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SUMMARY

Displacement experiments have demonstrated that experienced migratory birds translocated thousands of kilometers away from their migratory corridor can orient towards and ultimately reach their intended destinations [1]. This implies that they are capable of "true navigation", commonly defined [2–4] as the ability to return to a known destination after displacement to an unknown location without relying on familiar surroundings, cues that emanate from the destination, or information collected during the outward journey [5–13]. In birds, true navigation appears to require previous migratory experience [5–7, 14, 15, but see 16, 17]. It is generally assumed that, to correct for displacements outside the familiar area, birds initially gather information within their year-round distribution range, learn predictable spatial gradients of environmental cues within it and extrapolate from those to unfamiliar magnitudes – the gradient hypothesis [6, 9, 18–22]. However, the nature of the cues and evidence for actual extrapolation remains elusive. Geomagnetic cues (inclination, declination and total intensity) provide predictable spatial gradients across large parts of the globe and could serve for navigation. We tested the orientation of long-distance migrants, Eurasian reed warblers, exposing them to geomagnetic cues of unfamiliar magnitude encountered beyond their natural distribution range. The birds demonstrated re-orientation towards their migratory corridor as if they were translocated to the corresponding location but only when all naturally occurring magnetic cues were presented, not when declination was changed alone. This result represents direct evidence for migratory birds' ability to navigate using geomagnetic cues extrapolated beyond their previous experience.

KEYWORDS: magnetic sense, animal navigation, magnetic map, bird migration, magnetoreception, extrapolated map, true navigation, position determination, bicoordinate navigation.

RESULTS

Testing the gradient map hypothesis

The gradient map (or extrapolated map) hypothesis assumes that once birds have learned the spatial gradients of some environmental cues in their familiar year-round distribution range, they should be able to respond to such cues even outside their familiar range of magnitude if displaced to unfamiliar areas (Figure 1) [19–22]. However, the gradient map hypothesis and the nature of potential environmental cues providing the spatial gradients are topics of intense debate. Currently, several different environmental cues are proposed, but due to its global nature, the Earth's magnetic field remains amongst the most discussed [1, 9, 10]. The magnetic navigation hypothesis proposes that animals use some cues derived from the Earth's magnetic field, which shows a relatively predictable spatial distribution [21, 23]. Depending on where on the globe such cues are sampled, they have the potential to provide different information on geographic position [24–26]. In many parts of the world, the total intensity of the Earth's magnetic field (magnetic field strength) and magnetic inclination (dip angle between magnetic field lines and the horizon) generally vary along a north-south axis whereas magnetic declination (the angle between directions to geographic and magnetic North) varies mainly along an east-west axis [24]. However, this is by no means a perfect global grid, and in some areas, such as north-eastern Europe and western Asia, this simple relationship breaks down, such that birds would have to learn a more complex spatial relationship between the cues to navigate accurately [25]. The aim of this experimental study is to explore the hypothesis of magnetic true navigation, i.e., true navigation based on geomagnetic cues, using the Eurasian reed warbler (*Acrocephalus scirpaceus*, hereafter reed warbler) as a model species representing migratory songbirds (the largest taxonomic group amongst avian migrants).

To overcome the challenge of accurately manipulating the magnetic field around a moving animal, virtual magnetic displacements, i.e., experiments in which captive animals are exposed to simulated geomagnetic conditions of a different location while tested in orientation cages at the capture site, have become the preferred method to investigate the role of the Earth's magnetic field for navigation purposes [26–29]. As well as studies on true navigation using magnetic cues, the method has been used successfully to reveal the innate signpost mechanisms used by hatchling sea turtles, eels and salmonids [e.g., 26, 27, 30–33]. The results of virtual magnetic displacement experiments with reed warblers suggest that they can respond to such treatments as if they had been physically displaced to the respective magnetically simulated unfamiliar locations, despite the fact that they are physically located at the site of their capture, which suggests true navigation ability [28, 29, 34–36]. However, in these previous virtual magnetic displacement studies, reed warblers were presented with inclination, declination and intensity values they could have experienced during their year-round movements, even if not in the specific combinations used in the experiments (Figure S1) [28, 29, 35] and so do not necessarily support the use of a map extrapolated to unfamiliar values of the magnetic field. In this study, we tested whether reed warblers can indeed navigate by an extrapolated gradient map using the Earth's magnetic field, i.e., whether or not they are able to show a navigational response (re-orientation towards their known migratory corridor) when exposed to magnetic parameters which they have never previously encountered in their familiar range.

Experiment 1: Declination-only virtual magnetic displacement

In this experiment, we intended to assess whether reed warblers can use the magnetic declination alone as an indication of an eastward displacement beyond their year-round distribution range. Given the way declination varies in relation to other magnetic parameters to the east of the capture site (Figures 2, 3A, B), this would give insights into the way the birds perceive the relationship between the different magnetic cues (inclination, declination and intensity). This experiment drew on a previous study in which we used experienced reed warblers from the Baltic population and exposed them to a change in declination (all other magnetic cues stayed unchanged) during their fall migration. This corresponded to a westward virtual displacement from the Kaliningrad region, Russia, to southern Scotland to which the birds responded with a re-orientation towards their migratory corridor in Central Europe [29 but see 37]. For this experiment, we captured experienced reed warblers near the Biological Station Lake Neusiedl in Illmitz, south-eastern Austria (Figure 2; see Methods for details) before the onset of their fall migration. The band recoveries from this population provide evidence for a year-round distribution range covering southern Europe and Africa to the north of the equator (Figure 2; the potentially familiar range of this population). Orientation tests were performed in orientation cages (modified Emlen funnels, Figure S2A, B) [38] placed in an outdoor magnetic coil system on clear starry nights within the fall migration season. In the natural magnetic field (NMF: total intensity 48,512 nT, inclination 64.2°, declination +4.2°; see Methods for details), the birds were oriented in the population-specific, seasonally appropriate south-eastern direction (Figure 3C; mean group direction α=113°, 95% confidence interval (CI) 82°–144°, n=52, the Rayleigh test of uniformity: r=0.34, P=0.0021). Subsequently, from the significantly oriented individuals, we chose a random subsample which was exposed to a declination-only changed magnetic field (dCMF) with declination increased by 10° with respect to the local field, but the total intensity and inclination unchanged (see Methods for details). Exposure to the dCMF did not significantly change the birds' mean orientation (Figure 3C; α=142°, 95% CI 101°–184°, n=32, the Rayleigh test of uniformity: r=0.33, P=0.029; 95% CIs of NMF and dCMF broadly overlap; the Mardia-Watson-Wheeler [MWW] test: W=1.8487, P=0.3968). This result is at variance with the re-orientation response of the experienced reed warblers from the Baltic population [29 but see 37]. The declination simulated in the dCMF naturally occurs beyond this species' distribution range, however, the combination of the changed declination and the other unchanged magnetic parameters does not occur anywhere on the globe (Figure 3A). Therefore, one possible interpretation for the lack of re-orientation could be that the combination of geomagnetic cues presented did not make sense and was neglected by the birds, as in a natural situation they do not co-vary spatially in that way (see Discussion for further interpretations).

Experiment 2: All parameters virtual magnetic displacement

For this experiment, we changed all parameters of the magnetic field so that the cues matched a real geographic location to the north-east of the species' distribution range, to test if this was recognized as a displacement. Again, we used experienced reed warblers captured at the same site and during the same season as for Experiment 1. The birds were tested using the same protocol. In the NMF, the birds were again oriented in the population-specific, seasonally appropriate south-eastern direction (Figure 3D; α=133°, 95% CI 110°–156°, n=24, the Rayleigh test: r=0.62, P<0.001), which was not significantly different from the NMF direction in Experiment 1 (MWW test, W=3.4867, P=0.1749). Subsequently, as in Experiment 1, we randomly chose a subsample from the significantly oriented individuals (see the Methods for details) which was exposed to a changed magnetic field with *all* the parameters changed (aCMF), including the same change in declination as in Experiment 1. These birds showed a mean direction towards the southwest (Figure 3D; α=228°, 95% CI 196°–265°, n=15, Rayleigh test: r=0.54, P=0.01). There was a significant difference in the birds' orientation when tested under the NMF and aCMF conditions in Experiment 2 (95% CIs do not overlap; MWW test:

W=16.991, P<0.001). We also tested for a potential seasonal effect that could theoretically explain the shift of the birds' orientation simply due to a time-dependent change of migratory orientation which has been reported for some bird migrants [39, 40]. However, we did not find any evidence for any seasonal effect in our data (see the Methods for details). The changed magnetic parameters fully corresponded to the Earth's magnetic field naturally occurring near the City of Neftekamsk in the Kirov region, Russia. Thus, this experiment represents a virtual magnetic displacement of approximately 2,700 km to the north-east of the study site, i.e., to an area beyond the population's and even the species' distribution range (Figures 2, 3B). All the geomagnetic cues available under the aCMF condition should have been of completely unfamiliar magnitudes to any reed warbler belonging to our study population (Figure 2). The observed change of the mean group direction is consistent with a re-orientation towards the natural migration corridor and/or the capture site (Figures 2, 3B, D).

DISCUSSION

Our study shows that reed warblers can use a combination of cues derived from the Earth's magnetic field to detect a displacement, even if all of these cues are of unfamiliar magnitude, and adjust their migratory direction accordingly, i.e., they are able to perform magnetic true navigation (see the definition in Results). This is consistent with the hypothesis postulating a map (*sensu* cognitive representation of a large-scale geographic context) in which the spatial variation of cues can be extrapolated beyond the familiar range to allow navigation from unfamiliar areas where these cues occur in unknown magnitudes. This hypothesis, which was first suggested by Wallraff [18] and further developed by others [e.g., 6, 9, 19–23], is usually called a gradient map hypothesis. As proposed by some authors, this mechanism could theoretically allow determining precise locations relative to a desired destination so that the distance of displacement could be calculated based on the magnitude of change in certain cues, i.e., a theoretical mechanism comparable to the Cartesian coordinate system [18]. However, whether or not birds or other animals have the cognitive, sensory and computational capacity to develop and use a cognitive map with such accuracy and complexity is questioned by other authors [e.g., 27]. A simpler and less cognitively demanding alternative could be that the birds use a "rule of thumb" mechanism. In this case, rather than determining a precise geographic position and its relation to a destination, an increase or decrease outside of the previously experienced range of magnitudes simply tells the bird their approximate direction of displacement, which may be accurate enough to return them to familiar areas such as the migratory corridor (Figure 1).

Taken together, the virtual magnetic displacement studies on reed warblers provide evidence for compensatory orientation from two separate study sites and migratory populations, displaced east [28, 35], west [29], and north-east (the present study) of their sites of capture. On this basis, the evidence is now very strong that adult night-migratory reed warblers have a magnetic map, and that they can use it to compensate for large geographical displacements. Also of note is that, although different environmental cues have been shown or suggested to be important for true navigation in other bird species [e.g., 41–45], in all the virtual magnetic displacement studies with reed warblers [this study, 28, 29, 35] all other environmental cues were unchanged, accessible and would indicate that the birds had not been displaced from the capture site. Thus, the compensatory responses we observe in adult reed warblers in response to the changed magnetic field and in conflict with local cues does not support a strong role for other environmental cues in the true navigation map of this species (cautiously, we do not generalise this conclusion to all avian taxa or even to all passerine species.).

In addition to these key findings, the lack of response to the declination only treatment is, at first glance, at odds with a previous study on the same species [29]. However, it is possible that the declination change was ignored by the birds because, unlike in the prior study [29], the changed declination did not match up with any likely location considering the experiences the tested reed warblers are likely to have had with the spatial variation of the other magnetic parameters. Therefore, the birds might have trusted the two parameters (magnetic intensity and inclination) that matched the capture site more than the detected declination and determined their position using the first two parameters only ignoring the last one. Alternatively, it is possible that the birds could not detect the change in declination. The lack of response to the declination only manipulation is consistent with other recent results obtained at Rybachy in which adult European robins (*Erithacus rubecula*), a short-distance migrant, and adult garden warblers (*Sylvia borin*), a long-distance trans-Saharan migrant similar to the reed warbler, also did not react to the declination only manipulations [37]. Our study together with the two above mentioned [29, 37] suggest that the role of magnetic declination in the map of birds is not yet fully understood.

In conclusion, our experiments show that magnetically displaced reed warblers demonstrate re-orientation towards their natural migratory corridor as if they were translocated over a large distance to the corresponding geographic location when all naturally occurring geomagnetic cues are presented, but not when only one cue, i.e., magnetic declination, is changed. To the best of our knowledge, this is the first direct evidence suggesting that migratory birds can navigate based on positional estimates calculated from geomagnetic cues entirely extrapolated beyond the range of magnitudes they previously experienced during their individual year-round movements.

SUPPLEMENTARY INFORMATION

Supplementary figures can be found online with the publication. The dataset used for Figure 3 with the main results can be found at Mendeley Data at http://dx.doi.org/10.17632/k4prgc5gdw.1.

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AUTHOR CONTRIBUTIONS

Conceptualization: D.K., R.H., F.P., N.C., H.M. Data curation: D.K., F.P. Formal analysis: D.K., F.P. Funding acquisition: main funding - R.H., additional funding - D.K., N.C., H.M. Investigation: orientation tests - D.K., F.P. Methodology: logistics of the magnetic set-up and personnel training - D.K., R.H., F.P.; the methods of declination change - N.C., D.K.; the general guidance for magnetic field operations - H.M. Project administration: R.H., H.-C. W. Resources: main funding - R.H., access to the study site and logistical support - T.Z., logistical support and catching birds - H-C. W., D.K., F.P., additional staff and access to the magnetic set-up - H.M. Supervision: R.H. Visualization: figures - F.P., D.K. Writing – original draft: D.K., F.P., R.H. Writing – review and editing: D.K., F.P., R.H., N.C., H.M.

DECLARATION OF INTERESTS

The authors declare no competing interests.

MAIN FIGURE TITLES AND LEGENDS

Figure 1. The hypothesis of a bi-coordinate map formed by extrapolation from two gradients learnt through year-round experience. The dotted line outlines a familiar range of a hypothetical bird explored during post-fledgling movements at the breeding site (B), movements to the wintering site (W) via fall migration stopover sites (F) and its return to the breeding site passing through spring migration stopover sites (S). The two hypothetical gradients are increasing from west to east (Gradient 1, red) and from south to north (Gradient 2, blue). A fictional animal displaced to an unfamiliar site situated to the north-east beyond its year-round distribution range (? indicates an unfamiliar site) perceives changes in both gradients and realizes that they exceed the maximum ranges of magnitude the animal has ever encountered. This could be interpreted by a simple rule of thumb: "According to Gradient 1, the current position is further east from the most eastern familiar site so one needs to move westward. According to Gradient 2, the current position is further north from the breeding site so one needs to move southward. The resultant goal-ward direction (R) is the mean of the two above, i.e., one needs to move south-west".

Figure 2. Map of the year-round distribution range of Eurasian reed warblers breeding at the study site. Note that, as we could not know the previous experience of individuals included in the study, we used the population range derived from the band recoveries as a conservative proxy for individual experience of birds from Lake Neusiedl and the surrounding areas. The white dot depicts the study site near Illmitz, Lake Neusiedl, south-eastern Austria. The triangles show bird band recoveries from reed warblers captured at or near the study site by the Austrian and Hungarian banding schemes during the breeding season (late May-August) and found elsewhere (>100 km) during fall (September-November; downward triangles) or spring migration (March-May; upward triangle). Fall recoveries of the same calendar year are

depicted as filled symbols and connected with the banding site by great circle lines. The species' breeding and wintering distribution ranges are shown in solid green and yellow, respectively. The transparent yellow polygon represents the potential migratory distribution range including all known bird band recoveries and limited by the northern border of the species' wintering range in Africa. Magnetic inclination (blue), declination (red) and total intensity (dark gray) isolines are depicted as solid lines if crossing the potential year-round distribution range comprised by breeding (green), migratory (transparent yellow) and wintering (solid yellow) ranges (i.e., these values may be familiar to at least some birds included in the study), and as dashed lines if not crossing the year-round distribution range (i.e., these values should be unfamiliar to all birds included in this study). All isolines are based on data obtained from the US NOAA National Geophysical Data Center and Cooperative Institute for Research in Environmental Sciences [46, 47]. Eurasian reed warbler distribution data were provided by BirdLife International and Handbook of the Birds of the World [48]. Bird banding data can be requested via www.euring.org. The map represents an orthographic projection with the study site as the projection center. For information on the estimated population range of Eurasian reed warblers used in previous studies see Figure S1.

Figure 3. Predictions and results for the virtual magnetic displacements. (A, B) Maps illustrating the natural migratory direction (the black arrow from the study site depicted as the white dot) and the predicted migratory directions under changed magnetic field conditions if birds do (white arrows) or do not respond (black arrows) respond to the magnetic changes and re-orient towards the initial capture site (solid white arrows) or towards the natural migratory corridor (striped white arrows). Magnetic inclination (blue), declination (red) and total intensity (dark gray) isolines are shown, with broad isolines giving those values used in the virtual magnetic displacements. For information on magnetic inclination, declination and total intensity values used in the previous studies see Figure S1. Maps represent an orthographic

projection with the study site as the projection center. (C) Orientation of birds in the experiment when they were tested under the natural magnetic field conditions (NMF) and under the declination-only changed magnetic field condition (dCMF). (D) Orientation of birds in the experiment when they were tested under the NMF conditions and when all magnetic field parameters were changed (aCMF). Circular diagrams: dots at the periphery of each circle indicate individual mean directions; arrows show mean group directions and their concentrations; dashed line circles indicate the minimum radius a mean group vector needs to reach the 5% (inner circle), 1% (middle circle), or 0.1% (outer circle) levels of significance, respectively, according to the Rayleigh test of uniformity; solid lines flanking mean group vectors show 95% confidence intervals for the mean group directions.

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STAR METHODS

RESOURCE AVAILABILITY

Lead Contact

Further information and requests for methods and materials may be directed to and will be fulfilled by the lead contact, Dmitry Kishkinev (dmitry.kishkinev@gmail.com; d.kishkinev@keele.ac.uk).

Materials Availability

This study did not generate new unique reagents.

Data and Code Availability

The pre-processed data used to generate the figure with the main result (Figure 3) have been deposited to Mendeley Data at http://dx.doi.org/10.17632/k4prgc5gdw.1. The data used for other figures, the raw data generated by orientation tests and the R code used to process the data are available on request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Ethical statement

All applicable international, national and/or institutional guidelines for the care and use of animals were followed. The experiments were conducted in accordance with the national animal welfare legislation of Austria where all the provincial permits from the relevant authorities of the Burgenland had been secured before the experiments were conducted. Additionally, the experiments received local ethical approval by the animal welfare ethics review body (AWERB) of Bangor University as the core research team (D.K., F.P. and R.H) were employed by the organization during the period of data collection.

Experimental birds

To be consistent with previous real and virtual displacement experiments, we used Eurasian reed warblers as a model for migratory songbirds [8, 28, 29, 34–37, 49]. Reed warblers are common long-distance migrants breeding in Europe and overwintering in sub-Saharan Africa (Figure 2 for the bird band recoveries of the population used) [48]. We captured a total of 100 reed warblers (n=68 for Experiment 1, 2015-2016; n=32 for Experiment 2, 2018) near the Biological station Lake Neusiedl in Illmitz, south-eastern Austria (47° 46' 10.7"N, 16° 45' 21.6"E). All birds were caught with mist-nets in reed beds near the Biological station. We aimed for locally breeding individuals with the known direction of fall migration based on the bird band recoveries (Figure 2). Therefore, we captured birds from the end of July to mid-August, which is the period when their breeding season ends (late May – late July) and birds prepare for the onset of their fall migration (mid-August through early October). This study is on the individuals' ability to correct for virtual magnetic displacement when they are presented with magnetic cues outside their range of individual experience. Because we could not know the previous experience of each individual, we used the population range derived from the band recoveries (Figure 2) as a conservative proxy for individual experience of birds from Lake Neusiedl and the surrounding areas. We were unable to identify sex based on morphology but it is reasonable to assume an approximately equal distribution of the two sexes. All birds were adults aged 1 year or older (age was determined by wear of plumage during this period according to [50]). Thus, all tested individuals had gained migratory experience before the experiments (i.e., they must have performed at least one fall and spring migration before the time of capture) and developed navigational skills because the latter requires migratory experience [5–7, 14, 15, but see 13, 16, 17]. At the time of capture, all the birds were lean and not in the migratory state. During the period of orientation tests, the birds were in a well-developed migratory state (see the sub-section "Orientation tests" below). The development of migratory status was confirmed by an increased weight (compared to the lean weight at the times of capture) and accumulation of subcutaneous fat deposits starting from the second half of August through the end of the experiments in late September or early October. Another confirmation of the migratory status of birds was the observed migratory restlessness, which coincided with the period of a gradual disappearance of local reed warblers from mist-net catches during the end of August and September.

Before and after the periods used for virtual magnetic displacements, the captured birds were kept in outdoor aviaries placed near the capture site with a clear view of the surrounding habitat to facilitate the access to local orientation cues (e.g., the sun and sun-related cues, stars, the Earth's magnetic field) as well as the local photoperiod, odors, temperature and humidity. There were two aviaries with two cages (cage dimensions: 90 x 80 x 40 cm), each equipped with perches, feeders and drinkers. Each cage hosted up to 10 birds (usually 5-8). During the virtual magnetic displacements, the birds were kept and tested within the magnetic set-up (see the "Magnetic set-up" section below). During the magnetic displacement treatments, up to 8 birds were living in a cubic-shaped cage (inner dimensions: 80 x 80 x 80 cm) positioned in the center of the magnetic coil system where the manipulated magnetic field was most homogeneous (Figure S2C, D). During virtual magnetic displacements, the birds were exposed to the natural photoperiod and local celestial cues. During rainy or windy periods, the cage was covered with a light-transparent plastic foil to protect the birds. As soon as the weather conditions improved the cover was removed to allow an unobstructed view. All aviaries and cages were made of non-magnetic (wood and plastic) or weakly magnetic materials (e.g., stainless steel screws) to minimize distortion of the magnetic field around the birds. The birds were provided with food (mealworms, dried insect mixture) and water *ad libitum*.

METHOD DETAILS

Orientation tests

Each test lasted for approximately 30 min and started shortly after the end of astronomical twilight when the stars were already clearly visible. Orientation tests were performed only during moonless periods when at least 50% of the starry sky was visible; usually, 90% – 100% of the sky was clear during the tests. As a behavioral paradigm, we used modified Emlen funnels – the classical approach for testing migratory orientation in songbirds since the establishment by S. Emlen and J. Emlen [38]. The funnels were made of aluminum (Figure S2A; top 350 mm, bottom 100 mm, slope 45°) with the top covered by a net allowing the birds to see the stars. The directionality of birds' activity was recorded as scratch marks left by the birds' claws on a print film covered with a dried mixture of whitewash and glue (Figure S2B). When such a print film is fitted inside a funnel, its two ends slightly overlap. During orientation tests, the alignment of the different funnels was alternated, with the overlap point facing in different cardinal directions (e.g. north and south). This funnel alignment was unknown to the researchers who estimated the birds' mean directions based on the distribution of the scratch marks from each orientation test. Instead, mean directions were estimated assuming an alignment to the North and later corrected according to the actual alignment from the record. This procedure was meant to avoid any observer bias with regard to directional estimations. Whenever it was logistically possible, at least two researchers independently estimated each bird's mean direction from the distribution of the scratch marks. The mean of the two observers' recorded directions was taken into further analysis. If both observers considered the scratch marks to be randomly distributed or their assessed directions deviated by more than 30º, a test was considered not to be oriented. Only tests with at least 40 scratch marks (the activity criterion) and clear unidirectional orientation were taken into analysis. Birds' individual directions were used to calculate individual mean directions for each magnetic field condition they were tested in by means of vector addition [51]. From individual mean directions, group mean directions were calculated for the different magnetic field conditions. Control tests were performed inside the magnetic coil system or a wooden replica of the system (the latter to control for the effect of parts of the magnetic set-up visible from the inside of the Emlen funnels). During the controls tests, power supplies near the funnels were running but not connected to the magnetic coil system to control for potential effects of the power supplies (e.g., the effect of noise) on birds' behavior.

Magnetic set-up and magnetic field measurements

To manipulate magnetic fields, we used direct currents running through a three-dimensional custom-built magnetic coil system which looks like a cuboid with a total of 6 square-shaped frames – 2 in each of the 3 orthogonal sets (Figure S2C, D). The system was originally donated by the Niels Bohr Institute, University of Copenhagen to H.M. It consists of two quadratic and one rectangular coil-pair with dimensions of 2.040 x 2.040, 2.040 x 2.000 and 2.070 x 2.070 m in the X-, Y-, and Z-axis directions, respectively (48, 48 and 80 copper wire turns, respectively). The aluminum profiles of the coils were wound up with single-wrapped wirings and waterproofed. The system was modified for greater stability and outdoor use by the Institute of Mechanical Engineering at the Aalborg University. Previously, it was successfully used in a series of outdoor studies with magnetic field manipulations using songbirds and monarch butterflies [52–54]. The magnetic field inside the set-up was operated by direct electrical currents supplied by 3 precision bipolar operational DC power supplies (model BOP 50-2M, Kepco Inc., Flushing, NY, USA). Magnetic fields were measured and set using a 3-axis milli-gaussmeter with the accuracy of 10 nT for each axis (trifield.com, AlphaLab Inc., Salt Lake City, Utah, USA). For the NMF values presented in Results, we queried the NOAA EMM model (2000-2019) [55] using the coordinates and altitude (113 m) of the Illmitz field site and the mean dates of each field season (Sept 15th 2015; Sept 15th 2016; Sept 25th 2018). The magnetic field parameters for the magnetic displacements were calculated using NOAA website calculators using WMM model for 2015, 2016 and 2018 [55]. We performed fine adjustments and regular checks of the magnetic field inside the set-up before and after each group of experimental birds was placed into the system to ensure that the desired magnetic field was maintained inside the center of the system. Because the space covered by the cages (Emlen funnels and/or a cubic cage for housing magnetically displaced birds), and thereby the possible positions of the birds, in both cases remained within the central 50% of the radius of the coils (100 cm), the heterogeneities of all our artificial magnetic fields were <1% of the applied field strength, that is <200 nT (slightly more than the natural daily variations of the local geomagnetic field, which are typically in the order of 30-150 nT for total intensity as per the data for the closest, ca. 15 km distance to the field site, geomagnetic observatory at Nagycenk, Hungary) [56]. During magnetic displacement experiment tests up to 4 funnels were placed in the center of the system (Figure S2C) to make sure that the birds were exposed to the most homogeneous magnetic field. Magnetically displaced birds were never leaving the above mentioned 1% homogeneity area during magnetic displacement treatments while being transferred between a housing cage and Emlen funnels to ensure that they remained exposed to constant magnetic conditions during experimental treatments.

Virtual magnetic displacement experiments

Experiment 1: Declination-only condition

Before the start of the declination-only magnetic displacement, control tests were conducted with all the captured birds (from Sept 8th to Sept 12th 2015, and from Aug 23rd to Sept 24th 2016; a total of 68 birds: 32 in 2015 and 36 in 2016; on average 3.4 tests per bird). These tests were performed under the NMF conditions (the geomagnetic field of Illmitz, Austria; magnetic inclination 64.2°, magnetic declination +4.0°, total intensity 48,550 nT). From all the birds which had shown significant orientation during the NMF tests (a total of 52: 19 (59.4%) in 2015 and 33 (91.7%) in 2016; Figure 3) 40 individuals (77%) of the individuals with significant orientation (16 in 2015 and 24 in 2016) were randomly chosen and then used in the tests with changed declination (weather conditions during the field season did not allow to test all the birds with significant orientation during control tests in the experimentally changed fields). The subsequent treatment tests were conducted immediately after the control tests, and in 2016 they partly overlapped with the last control tests (from Sept 12^{th} to Sept 23^{rd} 2015; and from Sept 21^{st} to Sept 27^{th} 2016). These tests were performed under the dCMF conditions, with magnetic declination increased by 10° with regard to the local magnitude of magnetic declination but magnetic inclination and total intensity were unchanged (magnetic inclination 64°, magnetic declination +14°, total intensity 48,550 nT). During the dCMF treatment tests, 32 individuals (80% of 40 tested birds; 14 birds in 2015; 18 in 2016; on average 2.6 tests per bird) showed significant orientation (Figure 3).

Experiment 2: All magnetic parameters changed condition

In Experiment 2 (2018), a total of 32 birds were captured and tested under the NMF conditions (on average 3.6 tests per bird) and 24 birds (75%) of the tested individuals showed significant orientation (Figure 3). From these significantly oriented 24 birds, 19 individuals (79% of the total with significant control orientation) were randomly chosen and then used in the following magnetic displacement tests (as in Experiment 1, weather conditions during the field season did not allow testing all the birds with significant orientation during the control tests in the manipulated magnetic field condition). The virtual magnetic displacement tests were conducted under the magnetic conditions when *all* geomagnetic parameters, not just declination as in Experiment 1, were changed (aCMF condition), with magnetic declination increased by approximately 10° (the same change as in Experiment 1), magnetic inclination increased by approximately 9° and total intensity increased by approximately 6,560 nT (magnetic inclination 73°, magnetic declination +14°, total intensity 55,110 nT), simulating the geomagnetic field parameters naturally occurring near the City of Neftekamsk (56° 05' 51.5"N, 54° 15' 27.9"E; Kirov region, Russia; see the rationale for this displacement site below). During the aCMF treatment tests (on average 2.6 tests per bird), 15 individuals of the total 19 tested (79%) showed significant orientation and their results were taken into the further analysis (Figure 3). Note that the periods of NMF and aCMF tests partly overlapped: the NMF tests were conducted during the two periods (from Sept 8th to Sept 10th and from Sept 27th to Oct 5th) because these days allowed testing under the starry moonless sky (the period between these periods had moonlight), and the aCMF treatment tests were conducted during one period from Sept 30th to Oct 10th (a 6-day overlap with the NMF tests). The partly overlapping timelines of the NMF control and aCMF treatment tests suggest that a potentially possible alternative explanation of the results (an orientation shift in the aCMF treatment compared to the NMF direction) simply by the birds' innate migration program (i.e., the so-called "Zugknick" or "programmed change of migratory direction with time") [39, 40] appears to be highly unlikely (see the section "Testing the effect of time within the season on birds' orientation in Experiment 2" below).

The rationale of magnetic displacement site

While choosing a site for virtual magnetic displacements, one should bear in mind species- and population-specific distribution, expected response, and geographical and geophysical constraints. For example, for the reed warbler population from Lake Neusiedl migrating primarily south-east during fall migration (Figure 2), long-distance displacement to the north-west of the study site (e.g., near Iceland) would not only magnetically translocate the birds to an unusual (given that the reed warbler is a landbird species) location in the middle of Atlantic but also a compensatory response in this case would be expected towards the south-east, which is close, if not identical, to the normal south-eastern direction during fall migration shown in the control tests (Figure 3). Therefore, such a response could probably not be distinguished from the control direction. Displacements to any site in Sub-Saharan Africa would potentially expose at least some birds to familiar values of geomagnetic cues (see Figure 2), whereas the key point of the experimental design is to ensure that a magnetic displacement location is realistic, i.e., it exists on the planet's surface, but is unfamiliar to experimental birds unlike in previous virtual displacement experiment on this species (Figure S1). Given the above rationale, the displacement to the north-eastern part of the European part of Russia (the inland dashed magnetic isolines in the upper right corner of Figure 2) appeared to be most suitable for this study.

QUANTIFICATION AND STATISTICAL ANALYSIS

Circular statistics

The circular statistical analyses were conducted using both the software R version 3.5.2 [60], package "circular", and Oriana (version 4.01; http://kovcomp.co.uk; Pentraeth, UK). We used the standard Rayleigh test of uniformity [51] to assess if data of the individuals' tests and mean group directions significantly differed from the uniform distribution (the null hypothesis). To compare mean group directions between treatments, both the 95% confidence intervals around mean group directions and the non-parametric Mardia-Watson-Wheeler test were used. We used a non-parametric test because the assumptions for more powerful parametric tests (e.g., the Watson-Williams) were not fulfilled [51]. The assumptions are automatically tested by the used version of the circular statistics program ''Oriana'' (version 4.01).

Testing the effect of time on birds' orientation

As mentioned before, the birds included in the experiments were tested for their orientation under the NMF conditions first. Then we chose a random subsample from the oriented birds, which were subsequently tested for their orientation under the aCMF conditions. The periods used for NMF and aCMF tests for Experiment 2 partly overlapped: the NMF tests were conducted during the two periods (from 8th to 10th Sept and from 27th Sept to 5th Oct) because these days allowed testing under the starry moonless sky (the period between these periods had moonlight), and the aCMF treatment tests were conducted during one period from 30th Sept to 10th Oct which had a 6-day overlap with the NMF tests.

In order to test the possibility that the change in birds' orientation observed in Experiment 2 could be explained as a function of time within the season (i.e., an "endogenously controlled change of migratory direction" or "Zugknick"; [39, 40]), we applied two modelling approaches using either the daily mean directions or the individual directions obtained during each test night of the season. As birds' orientation was found to change mainly in the east-west component (from 133° (SE) to 228° (SW)), we chose to model the effect of time within the season on the sine of the direction (either daily mean or individual). The sine of a direction is bound between -1 (sine of 270° (W)) and 1 (sine of 90° (E)). We linearly transformed the sine from its original scale to the open unit interval (0, 1) following [57] by first taking $y' = (y - a)/(b - a)$, where "b" is the highest possible value (1) and "a" is the smallest possible value (-1), and then compressing the range to avoid highest and lowest possible values by taking $y'' = [y'(n - 1) + 1/2]/n$, where "n" is the sample size. This transformation allowed the application of Generalized Additive Models (GAMs) of the family "betar" (beta regression) for our modelling approaches. We used the function "gam" implemented in the R package "mgcv" [58] to fit the GAMs with the day of year as a smoothing term and the magnetic condition as an additional explanatory factor with two levels: NMF and aCMF. The GAM used to explain the effect of time within the season (the day of year) on the sine of the individual directions included the birds' ID as an additional random effect to account for the non-independence of data from repeated orientation tests of the same individuals. Further we used this GAM as a "global model" to conduct an automated model selection and find the best, i.e., the most parsimonious, model by means of the "dredge" function implemented in the R-package "MuMIn" [59]. The GAMs validation was checked using diagnostic plots generated with the function "gam.check" implemented in the R package "mgcv" [58] and no serious violations of the models' assumptions could be found.

As a result, we found no evidence for the day of year effect on either the sine of the daily mean directions or the sine of the individual directions (Table S1). If there was a confounding time-dependent effect explaining the seasonal shift in birds' orientation by the order of experiment and/or by the day of year alone, we would expect a significant smoothing term (different from zero). Contrary to that, the automated model selection revealed that the most parsimonious model does not include the day of year as a significant smoothing term (Table S2). At the same time, the effect of the magnetic conditions (NMF or aCMF) on the birds' orientation was significant (see Table S1 and Figure S3). This result strongly suggests that an "endogenously controlled change of migratory direction" or "Zugknick" [39, 40] is to be an unlikely explanation for the change in birds' orientation observed in Experiment 2. Altogether, this result strongly supports the hypothesis that the observed change in the mean orientation represents a navigational response triggered by the magnetic conditions (re-orientation following the change of the magnetic conditions in Experiment 2).