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Migratory Eurasian reed warblers can use magnetic declination to solve the longitude problem

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Summary

The longitude problem (determining East-West position) is a classical problem in human sea navigation. Prior to the use of GPS satellites, extraordinarily accurate clocks measuring the difference between local time and a fixed reference (e.g., GMT) [1] were needed to determine longitude. Birds do not appear to possess a time-difference clock sense [2]. Nevertheless, experienced night-migratory songbirds can correct for East-West displacements to unknown locations [3-9]. Consequently, migratory birds must solve the longitude problem in a different way, but how they do so has remained a scientific mystery [10]. We suggest that experienced adult Eurasian reed warblers (*Acrocephalus scirpaceus*) can use magnetic declination to solve the longitude-problem at least under some circumstances under clear skies. Experienced migrants tested during autumn migration in Rybachy, Russia were exposed to an 8.5° change in declination while all other cues remained unchanged. This corresponds to a virtual magnetic displacement to Scotland if and only if magnetic declination is a part of their map. The adult migrants responded by changing their heading by 151° from WSW to ESE, consistent with compensation for the virtual magnetic displacement. Juvenile migrants that had not yet established a navigational map also oriented WSW at the capture site, but became randomly oriented when the magnetic declination was shifted 8.5°. In combination with latitudinal cues, which birds are known to detect and use [10-12], magnetic declination could provide the mostly east-west component for a true bi-coordinate navigation system under clear skies for experienced migratory birds in some areas of the globe.

Keywords: magnetic compass, star compass, magnetic sense, magnetic map, bird migration
Results and Discussion

Sea turtles inhabiting the North Atlantic Gyre system are known to use the oblique grid between magnetic inclination and magnetic intensity [13,14], and a similar mechanism has been suggested to be used in pigeon homing [10,15]. However, this mechanism is difficult to use in many parts of the world including most of Western Europe and North America where the magnetic inclination and magnetic intensity isolines run almost parallel [16]. In these areas magnetic declination would be ideally suited as a magnetic map parameter, but no evidence exists that migratory birds could detect and use magnetic declination for navigation. Therefore, we experimentally tested whether adult Eurasian reed warblers can use magnetic declination as part of their map sense.

We captured 15 adult experienced Eurasian reed warblers during autumn migration in Rybachy (55°09' N, 20°52' E, Fig. 1). We housed the birds in wood and cloth-net outdoor cages [8] located inside a three-dimensional, double-wrapped, Merritt-4-coil system that we could use to change any magnetic field parameter very accurately and homogeneously [17,18]. The birds' spontaneous night-time migratory orientation was recorded in Emlen funnels [19], which were placed on top of the housing cages during testing. In this way, the birds never left the highly homogeneous magnetic field area in the centre of the coil system at any time. Both the housing cages and the Emlen funnels provided the birds with free access to all non-magnetic cues including unaltered photoperiodic, celestial, olfactory, and landmark cues. When we tested the adult reed warblers under clear moonless starry skies in the natural magnetic field of Rybachy (NMF: total magnetic field intensity 50,118 nT, inclination 70.1°, declination +5.5°), they oriented in their seasonally appropriate WSW direction ($\alpha = 253^\circ$ [all directions indicated relative to geographic North], $r = 0.59$, $p = 0.004$, $n = 15$, 95% confidence interval of the group mean direction [CI_mean] = 221°–284°, Fig. 1).

Following the control tests, instead of a real displacement, we virtually displaced the birds. This has the advantage over a real displacement that we could change a single magnetic parameter while all other parameters remained completely unchanged. We rotated the magnetic

field 8.5° counter clockwise with the coils while keeping the magnetic intensity and magnetic inclination constant (CMF: total magnetic field intensity 50,118 nT, inclination 70.1° , declination -3°). The changed magnetic field approximately matched the natural geomagnetic field parameters found around Dundee in Scotland (at $\sim 56^\circ 30' \text{ N}$, $3^\circ 20' \text{ W}$, Fig. 1). Magnetic intensity and magnetic inclination are the same in Rybachy and in Southern Scotland. Thus, only magnetic declination indicated that the birds tested in the CMF condition had been displaced to a location far ($\sim 1450 \text{ km}$) WNW of their normal migratory route. All other environmental cues such as olfactory, celestial, and photoperiodic cues indicated that the birds were still in Rybachy.

When the same adult Eurasian reed warblers previously tested in the NMF condition were constantly exposed to the 8.5° shifted declination and tested in Emlen funnels under clear, moonless, starry skies over the next 4-6 days, they responded with a dramatic 151° change in their mean orientation from WSW to ESE ($\alpha = 102^\circ$, $r = 0.51$, $p = 0.017$, $n = 15$, 95% CI_{mean} = 62° – 141° , Fig. 1). The difference between the mean orientation in the NMF and CMF conditions was highly significant (Mardia-Watson-Wheeler [MWW] test: $W = 14.3$, $p < 0.001$, 99% confidence intervals did not overlap). This re-orientation is consistent with compensatory orientation in response to the virtual magnetic-declination-only displacement from Rybachy to Scotland. This result suggests that a small change in magnetic declination is sufficient to elicit a dramatic re-orientation response and that Eurasian reed warblers can use magnetic declination to determine their approximate east-west position within Europe (Fig. 1 and 2).

If magnetic declination provides the east-west component of the navigational map in adult experienced reed warblers, one key prediction is that young inexperienced migrants, which have not yet established a functional map [3-5,9], should either continue to orient in their normal WSW migratory direction when they are exposed to a -3° magnetic declination, or they might become confused because young reed warblers caught in Rybachy in autumn only have experience with positive magnetic declination values (Fig. 1 and 2).

We therefore captured 25 juvenile (first-year) Eurasian reed warblers during autumn migration in Rybachy. The inexperienced reed warblers also oriented in the seasonally appropriate WSW direction when they were exposed to the natural magnetic field in Rybachy (NMF: $\alpha = 233^\circ$, $r = 0.60$, $p < 0.001$, $n = 25$, 95% CI_mean = 210° – 256° , Fig. 1). When the juvenile reed warblers were exposed to the 8.5° counter-clockwise change in magnetic declination, they did not re-orient towards ESE like the adult birds did, but showed random orientation (CMF: $\alpha = 87^\circ$, $r = 0.04$, $p = 0.97$, $n = 25$, Fig. 1). The lack of a preferred direction in juvenile reed warblers tested in the CMF condition was different from the same birds' significant orientation in the NMF condition ($p < 0.001$; the bootstrapped 99.9% confidence interval for the r-value in the juvenile NMF condition is $0.31 < r < 0.86$, and this does not overlap with the r-value of 0.04 found when the same juvenile birds were tested in the CMF condition) and was also significantly different from the significantly oriented behaviour of the adult reed warblers in the CMF condition ($p < 0.001$; the bootstrapped 99.9% confidence interval for the r-value in the adult CMF condition is $0.07 < r < 0.91$, and this does not overlap with the r-value of 0.04 found in the juvenile CMF condition).

The re-orientation response of the adult experienced Eurasian reed warblers is remarkable because an 8.5° counter-clockwise rotation of magnetic north triggered a 151° shift in the adult reed warblers' migratory heading. This response cannot be a simple magnetic compass response or a cue calibration response when magnetic compass is calibrated by astronomical cues [23], since an 8.5° shift in orientation would be predicted (the 99.9% confidence intervals for the orientation in the shifted field (CMF) does not include $253^\circ - 8.5^\circ = 244^\circ$). Thus, the re-orientation response of the adult experienced Eurasian reed warblers seems to be a compensatory response towards their normal migratory corridor, similar to the compensatory responses shown by translocated common cuckoos (*Cuculus canorus*) [24], and the only map parameter that was changed was magnetic declination. The reed warblers' easterly orientation in the -3° magnetic declination condition might not necessarily reflect a reaction to a

specific estimated map location, but could simply be a reaction to the birds having detected that they are likely to be far too far west of their expected current location.

While adult avian migrants have a map sense and are capable of true navigation from their first spring onwards [3-9], inexperienced migrants on their first autumn migration are thought to follow a clock-and-compass strategy, also called the vector navigation strategy, which does not involve a map [3-5,25,26]. However, some experiments suggest that also inexperienced birds and certainly sea turtles have inherited at least some simple magnetic signposts which can trigger adaptive changes in behaviour [11,13,14,27,28]. Our data seem to indicate that if inexperienced reed warblers encounter a sudden change in magnetic declination, which they have not yet experienced on their migratory routes (a combination of negative declination values, suggesting Iberian peninsula or West Africa, and steep inclination and high total intensity, suggesting northern-central Europe), they are perplexed and become disoriented. The strikingly different responses of the adult and juvenile reed warblers to a change in magnetic declination and the virtual nature of the displacement effectively exclude any non-specific stress response or a clock-and-compass effect as explanations of our results .

In many previous studies, symmetrical displacement locations have been used to study magnetic map responses in other animals (e.g. sea turtles [14], newts [29], and lobsters [30]). In our Rybachy system, we could, however, only sensibly do a westward displacement. Any predicted reaction of the reed warblers to an eastward displacement would be impossible to detect, because the control direction observed in the adult reed warblers tested in Rybachy was already very westerly (253°). Furthermore, it is impossible to simulate an eastward displacement from Western or Central Europe to a location that actually exists by only changing magnetic declination. As one moves into Russia, the declination isolines no longer run regularly NorthSouth and the magnetic intensity and magnetic inclination isolines start to diverge substantially and to form a grid. Therefore, any results of such a displacement would be very difficult to interpret.

Since all other potential magnetic map parameters (inclination and intensity) as well as all olfactory and visual cues were kept constant, and since magnetic declination can only be determined by comparing magnetic and celestial compass information, a part of the reed warblers' map system seems to rely on compass information at least under some circumstances. Consequently, the traditional [15,31] strict separation between map and compass information needs to be reconsidered on the sensory mechanistic level. Our results also mean that the angular accuracy of both the birds' magnetic and celestial compasses must be very good ($\leq 1^\circ$). This suggestion is supported by experimental and theoretical evidence [32-34]. Furthermore, declination can only provide map information when celestial compass information is available, and thus should not work under complete overcast or deep in the ocean. Since some animals, such as sea turtles, can use their magnetic map in the absence of celestial cues [13,14], magnetic declination is certainly not the single universal solution for determining longitudes used by all animals in all locations. For animals that respond accurately to virtual magnetic displacements without a view of the sky, another magnetic parameter has to be available to determine longitude when celestial cues are unavailable.

In conclusion, we suggest that the detection of magnetic declination could settle the longitude problem, at least for some bird species when both geographic and magnetic North are detectable. Other mechanisms are obviously important in other animals experiencing different orientation environments and several map mechanisms are likely to work in concert in nature. Nevertheless, we predict that detection of magnetic declination is an important part of the map enabling experienced Eurasian reed warblers and probably other long-distance migrants to perform true navigation [3-9]. Magnetic declination in combination with magnetic inclination and/or total magnetic intensity [11,12,27,28] and/or celestial latitudinal cues [35] has the advantage that they provide a bicoordinate grid useful for successful true navigation on some parts of the Earth, thereby reducing the number blind zones compared to if only magnetic inclination and total magnetic intensity are used [16] (Fig. 2).

Author contributions

N.C., H.M., D.Ki. and R.H. designed research; N.C., A.P. and D.Ko. performed orientation tests and analyzed the data; H.M. and N.C. wrote the paper. All authors commented on the manuscript.

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References

1. Andrewes, W.J.H. (ed.) (1996). The Quest for Longitude. The Proceedings of the Longitude Symposium, Harvard University, Cambridge, MA, November 4-6, 1993. (Cambridge, Massachusetts: Collection of Historical Scientific Instruments, Harvard University).
2. Kishkinev, D., Chernetsov, N., and Mouritsen, H. (2010). Double-clock or jetlag mechanism is unlikely to be involved in detection of east-west displacement in a long-distance avian migrant. *Auk* 127, 773–780.
3. Perdeck, A.C. (1958). Two types of orientation in migrating *Sturnus vulgaris* and *Fringilla coelebs* as revealed by displacement experiments. *Ardea* 46, 1–37.

4. Mouritsen, H. (2003). Spatiotemporal orientation strategies of long-distance migrants. In *Avian Migration*, Berthold, P., Gwinner, E., and Sonnenschein, E., eds. (Berlin, Heidelberg, New York: Springer), pp. 493–513.
5. Thorup, K., Bisson, I.-A., Bowlin, M.S., Holland, R.A., Wingfield, J.C., Ramenofsky, M. and Wikelski, M. (2007). Evidence for a navigational map stretching across the continental U.S. in a migratory songbird. *Proc. Natl. Acad. Sci. USA* *104*, 18115–18119.
6. Chernetsov, N., Kishkinev, D., and Mouritsen, H. (2008). A long-distance avian migrant compensates for longitudinal displacement. *Curr. Biol.* *18*, 188–190.
7. Kishkinev, D., Chernetsov, N., Heyers, D., and Mouritsen, H. (2013) Migratory reed warblers need intact trigeminal nerves to correct for a 1,000 km eastward displacement. *PLoS ONE* *8*, e65847.
8. Kishkinev, D., Chernetsov, N., Pakhomov, A., Heyers, D., and Mouritsen, H. (2015). Eurasian reed warblers compensate for virtual magnetic displacement. *Curr. Biol.* *25*, R822–R842.
9. Holland, R.A. (2014) True navigation in birds: From quantum physics to global navigation. *J. Zool.* *293*, 1–15.
10. Gould, J.R. (2008). Animal navigation: the longitude problem. *Curr. Biol.* *18*, R215–R216.
11. Henshaw, I., Fransson, T., Jakobsson, S., and Kullberg, C. (2010). Geomagnetic field affects spring migratory direction in a long distance migrant. *Behav. Ecol. Sociobiol.* *64*, 1317–1323.
12. Mora, C.V., Acerbi, M.L., and Bingman, V.P. (2014). Conditioned discrimination of magnetic inclination in a spatial-orientation arena task by homing pigeons (*Columba livia*). *J. Exp. Biol.* *217*, 4123–4131.
13. Putman, N.F., Endres, C.S., Lohmann, C.M.F., and Lohmann, K.J. (2011) Longitude perception and bicoordinate magnetic maps in sea turtles. *Curr. Biol.* *21*, 463–466.

14. Lohmann, K.J., Putman, N.F., and Lohmann, C.M.F. (2012). The magnetic map of hatchling loggerhead sea turtles. *Curr. Opin. Neurobiol.* 22, 336–342.
15. Gould, J.R. (1982). The map sense of pigeons. *Nature* 296, 205–211.
16. Boström, J.E., Åkesson, S., and Alerstam, T. (2012). Where on earth can animals use a geomagnetic bi-coordinate map for navigation? *Ecography* 35, 1039–1047.
17. Zapka, M., Heyers, D., Hein, C.M., Engels, S., Schneider, N.-L., Hans, J., Weiler S., Dreyer, D., Kishkinev, D., Wild, J.M. *et al.* (2009). Visual, but not trigeminal, mediation of magnetic compass information in a migratory bird. *Nature* 461, 1274–1277.
18. Engels, S., Schneider, N.-L., Lefeldt, N., Hein, C.M., Zapka, M., Michalik, A., Elbers, D., Kittel, A., Hore, P.J., and Mouritsen, H. (2014). Anthropogenic electromagnetic noise disrupts magnetic compass orientation in a migratory bird. *Nature* 509, 353–356.
19. Emlen, S.T., and Emlen, J.T. (1966). A technique for recording migratory orientation of captive birds. *Auk* 83, 361–367.
20. Bolshakov, C.V., Shapoval, A.P., and Zelenova, N.P. (2001). Results of bird trapping and ringing by the Biological Station “Rybachy” on the Courish Spit: Long-distance recoveries of birds ringed in 1956–1997: Part 1. *Avian Ecol. Behav. Suppl.* 1, 1–126.
21. Bolshakov, C.V., Shapoval, A.P., and Zelenova, N.P. (2002). Results of bird trapping and ringing by the Biological Station “Rybachy” on the Courish Spit: Controls of birds ringed outside the Courish Spit in 1956–1997. Part 1. *Avian Ecol. Behav. Suppl.* 5, 1–106.
22. BirdLife International, and NatureServe (2015). Bird species distribution maps of the world (BirdLife International, Cambridge, UK and NatureServe, Arlington, USA).
23. Cochran, W.W., Mouritsen, H., and Wikelski, M. (2004). Migrating songbirds recalibrate their magnetic compass daily from twilight cues. *Science* 304, 405–408.
24. Willemoes, M., Blas, J., Wikelski, M., and Thorup, K. (2015). Flexible navigation response in common cuckoos (*Cuculus canorus*) displaced experimentally during migration. *Sci. Rep.* 5, 16402.

25. Mouritsen, H., and Mouritsen, O. (2000). A mathematical expectation model for bird navigation based on the clock-and-compass strategy. *J. Theor. Biol.* 207, 283–291.
26. Wiltschko, R. (2017). Navigation. *J. Comp. Physiol. A*: in press. doi 10.1007/s00359-017-1160-1. Published online 14 March 2017
27. Fransson, T., Jakobsson, S., Johansson, P., Kullberg, C., Lind, J., and Vallin A. (2001). Magnetic cues trigger extensive refuelling. *Nature* 414, 35–36.
28. Bulte, M., Heyers, D., Mouritsen, H., and Bairlein, F. (2017). Geomagnetic information modulates nocturnal migratory restlessness but not fuelling in a long distance migratory songbird. *J. Avian Biol.* 48, 75–82.
29. Fischer, J.H., Freake, M.J., Borland, S.C., and Phillips, J.B. (2001). Evidence for the use of the magnetic map information by an amphibian. *Anim. Behav.* 62, 1–10.
30. Boles, L.C., and Lohmann, K.J. (2003). True navigation and magnetic maps in spiny lobsters. *Nature* 421, 60–63.
31. Kramer, G. (1957). Experiments in bird orientation and their interpretation. *Ibis* 99, 196–227.
32. Åkesson, S., Morin J., Muheim, R., and Ottosson, U. (2001). Avian orientation at steep angles of inclination: experiments with migratory white-crowned sparrows at the magnetic North pole. *Proc. R. Soc. Lond. B* 268, 1907–1913.
33. Lefeldt, N., Dreyer, D., Steenken, F., Schneider, N.-L., and Mouritsen, H. (2015). Migratory blackcaps tested in Emlen funnels can orient at 85 but not at 88 degrees magnetic inclination. *J. Exp. Biol.* 218, 206–211.
34. Hiscock, H.G., Worster, S., Kattinig, D.R., Steers, C., Jin, Y., Manolopoulos, D.E., Mouritsen, H., and Hore, P.J. (2016). The quantum needle of the avian magnetic compass. *Proc. Natl. Acad. Sci. USA* 113, 4634–4639.
35. Emlen, S.T. (1980). Decision making by nocturnal bird migrants: the integration of multiple cues. In *Acta XVII Congr. Intern. Ornithol.*, R. Nohring, ed. (Berlin: Deutsche Ornithologen-

Gesellschaft), pp. 553–560.

36. Svensson, L. (1992). Identification Guide to European Passerines, 4th ed. (Stockholm, published by the author).

Figure legends

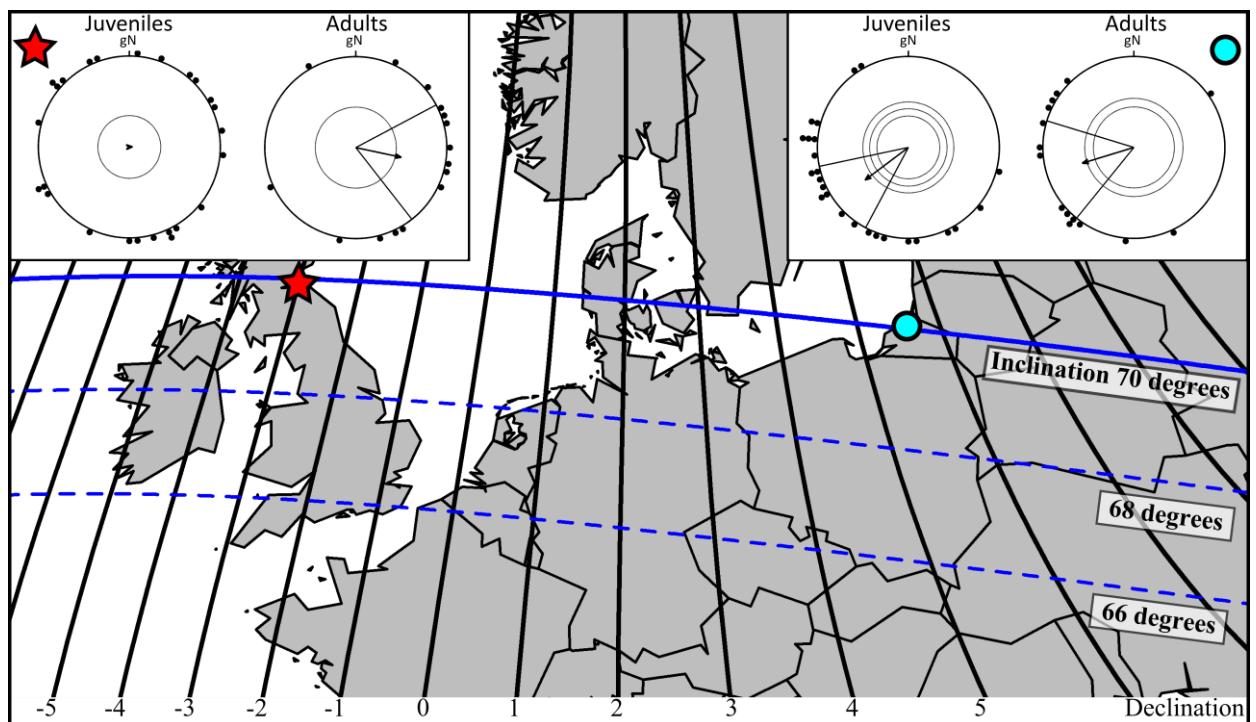


Figure 1. Results of a virtual magnetic-declination-only westward displacement from Rybachy. Right: Orientation of adult and first-year Eurasian reed warblers in Rybachy (blue dot) in the unchanged geomagnetic field. Left: Orientation of the same birds tested in Rybachy, but in a magnetic field where magnetic North was rotated 8.5° anticlockwise. If birds used magnetic declination as part of their map, this manipulation is equivalent to a virtual displacement to SE Scotland (red star). All other cues indicated that the birds were in Rybachy. Each dot at the circular diagram periphery indicates the mean orientation of one individual bird. The arrows show group mean directions and vector lengths, and the dashed line circles indicate the length of the group mean vectors needed for significance at the 0.05, 0.01, and 0.001 levels according to the Rayleigh test; solid lines flanking mean group vectors give 95% confidence intervals for the significant group mean directions. The map of Northern Europe shows the isolines for magnetic declination in black. gN = geographic North. The map is a Mercator projection of the WGS84 geographic coordinate system.

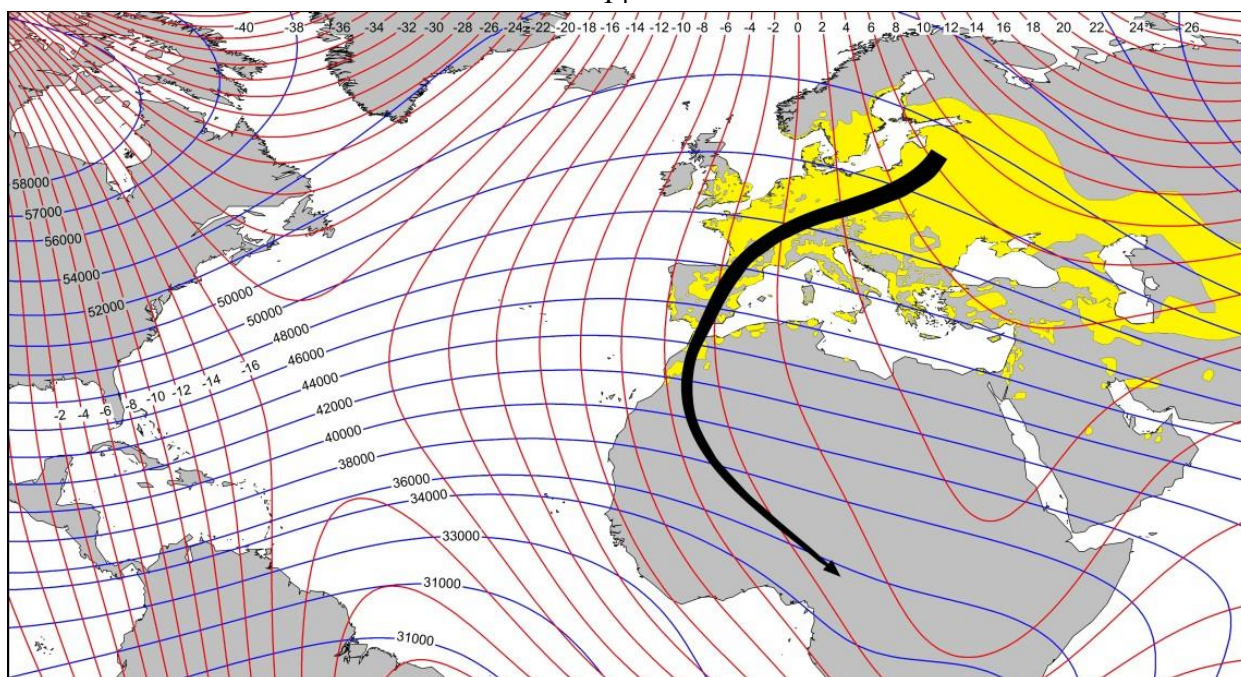


Figure 2. Magnetic intensity and magnetic declination form an excellent bi-coordinate grid in some parts of the World. The map shows magnetic declination isolines (red, degrees) and total intensity isolines (blue, nT) based on U.S. NOAA National Geophysical Data Center and Cooperative Institute for Research in Environmental Sciences (source: <http://www.ngdc.noaa.gov/ftp/geomag/wmm/wmm2010/shapefiles/2014/>). The breeding range of Eurasian reed warblers is shown in yellow [20]. The black curve indicates the autumn migratory route of a typical Eurasian reed warbler from the Baltic region based on ringing recoveries [21,22]. The map is a Mercator projection of the WGS84 geographic coordinate system.

STAR METHODS

CONTACT FOR REAGENT AND RESOURCE SHARING

“Further information and requests for resources should be directed to and will be fulfilled by the corresponding author Nikita Chernetsov (nikita.chernetsov@gmail.com).”

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Experimental birds and site

We captured 15 adult (at least one year old, sex unknown) and 25 first-year (2–3 month old, sex unknown) Eurasian reed warblers (*Acrocephalus scirpaceus*) on their autumn migration during late July and August 2013–2015 at Rybachy on the Courish Spit. The birds were aged on the basis of feather abrasion which is a reliable character in this species [36]. The sample size in the juvenile group was higher because one of two alternative predicted responses of this group to a change in magnetic declination was random orientation and a larger sample size is usually needed to provide the statistical power to identify a random distribution. According to ringing recoveries, Eurasian reed warblers that migrate through Rybachy in late summer and autumn fly via central and southwestern Europe and spend their winter in West Africa [20,21] (Fig. 2). The birds were provided with food (mealworms) and water ad libitum and were kept outdoors in individual cages (40 × 30 × 30 cm; see photo in Ref. [8]), which provided them with a clear view of celestial orientation cues and the cages had good air circulation so that local Rybachy olfactory information was continuously available both before and after the virtual magnetic displacement. Due to the remote nature of the location [8], it is almost certain that the level of anthropogenic electromagnetic noise was far below the level that can disrupt the birds' magnetic compass [18], and any putative left-over electromagnetic noise will have been similar for the control and experimental conditions [8,18]. The birds were released back into the wild after all experiments had been completed. All animal procedures (in this case, capture of the birds and simple, noninvasive, behavioural experiments) were approved by the appropriate authorities: Permit 2013-08 by Kaliningrad Regional Agency for Protection, Reproduction and Use of Animal World and

Forests; and Permit 2014-12 by the specialized committee of the Scientific Council of the Zoological Institute, Russian Academy of Sciences.

METHOD DETAILS

Manipulations of the magnetic field

The 8.5° counter-clockwise rotated magnetic field (CMF) was produced by running electric current in the same direction through the two subsets of windings in the double-wrapped, threedimensional, Merritt four-coil system described in detail elsewhere [17,18]. An 8.5° counterclockwise turn of the magnetic field corresponds to an approximate virtual displacement location in Southern Scotland or Northern England no matter which cue Eurasian reed warblers would use to determine approximate latitude (e.g. by magnetic inclination, magnetic intensity, height of the celestial centre of rotation above the horizon, and/or day length). The currents needed to rotate the field 8.5° counter-clockwise while keeping magnetic intensity and inclination absolutely constant were minimal, and all holding cages and experiments took place inside the ca. 1 × 1 × 1 m cube, where the field applied by the coils was >99% homogenous. In the NMF condition, the same amount of current that was needed in the CMF condition ran through the coils, but in anti-parallel directions through the two identical subsets of windings so that the coils generated no net magnetic field change ($\Delta < 5$ nT).

Orientation tests

Orientation tests (3-6 tests per bird) were performed with modified Emlen funnels [19] made of aluminium (top diameter 300 mm, bottom diameter 100 mm, slope 45° with the top opening covered by netting) [2, 6-8]. All tests with adult birds were performed between 15 August and

September (mean date for NMF tests: 27 August \pm 3.4 days [SD], mean date for CMF tests: 8 September \pm 5.9 days), which is well before October, when juvenile Eurasian reed warblers might naturally change their orientation from SW to SSE as part of their clock- and-compass strategy (the so-called “*Zugknick*”-effect). Furthermore and even more importantly, adult birds, which have developed a map, are not expected to show a *Zugknick* as a mere function of time at a consistent location (here Rybachy), but only when their map location is changed [4,9]. Since the idea of a virtual displacement experiment is to simulate a real displacement, the control tests (pre-displacement) and treatment tests (post-displacement) were done consecutively. During testing, the Emlen funnels were placed inside the coil system on top of the holding cages so that the birds never left the central ca. $1 \times 1 \times 1 \text{ m}^3$ >99% homogeneity area in the centre of the coils [8]. All tests were performed when at least 50% of the starry sky was visible; in most tests, the sky was 95% – 100% clear. Each test lasted 40 min and started at the beginning of astronomical twilight. The directionality of the birds’ activity was recorded as scratches left as birds were hopping in the funnels on a print film covered with a dried mixture of whitewash and glue. Two researchers (AP and DKo or AP and NC) independently determined each bird’s mean direction from the distribution of scratches. Even though the observers could not be blinded for logistical reasons, the two completely independent evaluations of the scratches secured that subjective errors were minimized. If both observers considered the scratches to be randomly distributed or if the two mean directions deviated by more than 30° , the bird was considered disoriented in the given test. We included the results of all birds that were tested at least three times, showed at least two sufficiently active results (i.e. left at least 40 and nearly always >100 scratches on the print film), and were significantly directed according to the Rayleigh test at the 5% significance level. Inactive birds (less than 40 scratches) and disoriented individuals (the mean vector not significant) were excluded from analysis. The exclusion criteria were pre-established and identical criteria have been used in several preceding papers using the same behavioural technique [2, 6-8]. The mean orientation of the individual bird’s directions was recorded as an

orientation data point. The group mean vectors for each condition were calculated by vector addition of unit vectors in each of the individual birds' mean directions.

QUANTIFICATION AND STATISTICAL ANALYSIS

The nonparametric Mardia-Watson-Wheeler test performed with ORIANA (Kovach Computing Services, version 3.21) was used to test for differences in the mean orientation direction between experimental groups. We did not use the more powerful parametric Watson-Williams test because the r -values for our group mean vectors in most cases were < 0.75 . An r value > 0.75 is a crucial assumption for the Watson-Williams test. In order to statistically test whether significantly oriented groups showed significantly more directed behaviour than non-significantly oriented groups, we tested if the length of the group mean vector (r -value; a measure of directedness) were significantly higher in the directed groups than in the disoriented groups by the bootstrap technique. With this technique, a random sample of n orientation directions is drawn with replacement from the sample of n orientation angles present in the significantly oriented experimental group ($n = 15$ for the adult birds, $n = 25$ for the juveniles). Based on these 15 or 25 orientation angles, the corresponding r -value is calculated. This procedure is repeated 100,000 times, each time with a new randomization. In the next step, the resulting 100,000 r -values are ranked in ascending order. The r -values at rank 2500 and 97500, at rank 500 and 99500, and at rank 50 and 99,950 define the 95%, the 99%, and 99.9 % confidence limits for the actually observed r -value of the significantly oriented group, respectively. If the actually observed r -value of the disoriented group lies outside these confidence intervals, the significantly oriented group is significantly more directed than the non-significantly oriented

group with a significance of $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. All maps were produced in R (R Foundation for Statistical Computing, <http://www.R-project.org/>) using packages "maptools", "mapproj" and "rgdal".