and III of the experiment were checked using the non-parametric MWW test. The orientation of the birds before and after exposure was statistically indistinguishable (W=2.26, P=0.32), and their 95% CI overlapped widely.

Analysis of intraindividual angular differences between the same individual spotted flycatchers under two conditions (before and after exposure to an artificially generated horizontal magnetic field) showed that a proportion of birds maintained a consistent directional preference in the two trials. Values showed a unimodal distribution with a mean direction of 347 deg (r=0.41, P=0.3; see Fig. S1C); the 95% CI=334–52 deg includes 0 deg.

We found that with the concentration parameter of the pooled sample preserved, the difference of 97 deg in the mean values of the two general populations would result in the type II error probability below 0.05 (Fig. S1E).

DISCUSSION

Because the difference in the directional orientation of birds before and after exposure was 19 deg for marsh warblers and 16 deg for spotted flycatchers, our data clearly show that neither species changed its orientation after exposure in the horizontal magnetic field as appropriately (by 173 deg) as garden warblers did (Wiltschko and Wiltschko, 1992). Beason (1987, 1989, 1992) and Wiltschko and Wiltschko (1992) suggested that the zero inclination of the magnetic field at the magnetic equator may trigger reorientation from equatorward to poleward. Our data do not support this idea: after a 2-day exposure to a horizontal magnetic field without access to celestial cues, marsh warblers and spotted flycatchers in orientation tests did not change their migration direction and continued to orient towards the southwest as before the exposure. These results are in sharp contrast to the results obtained by Wiltschko and Wiltschko (1992) in garden warblers.

A possible explanation for the lack of a reorientation response in marsh warblers and spotted flycatchers is that our simulation of crossing the magnetic equator was not sufficiently realistic (Kishkinev et al., 2021); there is no real place on our planet with the magnetic field parameters used in this study, according to the ViDMAL tool (Schneider et al., 2023). In all areas with the horizontal field, the field is much weaker than in our simulation. However, it may be noted that in a recent virtual displacement study, Eurasian reed warblers (*Acrocephalus scirpaceus*) disregarded field intensity as a positional cue and only relied on inclination and declination values (Packmor et al., 2024). Therefore, this pitfall might be less critical than often assumed.

As in the original study by Wiltschko and Wiltschko (1992) with garden warblers, we exposed the birds to a horizontal magnetic field for 2 days and 3 nights, rather than exposing them to a series of successively alternating artificial magnetic fields, corresponding to the natural magnetic fields on the migration path to the Southern Hemisphere through the equator (e.g. Fransson et al., 2001; Bulte et al., 2017). The duration of this exposure period in the artificial horizontal magnetic field can be justified by the fact that the average flight speed of the marsh warbler and spotted flycatchers ranges from 8.8 to 13.4 $\mathrm{m\,s^{-1}}$ (Bruderer and Boldt, 2001). Blackcaps (*Sylvia atricapilla*) were shown to be able to orient in the magnetic field with a shallow tilt angle of only 5 deg as efficiently as in a normal geomagnetic field of the Northern Hemisphere (67 deg inclination) (Schwarze et al., 2016). This suggests that the region where these birds cannot orient is smaller than the area with ±5 deg inclination. The inclination in Earth's magnetic field varies by approximately 0.009 deg $\mathrm{km^{-1}}$, the upper limit of the extent of the magnetic compass blind spot around the equator being $(2\times5\ \mathrm{deg})/(0.009\ \mathrm{deg\ km^{-1}})=1110\ \mathrm{km}$. However, if the limit of inclination detection turns out to be ca. 2–3 deg, as seems to be the case for steep inclinations, the blind spot of the magnetic compass will be ca. 440–660 km wide (Åkesson et al., 2001; Schwarze et al., 2016). Based on the average flight speed mentioned above, marsh warblers and spotted flycatchers would traverse the magnetic equator's blind spot in approximately 3 nights during migratory flight. This would only hold true if the birds do not make any stopovers in the area.

It is also possible that the temporal factor is a key aspect that caused the birds not to show the expected response (Chernetsov et al., 2008). It is likely that the signal to change direction should be given at a certain stage of their spatiotemporal programme. Temporal components may suppress the effect of geomagnetic map signals on migration preparation both at the beginning of the season (Kullberg et al., 2007) and at the end of the season (Kullberg et al., 2003). In addition, the duration of exposure of marsh warblers to the artificially created horizontal magnetic field may have been insufficient. Under natural conditions, these birds spend several months in areas close to the magnetic equator, which may be a period of adaptation to the new conditions and may also affect the absence of changes in flight direction. Furthermore, it cannot be ruled out that when crossing the magnetic equator during migration, where the magnetic compass does not function, birds might use non-magnetic cues, such as information from the star compass (Emlen, 1967a,b). Our group previously showed that the sensitive period for learning the star compass is not limited to the time before the first migration, that is, in autumn; birds can also learn and use the star compass in spring (Zolotareva et al., 2021). During migration, they may observe the starry sky while making stops along their route. For example, marsh warblers make an extended stop in Kenya, where they can learn new star patterns.

In any case, our data show that the protocol used by Wiltschko and Wiltschko (1992) in their garden warbler study apparently did not work for marsh warblers or spotted flycatchers. It is also possible that the lack of response after exposure to a horizontal magnetic field is due to species-specific or even population-specific variation. In species with a wide distribution, different populations may use different migration routes, which could require distinct migration programs. For example, permanently remaining in the magnetic field of southern Germany was sufficient for garden warblers to execute their migration program. In order to obtain the same result in migrant pied flycatchers, the birds had to be exposed to a series of successively changing magnetic field values that normally occur along their migratory route (Beck and Wiltschko, 1988).

In conclusion, our results indicate that probably no single solution for the problem of crossing the magnetic equator exists. Birds that use the inclination magnetic compass during migration need to switch from flying towards the equator to flying towards the pole, but stimuli that tell them that it is time to do so may differ between species. This situation is not unlike the problem of integration of compass information from different cues, where no uniform solution seems to exist either (Pakhomov et al., 2022).

Acknowledgements

We express our gratitude to Vladimir Belyakov, Anna Pallak, Antonina Solopova, Dmitry Sannikov and Alisa Starodubova for their assistance in caring for the birds and conducting the experiments. We also thank Mikhail Markovets for help in capturing birds, and Fyodor Cellarius for independently checking the statistical methods we used for our results and for help with Monte Carlo modelling. Additionally, we acknowledge Henrik Mouritsen for granting permission to use the magnetic coils owned by his group, despite not being directly involved in the design and execution of this study. We are also deeply grateful to the two anonymous reviewers for their insightful and constructive comments. Their feedback played a crucial role in significantly enhancing the quality of our article and refining its clarity. Furthermore, their suggestions have helped us strengthen the reproducibility of the study, ensuring that the research can be more effectively replicated and built upon by other scientists in the field.

Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: G.U., A.P., N.C.; Methodology: G.U., P.G., A.G., A.P., N.C.; Validation: P.G., A.G., A.P., N.C.; Formal analysis: G.U., P.G., A.P.; Resources: N.C.; Data curation: A.P., N.C.; Writing - original draft: G.U.; Writing - review & editing: P.G., A.P., N.C.; Visualization: G.U.; Supervision: N.C.; Project administration: A.P., N.C.

Funding

Financial support for this study was made available by the Russian Science Foundation (grant no. 24-14-00085 to N.C.).

Data availability

All relevant data can be found within the article and its supplementary information.

ECR Spotlight

This article has an associated ECR Spotlight interview with Gleb Utvenko.

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