RESEARCH ARTICLE

Access to the sky near the horizon and stars does not play a crucial role in compass calibration of European songbird migrants

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ABSTRACT

Migratory birds use different global cues including celestial and magnetic information to determine and maintain their seasonally appropriate migratory direction. A hierarchy among different compass systems in songbird migrants is still a matter for discussion owing to highly variable and apparently contradictory results obtained in various experimental studies. How birds decide whether and how they should calibrate their compasses before departure remains unclear. A recent 'extended unified theory' suggested that access to both a view of the sky near the horizon and stars during the cue-conflict exposure might be crucial for the results of cue-conflict experiments. In this study, we performed cue-conflict experiments in three European songbird species with different migratory strategies (garden warbler, *Sylvia borin*; pied flycatcher, *Ficedula hypoleuca*; and European robin, *Erithacus rubecula*; juveniles and adults; spring and autumn migrations) using a uniform experimental protocol. We exposed birds to the natural celestial cues in a shifted (120 deg clockwise/counterclockwise) magnetic field from sunset to the end of the nautical twilight and tested them in orientation cages immediately after cue-conflict treatments. None of the species (apart from adult robins) showed any sign of calibration even if they had access to a view of the sky and local surroundings near the horizon and stars during cue-conflict treatments. Based on results of our experiments and data from previous contradictory studies, we suggest that no uniform theory can explain why birds calibrate or do not calibrate their compass systems. Each species (and possibly even different populations) may choose its calibration strategy differently.

KEY WORDS: Bird migration, Animal orientation and navigation, Compass calibration, Magnetic compass, Celestial cues, Hierarchy of compass systems

INTRODUCTION

Migratory birds are able to use different mechanisms for orientation during migration based on the geomagnetic field (Wiltschko and Wiltschko, 1972; Mouritsen, 2018) and celestial cues, such as the stars (Emlen, 1967, 1970), the sun and patterns of skylight polarization (Kramer, 1953; Able, 1982; Schmidt-Koenig, 1990;

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Able and Able, 1995). However, compass information obtained by birds from various cues can be absent, redundant and/or contradictory, so it would appear that birds should calibrate their compasses with respect to a common reference system (Muheim et al., 2006a; Liu and Chernetsov, 2012; Pakhomov and Chernetsov, 2020). However, it is also possible that different calibration algorithms could be advantageous to different species, at different times and stages of their migratory journey, and/or under different ecological circumstances (Mouritsen, 2018).

Even though the first compass calibration studies were performed over half a century ago (Emlen, 1967; Moore, 1982) and many cue-conflict experiments have been performed over the past 20 years, the hierarchy among different compass systems still remains poorly understood. Although astronomical cues seem to take a higher position in the hierarchy of compass systems in various songbird migrants from different continents and calibrate the magnetic compass during the pre-migratory period before the first migration in a bird's life (Bingman, 1983; Able and Able, 1990a,b; Alert et al., 2015; Prinz and Wiltschko, 1992), the strategies used during the migration period are more diverse and complicated. According to our recent review (Pakhomov and Chernetsov, 2020), migratory birds could show three different compass calibration strategies during migration after cue-conflict treatment: (1) the magnetic compass is used to calibrate celestial compasses; (2) celestial cues are used to calibrate the magnetic compass; or (3) simple dominance of the stellar or magnetic compass is observed. Although North American migrants calibrate their magnetic compass with respect to information from celestial cues (Cochran et al., 2004; Muheim et al., 2007; 2009) or vice versa (Sandberg et al., 2000), which is also true of Australian silvereyes, *Zosterops lateralis* (Wiltschko et al., 1998, 1999, 2001, 2008), the majority of European migratory birds show simple dominance of one of the compass systems (Gaggini et al., 2010; Chernetsov et al., 2011; Schmaljohann et al., 2013; Sjöberg and Muheim, 2016; Vanni et al., 2017; but see pied flycatcher in Giunchi et al., 2015 and dunlin in Vanni et al., 2017).

There are several suggestions as to why species with different migratory strategies on different continents choose different calibration strategies. Theoretically, long-distance migrants might need to orient more precisely and thus might have to calibrate their compass systems more frequently. In contrast to the long-distance migratory species, medium-distance migrants may reach their goals using only one compass without calibration and survive even if their compass systems sometimes work less than perfectly. However, some long- and medium-distance songbird migrants showed simple dominance of one of the compasses in experimental studies (Chernetsov et al., 2011; Schmaljohann et al., 2013). Another possibility is that because the range of declinations encountered by birds in North America is much broader than in Europe in recent times, North American species might need to calibrate their compass systems more strongly than their European relatives

1

(Liu and Chernetsov, 2012). However, some authors do not agree with this view (Sjöberg and Muheim, 2016).

It has been suggested that if birds have access to the sky near the horizon, they use information from celestial cues during sunset or sunrise (the band of maximum polarization of skylight, or BMP) to calibrate the magnetic compass (Muheim et al., 2006a). In contrast, without access to this part of the sky at sunset/sunrise, the geomagnetic field calibrates the celestial compass systems. Although the idea is simple and elegant, this hypothesis has not been supported in the latest studies, in which birds with access to the setting sun, the BMP, and a view of the sky and landmarks near the horizon during cue-conflict treatment did not show any type of calibration (Chernetsov et al., 2011; Vanni et al., 2017). In an extended version of their theory, Sjöberg and Muheim (2016) proposed that birds need to see the BMP and the sky near the horizon both at sunset and sunrise to fully recalibrate their magnetic compass and transfer this information to stars from the previously recalibrated magnetic compass when they become visible. The authors further suggested that the birds might provisionally calibrate the magnetic compass by celestial cues if the BMP is visible only at sunset or sunrise (Sjöberg and Muheim, 2016). Conversely, without access to the BMP near the horizon and nearby landmarks at sunset or sunrise, birds recalibrate celestial compasses using information from the geomagnetic field. According to the authors of the 'extended unified theory', access to stars or lack of it during cue-conflict treatment might explain contradictory results in previous studies (Schmaljohann et al., 2013; Giunchi et al., 2015; Sjöberg and Muheim, 2016).

An additional complication affecting cue-conflict studies performed during migration is a lack of a unified experimental protocol. Most researchers use different cue-conflict treatments (polarizing filters to create artificial polarization patterns or magnetic coils to rotate the horizontal component of the magnetic field). Different techniques are used to study bird orientation in the field and in lab-based cue-conflict experiments: radio-tracking of free-flying birds released immediately (or with a small delay) after cue-conflict treatment with access to all natural cues, compared with testing in orientation cages such as Emlen funnels for several days after cue-conflict without access to one of the cues (magnetic or celestial; for more information, see Pakhomov and Chernetsov, 2020). Additionally, there is a lack of studies in which the same species was tested during both autumn and spring migrations. Therefore, it is difficult to compare data obtained in these studies and obtain the whole picture of compass calibration in migratory birds. Another possibility is that the search for one unified theory to fit all birds and situations could be equivalent to chasing a fata morgana. Maybe different bird species simply do different things and might even change their strategy based on the ecological situation they face and/or the stage of their journey.

In the present study, we aimed to determine whether European long- and medium-distance songbird migrants show compass calibration after cue-conflict exposures with full access to all orientation cues and a free view of the sky near the horizon during both autumn and spring migrations. We used a unified protocol of cue-conflict treatments and analyzed the orientation of three model species immediately after cue-conflict exposures using a special experimental setup and video cameras to record their orientation responses. In field studies with free-flying birds, birds were usually released with radio-tags and tracked immediately after the cue-conflict treatment (Cochran et al., 2004; Chernetsov et al., 2011; Schmaljohann et al., 2013). In contrast, cue conflicts and orientation tests in lab-based studies mostly take place at a different time (the

following day after the cue conflict) and place (cue conflict: outdoor; orientation test: indoor in the natural magnetic field without stars or outdoor in the vertical magnetic field under stars). In our project, we tried to use the benefits and strengths of both lab-based (opportunity to control the experimental conditions and the birds' access to cues during a test) and field (opportunity to perform orientation tests immediately after the cue-conflict treatment at the end of nautical twilight) approaches.

MATERIALS AND METHODS
Study site, model species and bird keeping
The study was conducted at the experimental site of the Biological Station Rybachy (Courish Spit, Kaliningrad region, Russia; 55°09′ N, 20°52′E) during the spring and autumn migratory seasons 2018–2020. Two long- and one medium-distance songbird migrants were chosen as model species for this study: garden warbler, *Sylvia borin* (Boddaert 1783) (warbler hereafter, without sex identification; autumn – juveniles or first-year, spring – adults), pied flycatcher, *Ficedula hypoleuca* (Pallas 1764) (flycatcher hereafter, both males and females; autumn – juveniles or first-year, spring – adults), and European robin, *Erithacus rubecula* (Linnaeus 1758) (robin hereafter, without sex identification; autumn – juveniles or first-year, spring – adults), respectively. We captured the birds using mist nests from the beginning of April/August to the middle of May/October in spring/autumn, respectively. After being caught, the birds were kept in individual cages (60×40×40 cm), and fed *ad libitum* mealworms (*Tenebrio molitor*), mixed diet (cheese, eggs, carrot and unsweetened biscuits), elderberry and a vitamin supplement in pure water. Each bird was weighed using an electronic scale and we visually estimated their fat score every day for 3 days after trapping and released any bird that showed a constant decrease of body mass and fat deposits. Cages were placed in a windowless indoor aviary with artificial lightning, so experimental birds had no access to any astronomical cues (sun, polarized light or stars) before and between orientation tests. Thereby, there was no opportunity to transfer information from a previously successful calibration to another during our cue-conflict experiments. Artificial lights (ZooDa Bird Compact bulbs: CRI 90+, 5800 K, 12% UVA and 2.4% UVB, 1300 lm) were automatically switched on/off at local sunrise/sunset by IoT (Internet of Things) dimmers. The indoor aviary was equipped with two IP (internet protocol) cameras with infrared LEDs so that we could monitor the activity and behaviour of the birds in their cages in real time during the day and at night. As soon as the birds started to show typical nocturnal restlessness (by not earlier than 3–5 days after catching), we tested them in cue-conflict experiments. In previous studies, it was found that if migratory birds show a high level of migratory restlessness in captivity the night before a day of release, they will depart from a stopover site during the following night (Klinner and Schmaljohann, 2020). Using this information, we predicted that if our birds were active during the previous night, they would be active in an orientation test during the following night. All birds were released at the end of the experiments well before the migration of their conspecifics finished. All experiments and methods were performed in accordance with relevant guidelines and regulations. All procedures were approved by the Ethics Committee for Animal Research of the Scientific Council of the Zoological Institute, Russian Academy of Science (permit 2018-12) and the Kaliningrad Regional Agency for Protection, Reproduction and Use of Animal World and Forests (permit 24/2018-06). The current research was carried out in compliance with the ARRIVE guidelines (https://arriveguidelines.org).

The experimental setup and the manipulation with the magnetic field

Each experiment consisted of two phases: the cue-conflict period and the orientation test. We built a special experimental setup for this project (the CalOri setup hereafter; Fig. 1A) which allowed us to perform each phase of an experiment at the same place without transferring a bird to another testing site. The lower part of the CalOri setup (Fig. 1B, Fig. S3) was a modified plastic Emlen funnel (top diameter 420 mm, bottom diameter 130 mm, wall slope 45 deg) which we used to analyze the birds' orientation (Emlen and Emlen, 1966). The upper part of our setup consisted of three wooden circular frames (inside diameter 425 mm) connected to each other by four aluminium tubes (300 mm in height; Fig. 1B) and covered by nets. This part of the setup was used during the cue-conflict exposures. A thin plastic plate was placed between the circular frames in the lower part of the construction and it separated two parts of the CalOri setup from each other. During the cue-conflict phase, the birds were kept in one of two different experimental conditions: control [the natural magnetic field (NMF)+full access to all natural celestial cues] or cue-conflict exposure [an artificial magnetic field with magnetic north deflected 120 deg counterclockwise (CCW) in spring or 120 deg clockwise (CW) in autumn (CMF)+full access to natural celestial

Fig. 1. Three-dimensional model of the experimental setup. (A) The double-wrapped, three-dimensional Merritt four-coil system (Schwarze et al., 2016) equipped with four waterproof infrared cameras (above) and the CalOri setup (centre of the magnetic coils). (B) The CalOri experimental setup during cue-conflict treatments (right) and orientation tests (left). Pictures were drawn using Blender 2.90 (Blender Foundation, The Netherlands).

cues]. Only in the experiments with European robins did we use the same magnetic field manipulation (120 deg CW) for both the autumn and spring experiments. Experimental magnetic fields were produced by a double-wrapped, three-dimensional Merritt four-coil system ('magnetic coils' hereafter). This coil system was identical to the ones used by the Oldenburg and Rybachy animal navigation groups in many neurobiological and behavioural experiments (Zapka et al., 2009; Lefeldt et al., 2014; Kishkinev et al., 2015; Schwarze et al., 2016; Pakhomov et al., 2017a, 2018; Chernetsov et al., 2020). This Merritt coil system generates fields with >99% homogeneity within a space of ca. 110×110×110 cm. Therefore, there we used only four experimental setups inside our magnetic coils (Fig. 1A). Each of the three axes of the coils was driven by a separate constant current power supply BOP 50-4M (Kepco Inc., USA) placed along with the coils' control box in a shielded and grounded box. The box reduced the sound of working power supplies so it was by far below the level of natural environmental noise near the experimental setup and could not be an audible position cue for tested birds. We did not find any effect of working power supplies on bird orientation according to the results of our previous studies in which the same power supply box was used (Kishkinev et al., 2015; Pakhomov et al., 2017a; Chernetsov et al., 2017). The parameters of the magnetic field were measured using a FMV400 Vector Magnetometer (MEDA Inc., USA) and recalibrated before the beginning of each experiment. Identical CalOri setups with birds tested in the control condition were placed inside a wooden mockup of magnetic coils to mimic the view of the sky and the local landmarks that the birds could see from inside the real magnetic coils in the cue-conflict condition.

We used waterproof IP cameras with additional infrared lights to record the birds' behaviour in the tests for later analyses. Cameras were mounted outside the magnetic coils and above each CalOri setup (Fig. 1A). The cameras were connected to the PoE (Power over Ethernet) switch via a shielded pair cable (FTP or SFTP). All switches and power supplies were kept inside a shielded and grounded special box to reduce any radio-frequency and other electromagnetic noise fields produced by the equipment which could potentially affect the magnetic orientation of our birds (Ritz et al., 2004; Engels et al., 2014; Pakhomov et al., 2017b; Kobylkov et al., 2019). The video from the cameras was streamed via shielded cable to a surveillance monitor (which was placed in the main building of the station, >300 m from the experimental site; Fig. S1) for real-time observation of the bird's behaviour and was recorded to a Synology NAS system in parallel.

Experimental procedures and orientation tests

As described above, when birds started to show nocturnal restlessness in the indoor aviary, we tested them the following night. After capture, the birds were randomly divided into two groups (control or cue-conflict). We tried to test any given bird in one condition only. Only some garden warblers (seven birds; see Table S1) in autumn 2020 were tested in both the control and cue-conflict conditions; the gap between the control and cue-conflict experiments for these birds was at least 1 week, so presumably they could not transfer information from any calibration in the control condition to the cue-conflict condition or vice versa (if it could take place theoretically). The pre–post design in which the same birds are tested in both control and experimental conditions (Giunchi et al., 2015; Muheim et al., 2006b) is quite powerful and allows assessing the change of orientation on an individual level. However, we decided to use another design and exclude any effects of the progress of migratory season on orientation and perform tests in the
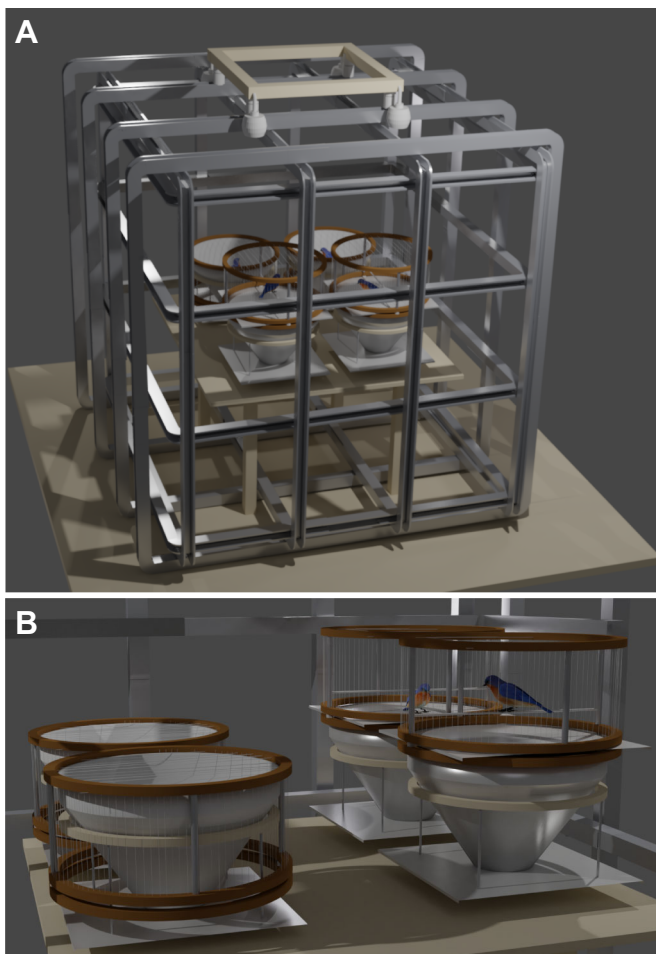
control and cue-conflict conditions at the same time. It minimizes the influence of various factors (progress of the season, different motivation of birds in different parts of migration season, etc.) on behavioural responses of birds to cue-conflict treatments.

Each test day, we carried out experiments with four birds from the cue-conflict group (we could use only one Merritt coil system for this project) and four to eight birds from the control group (in two wooden mockups of a magnetic coil system). All experiments started approximately 30 min before local sunset and were performed in good weather conditions: under a clear sky (minimum sky coverage, 0%; maximum, 50%; for most tests, 5–10%) and at a light wind (1–2 m s^{-1}). After measuring the magnetic field inside the coils, we placed birds inside the upper part of CalOri setups placed in the magnetic coils and the wooden mockup. The birds could move freely and had full access to all crucial calibration cues (the landscape, a good view of the setting sun, and the sky down to the horizon, stars, etc.). The magnetic coils were turned on 40 min before local sunset and turned off at the end of nautical twilight. We chose the end of nautical twilight (when the sun's disc is 12 deg below the horizon) as an endpoint for the cue-conflict phase because by that time most stars have become visible and all traces of the sun, including polarized light, have disappeared (Cochran et al., 2004; Chernetsov et al., 2011), so by this time, birds no longer have an opportunity to recalibrate their magnetic or stellar compasses using sunset cues.

At the end of the cue-conflict phase, we transferred the birds (without touching or catching them) from the upper part of our setup to the plastic Emlen funnel (the lower part) by removing the plastic plate between the two parts of the setup and slowly moving the upper part down. All procedures for each setup lasted <1 min. Using this technique, we tried to minimize any stress effects on bird orientation. After that, we turned off the magnetic coils, provided the birds with a short time period (approximately 10 min) to calm down and acclimate to a new magnetic field condition (the NMF), and started the video recordings (orientation test phase). Each orientation test lasted 40 min (for pied flycatchers and robins) or 55 min (for garden warblers). We chose the different duration of tests for different species because garden warblers need much more time to show significant orientation in Emlen funnels than robins and pied flycatchers according to data of our previous studies (Pakhomov et al., 2017a,b; Zolotareva et al., 2021) on these model species. Birds did not see the experimenters during either the cue-conflict or orientation phases. We visited our experimental site only when we changed the magnetic field and transferred birds from the upper part of our setup to the lower one at the end of the cue-conflict phase. Birds had full access to the NMF and stars during orientation tests.

Expectations

There are three main predicted orientation responses to cue-conflict treatments that model species with different calibration strategies could show in orientation tests according to Cochran et al. (2004) and Pakhomov and Chernetsov (2020): (1) the magnetic field dominates over celestial cues and calibrates the stellar compass, which is used by birds to maintain the preferred direction during a nocturnal migratory flight (Fig. 2A); (2) the magnetic compass is calibrated by celestial cues and birds use the magnetic compass as the main compass system to maintain the direction at night (Fig. 2B); and (3) the magnetic or stellar cues are used by birds separately without transferring information between the compasses (simple dominance; Fig. 2C).

For European robins, we used the same magnetic treatment protocol (magnetic field rotated 120 deg CW) in both migratory

seasons. For the other species, we used 120 deg CW magnetic rotations in autumn and 120 deg CCW magnetic rotations in spring), so that our birds should show the opposite shift in direction for responses 1 and 2 (Fig. 2A,B) in spring and autumn.

Data analysis and statistics

Recorded video was analyzed using BirdOriTrack, specialized software for analysis of animal behaviour in circular arenas (Muheim et al., 2014). This software tracks and analyses movements of birds inside the Emlen funnels and calculates the relative vector length for each movement (valid data point) from the centre of the funnels (resting area) toward the outside (outer circle of orientation cages). Based on these data and vector algebra, the mean orientation and the mean vector length are calculated from the direction of all valid points (see details in Muheim et al., 2014). We included the results of the birds tested at least two times with at least one result being sufficiently active (i.e. ≥20 jumps from the centre of a funnel to its outer circle) and used the 5% level according to the Rayleigh test (*P*, calculated by BirdOriTrack) as a criterion to use this data (mean direction) for further analysis. Inactive (less than 20 jumps) and disoriented trials (*P*>0.05) were excluded from calculations of the group mean vector and direction. For each bird, the mean orientation of all tests in a given condition (control and cue-conflict) was calculated. The group mean vectors for each condition were calculated by vector addition of unit vectors in each of the individual birds' mean directions. The group mean orientation was compared against uniformity with the Rayleigh test.

The nonparametric Mardia–Watson–Wheeler (MWW) test, which tests whether two samples are different from each other with respect to mean directions, variance or a combination of both measurements (Batschelet, 1981), was used to test the significance of difference between the control and the cue-conflict groups. Results were regarded as significant at *P*<0.05. Statistical tests were performed with the ORIANA software package (Kovach Computing Services, UK, version 4.02) and Python 3.7.

Additionally, to explore the probability of a type II error, i.e. that we failed to detect a change of direction after the cue-conflict exposure, e.g. owing to the low sample size, we performed Monte Carlo simulations (using a custom-written Python script), previously described in our recent study (Chernetsov et al., 2020). In our approach to type II error estimation, we took the control sample and estimated the probability of reaching results similar to ours (i.e. to reach the same or a higher *P*-value as we obtained in the MWW tests) in a comparison test with a random sample from an artificial von Mises population. To compute this estimation, we performed 10,000 comparisons of control sample versus random samples of size *N* from $V(\theta+\Delta\theta, \kappa+\Delta\kappa)$, where *N* is the size of the experimental sample and $V(\theta+\Delta\theta, \kappa+\Delta\kappa)$ is a von Mises population with parameters of mean and concentration $\theta+\Delta\theta$ and $\kappa+\Delta\kappa$, respectively. In this notation, θ and κ are the parameters of the control sample, $\Delta\theta$ and $\Delta\kappa$ are effect sizes for mean and concentration. The estimated probability can be close to the type II error probability for effects on mean and concentration $\Delta\theta$ and $\Delta\kappa$, with an assumption that the control sample is a perfect representation of a parent statistical population. Confidence intervals (CIs) were calculated using the bootstrap method.

RESULTS

Table 1 summarizes the results of all experiments in the control condition and after cue-conflict treatments (raw data from each bird are in Table S1). Garden warblers and pied flycatchers kept under control conditions (in the natural magnetic field) during the cue-
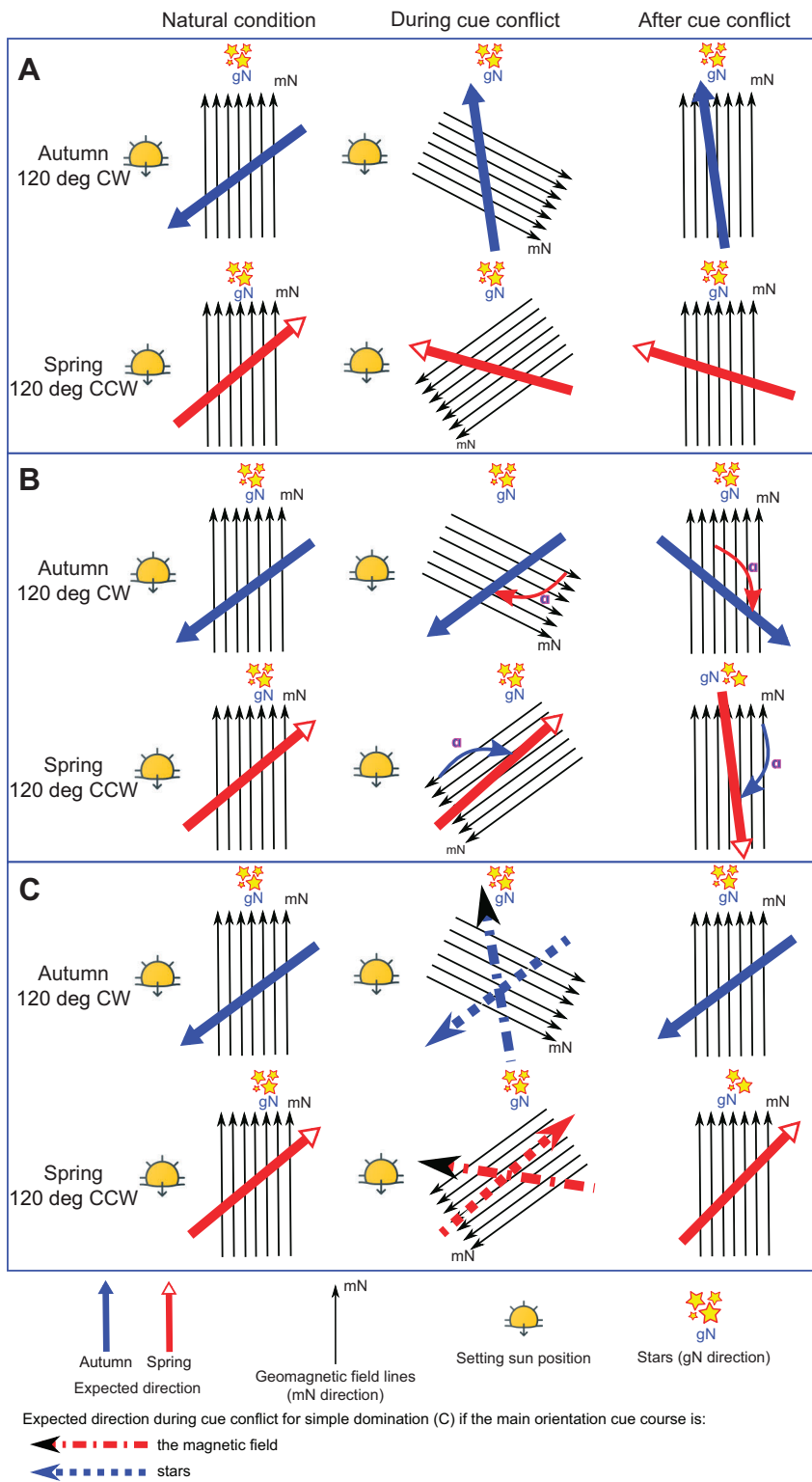
Fig. 2. Predicted orientation responses of birds exposed to an altered magnetic field [rotated 120 deg clockwise (CW) in autumn and 120 deg counterclockwise (CCW) in spring for garden warblers and pied flycatchers; rotated 120 deg CW in spring and autumn for European robins] during the cue-conflict phase of the experiments according to three hypotheses about compass calibration. (A) The magnetic field dominates over celestial cues and calibrates the stellar compass, which is then used by the birds to maintain the preferred direction during a nocturnal migratory flight. (B) The magnetic compass is calibrated by celestial cues and the birds use the magnetic compass as the main compass system to maintain their direction at night. (C) The magnetic or the stellar cues are used by birds separately without transferring information between compasses (simple dominance). Dashed arrows indicate the expected directions during cue-conflict treatments in the situation of simple dominance of stellar or magnetic compasses (C) depending on which of the compasses dominates (black arrowhead: the magnetic compass; blue or red arrowhead: the stellar compass). gN, geographic north; mN, magnetic north.

conflict phase of the experiments were oriented in their species-specific migratory direction in autumn (garden warblers, Fig. 3A; pied flycatchers, Fig. 4A) and spring (garden warblers, Fig. 3C; pied flycatchers, Fig. 4C). The directions shown by the control birds in our tests are not different from the migratory directions of these species during autumn migration on the Courish Spit according to ring recoveries (Bolshakov et al., 2001) and data from previous experiments (Kavokin et al., 2014; Pakhomov and Chernetsov,

2014; Pakhomov et al., 2017b). After cue-conflict treatments (the magnetic field deflected 120 deg CW/CWW in autumn/spring), the birds from the cue-conflict group chose a seasonally appropriate migratory direction very similar to the conspecific control birds in autumn (garden warbler, Fig. 3B; pied flycatcher, Fig. 4B) and spring (garden warbler, Fig. 3D; pied flycatcher, Fig. 4D). The 95% CIs for the groups overlapped in both seasons and the two relevant distributions (control and cue-conflict) were statistically

Table 1. Orientation of garden warblers, pied flycatchers and European robins in the control condition and after cue-conflict treatments

Season	Species	Condition	α (deg)	r	P	n	95% CI (deg)
Autumn	Garden warbler	Control	221	0.57	<0.001	25	196–246
		Cue conflict	259	0.46	0.005	24	226–292
	Pied flycatcher	Control	240	0.5	0.007	19	206–274
		Cue conflict	215	0.42	0.04	18	173–257
	European robin	Control	233	0.55	0.001	21	204–262
		Cue conflict	256	0.69	<0.001	21	235–278
Spring	Garden warbler	Control	44	0.38	0.03	23	1–86
		Cue conflict	45	0.53	0.004	19	13–77
	Pied flycatcher	Control	6	0.67	<0.001	22	344–27
		Cue conflict	31	0.52	0.002	21	0–62
	European robin	Control	348	0.73	<<0.001	21	329–8
		Cue conflict	272	0.45	0.004	27	240–304

α is the mean direction of birds in each condition, r is the length of a mean vector in each condition, P is the significance level according to the Rayleigh test, n is the number of birds in each group and 95% CI is the 95% confidence interval. Geographic north is 0 deg.

indistinguishable, according to the MWW test for garden warblers (autumn: $W=4.49$, $P=0.09$; spring: $W=1.04$, $P=0.60$) and pied flycatchers (autumn: $W=0.13$, $P=0.86$; spring: $W=5.44$, $P=0.07$). Despite the fact that results of the MMW test for spring tests in pied flycatcher are close to the significance threshold, both 95 and 99% CIs (95% control: 344–27 deg, cue-conflict: 0–62 deg; 99% control: 338–34 deg, 99% cue-conflict: 351–71 deg) for these distributions are overlapped so we could say birds chose the similar directions when they were tested in control and cue-conflict conditions (for more details, see the Discussion). There were birds that showed opposite direction in two sequential tests (the difference between two directions was >120 deg). We omitted such cases from the final analysis, but the result did not change: in both conditions (control tests and after cue-conflict), birds showed the seasonally appropriate migratory directions and no sign of calibration (Table S2). Heat maps that represent results of Monte Carlo simulations (Fig. S2) indicate that the probability of obtaining a similar or higher P-value

(higher than we obtained in the MWW test for each model species) in hypothetical situations when the mean direction in the cue-conflict group would differ from the mean direction in the control group by 40 deg or more, but we could not see this difference. This means that our sample size (or other factor) did not have an effect on results of the MWW test (we did not make type II errors) and the orientation of the control and cue-conflict birds actually do not differ from each other.

Whereas long-distance migrants did not show any type of calibration after cue-conflict treatment in both migratory seasons, middle-distance migratory European robins responded to the magnetic manipulation prior to the orientation tests differently depending on the migratory season. Young, inexperienced European robins showed a simple dominance of magnetic or stellar compasses (according to our scheme in Fig. 2C) during their first autumn migration, like garden warblers and pied flycatchers (control European robin, Fig. 5A; cue-conflict robin, Fig. 5B;
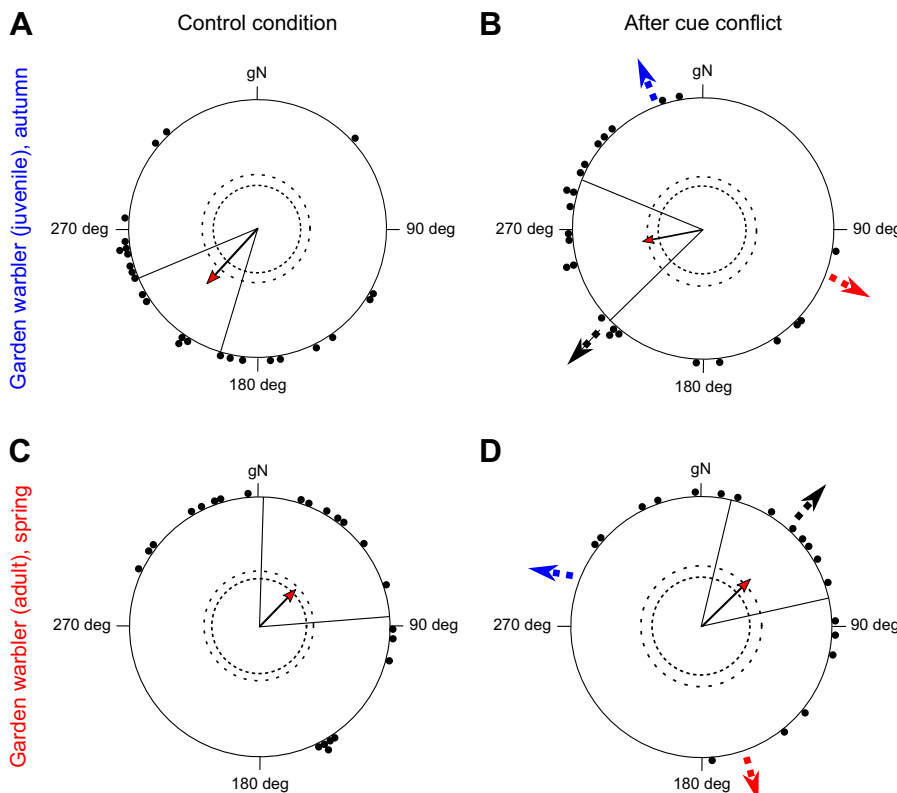

Fig. 3. Orientation of juvenile and adult garden warblers exposed to control (the unchanged geomagnetic magnetic field and all natural astronomical cues) or cue-conflict (a 120 deg CW/CCW rotated magnetic field in autumn/spring and natural astronomical cues) conditions. (A,B) Juveniles, autumn migration; (C,D) adults, spring migration. Each dot at the circle periphery indicates the mean orientation of one individual bird; the inner and outer dashed circles represent the 1 and 5% significance levels of the Rayleigh test, respectively. Geographic north corresponds to 0 deg, radial lines indicate the 95% confidence intervals (CIs) for the group mean orientation direction. Dashed arrows indicate the expected direction according to the different compass calibration strategies: blue: the magnetic field calibrates the stellar compass; red: the magnetic compass is calibrated by celestial cues; black: simple dominance of one compass system (stellar or magnetic).
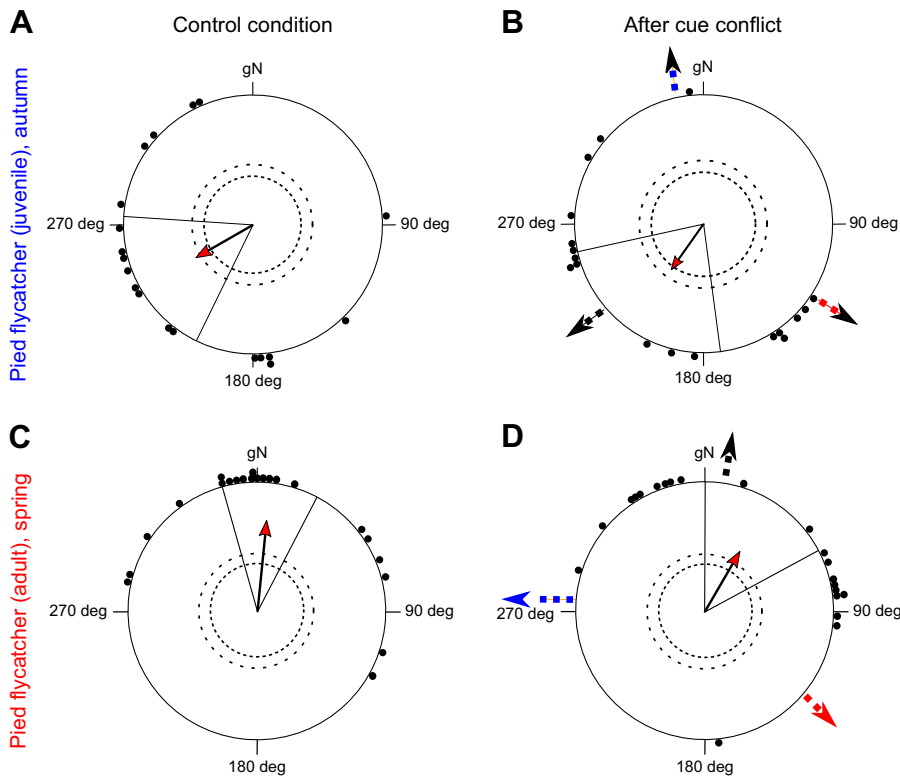
Fig. 4. Orientation of juvenile and adult pied flycatchers exposed to control (the unchanged geomagnetic magnetic field and all natural astronomical cues) or cue-conflict (a 120 deg CW/CCW rotated magnetic field in autumn/spring and natural astronomical cues) conditions. (A,B) Juveniles, autumn migration; (C,D) adults, spring migration. For a description of the circular diagrams, see the legend in Fig. 3.

MWW autumn: $W=2.05$, $P=0.36$). However, in spring, adult robins that had completed at least one migration changed their orientation in Emlen funnels after exposure to the magnetic field with magnetic north deflected 120 deg CW, and were oriented towards the southwest (Fig. 5D) in contrast to control birds, which showed a typical northerly direction (Fig. 5C). The difference between the mean orientation of the robins from the control and the cue-conflict groups was highly significant (MWW spring: $W=14.7$, $P<<0.001$; 99% CIs did not overlap). The mean orientation of the cue-conflict birds was 76 deg CCW relative to the direction of the birds in the control condition. The predicted change in orientation was a 120 deg CCW rotation after the cue-conflict and a 76 deg CCW
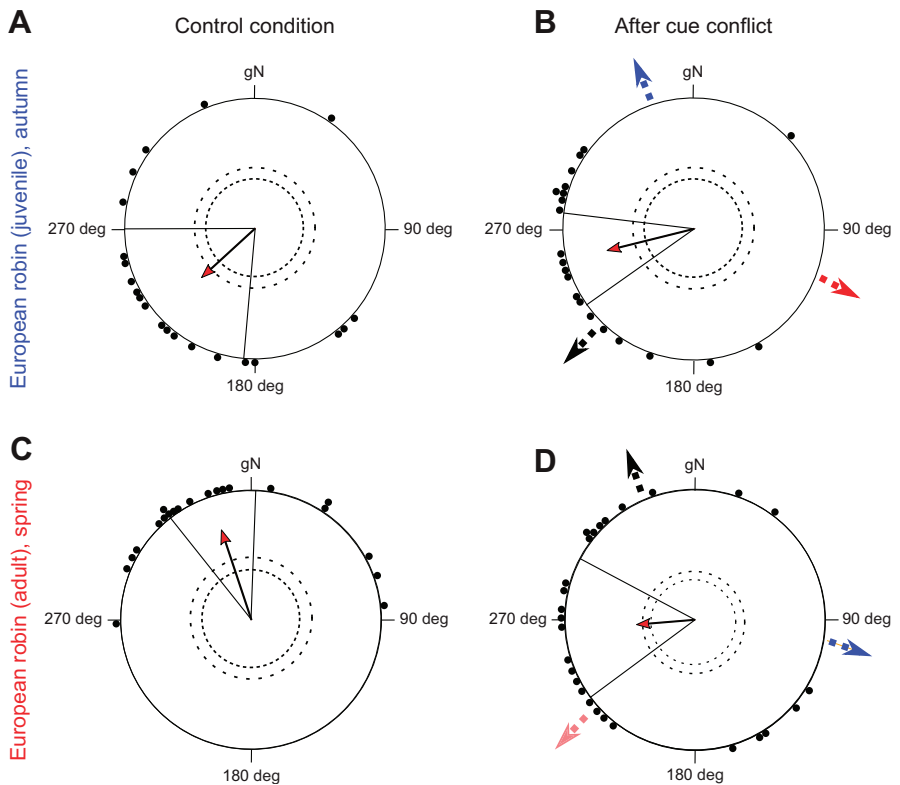

Fig. 5. Orientation of juvenile and adult European robins exposed to control (the unchanged geomagnetic magnetic field and all natural astronomical cues) or cue-conflict (a 120 deg CW rotated magnetic field in autumn and spring, and natural astronomical cues) conditions. (A,B) Juveniles, autumn migration; (C,D) adults, spring migration. For a description of the circular diagrams, see the legend in Fig. 3.

rotation is within the reasonable scenarios considering that the 95% CIs of the control and cue-conflict groups suggest a possible change in direction range of somewhere between 323–298 deg=25 deg and 362–234 deg=128 deg.

DISCUSSION

Experimental data obtained in this 3-year project strongly suggest that two long-distance songbird migrants, garden warblers and pied flycatchers, do not calibrate their compass systems and show a simple dominance of the magnetic or stellar compasses even if they have access to all orientation cues, including the band of maximum polarization (BMP) and a view of the sky near the horizon at sunset. The compass calibration strategy chosen by these songbird species does not depend on season and age: control and cue-conflict birds in our experiments showed a similar species-specific migratory direction in both spring (adult birds) and autumn (only first-year birds) migrations. Our results agree with data from previously published studies in the same species: free-flying garden warblers (Sjöberg and Muheim, 2016) and pied flycatchers (Giunchi et al., 2015) released with radio tags within 1 h or the following day after a cue-conflict treatment (manipulation of the geomagnetic field in autumn and the BMP in spring, respectively) did not shift their vanishing directions as they should have done if their magnetic compass were calibrated by celestial cues similar to North American thrushes (Cochran et al., 2004). Interestingly, the same flycatchers tested in Emlen funnels covered with opaque lids to prevent them from seeing celestial cues before releasing them with radio tags showed a recalibration of the magnetic compass, in contrast to the results collected in the previous lab-based study by the same authors (Gaggini et al., 2010).

However, it should be noted that our pied flycatcher results are not fully unambiguous. The 95 and 99% CIs for control and experimental groups during spring migration overlap, but the P-level for the MWW test (0.07) is too close to the commonly assumed threshold (0.05), which might indicate some kind of difference between groups of pied flycatchers. The MWW test is not very powerful in detecting differences between two distributions when they are not both unimodal (Landler et al., 2021). There is an asymmetric bimodal distribution for birds tested after the cue-conflict treatment, with two clusters (Fig. 4D): birds from the first cluster concentrate around the direction that indicates no calibration (the black arrow), whereas birds from the other cluster concentrate close to the red arrow, indicating calibration of the magnetic compass by celestial cues. We cannot say for sure what the main reason is for such a distribution: (1) some birds choose NW and N as a main migratory direction and other birds prefer NE and E (a type of artefact owing to experimental conditions/settings) or (2) some birds calibrate their magnetic compass by information from celestial cues, whereas others do not (different access to orientation cues inside the experimental setup, population-specific or individual variation, etc.). The results of previous studies on pied flycatchers are also conflicting: this species can show different responses to cue-conflict treatment depending to experimental design, access to cues during orientation tests, etc. (Gaggini et al., 2010; Giunchi et al., 2015). We still interpret our results in the sense that pied flycatchers do not calibrate their compass systems, but acknowledge the points discussed in this paragraph and emphasize that calibration strategy of this species requires further study.

Only adult European robins showed calibration of the magnetic compass by celestial cues in our spring experiments after cue conflict (120 deg CCW deflected magnetic field and natural celestial cues). The initial design of magnetic manipulation in our cue-conflict treatment was 120 deg CW in spring and CCW in autumn for all modal species. However, we made a mistake during the first spring season of 2018: we started to perform experiments in which the magnetic field was rotated 120 deg CCW (instead of 120 deg CW). When we detected that mistake, several robins had already been tested and we decided to not change that cue-conflict treatment for that species but use the initial design for other ones. We cannot say with certainty whether this difference in the magnetic protocol (CW or CCW) between European robins and other species influenced the results of cue-conflict treatment. To do so, we need to perform additional experiments in which we analyse orientation of European robins after CW magnetic cue-conflict treatment.

All cue-conflict studies can be divided into two main types: either orientation tests and cue-conflict treatment are performed at the same time (type I), or orientation tests are performed after the cue-conflict phase (immediately or the next day or migratory season; after-effects to cue-conflict treatments; type II). There was some asymmetry in response to different magnetic cue-conflict treatments (CW or CCW) in some previous studies: Prinz and Wiltschko (1992) obtained evidence for an asymmetry in the response to pre-migratory exposure to shifted magnetic fields (type II). They observed recalibration of the magnetic compass only when the magnetic field was shifted CCW relative to the celestial cues (and birds became disoriented when the field was shifted CW). Another situation was observed in type I cue-conflict studies, according to Muheim et al. (2006a): the birds exposed to CW shifts of the magnetic field generally followed the shift with remarkable accuracy compared with the birds exposed to CCW shifts. However, results of cue-conflict studies (type II) performed during migration seasons indicate no difference between CW and CCW magnetic treatments: song thrushes did not show any sign of compass calibration after pre-exposure to 120 deg CW (spring) and CCW (autumn) rotated magnetic fields before release (Chernetsov et al., 2011). In another study performed in North American songbird migrants, different model species were tested in different designs of magnetic cue-conflict treatment (CW: red-eyed vireo, *Vireo olivaceus*, and northern waterthrush, *Parkesia noveboracensis*; CCW: indigo bunting, *Passerina cyanea*, and grey catbird, *Dumetella carolinensis*) during the same migratory season (autumn) and showed calibration of the stellar compass system by the magnetic field (Sandberg et al., 2000). Based on these data, we suggest that the difference in magnetic treatment (in spring) between species is very unlikely to have affected the results of our cue-conflict experiments.

All theories and hypotheses about this phenomenon proposed in previous experimental studies and reviews have not been proven until now. A suggestion that the difference in the hierarchy of compass systems between North American and European species might be explained by the variation of parameters of the geomagnetic field (e.g. declination) that birds could expect to encounter during their migration in North America but not in Europe (Liu and Chernetsov, 2012) was not supported by the latest calculation of variation of the geomagnetic field in North America and Europe (Sjöberg and Muheim, 2016). In contrast, atmospheric radio-frequency fields produced by thunderstorms at levels that are sufficient to disturb a magnetoreceptive animal are a much more common phenomenon in central North America than in Europe (Granger et al., 2022), and could be another possible explanation for the difference in compass calibration strategies between species of North America and Europe. Such radio-frequency fields could disrupt magnetic compass orientation (Ritz et al., 2004; Kavokin et al., 2014; Pakhomov et al., 2017b), and North American species

should rely on more stable orientation cues (stars and the sun) and calibrate the magnetic compass if they use it as a compass system during nocturnal flight. If the assumption that long-distance migrants calibrate their compasses and short-distance migrants do not were true (Chernetsov et al., 2011), we would find a difference in compass calibration strategy between these two groups in our study. However, garden warblers and pied flycatchers did not show any type of calibration like that of their conspecifics and other long-distance songbird migrants in previous studies (Gaggini et al., 2010; Schmaljohann et al., 2013; Sjöberg and Muheim, 2016). In contrast, medium-distance European robins responded to cue-conflict treatment in an unexpected way: young, unexperienced birds did not respond to the conflict between the celestial and magnetic compasses during their first autumn migration, whereas their adult conspecifics changed the direction of their activity in circular arenas when the magnetic field was rotated 120 deg CW in spring. A possible explanation for this seasonal difference in the behaviour of birds of the same species is the difference in age and degree of development of links between various compass systems in juveniles and adults. Presumably, young, inexperienced European robins during the autumn migration may have fully developed compass systems, as shown in earlier experimental studies (Wiltschko and Wiltschko, 1972; Katz, 1985; Pakhomov et al., 2017a), but the hierarchy between them may not yet be fully formed. In contrast, adult birds in spring that have already performed at least one complete migration from breeding grounds to wintering locations in southern Europe might have set their hierarchy. However, juvenile and adult Savannah sparrows in autumn (Able and Able, 1995) and song thrushes in autumn and spring did not show differences in their compass calibration strategies (Chernetsov et al., 2011).

As mentioned above, we did not find any experimental evidence for the theory proposed by Muheim and co-authors in their review (Muheim et al., 2006a) and later updated to an extended unified theory (Sjöberg and Muheim, 2016) in which a view to the sky near the horizon at sunset (or sunrise) plays a crucial role determining whether a migratory bird calibrates its compass systems before departure from a stopover site. In our experiments, all birds were kept inside the upper part of the experimental setup during the cue-conflict treatment, which provided them with full access to the surroundings and the setting sun and the BMP down to the horizon. Only adult European robins showed calibration of the magnetic compass relative to the information from celestial cues in spring (but not in autumn). Previous studies performed in various European migratory species using a different experimental design (Gaggini et al., 2010; Chernetsov et al., 2011; Schmaljohann et al., 2013; Åkesson et al., 2015; Giunchi et al., 2015; Sjöberg and Muheim, 2016; Vanni et al., 2017) showed similar results (no calibration), whereas studies in North American songbird migrants mostly showed celestial calibration of their magnetic compass (Cochran et al., 2004; Muheim et al., 2006b, 2007, 2009). Trying to explain contradictory results of these studies, the authors of the extended unified theory (Sjöberg and Muheim, 2016) made several suggestions why most researchers who performed cue-conflict experiments in European songbird migrants failed to show calibration of the magnetic compass by celestial cues.

One suggestion was that a view of the surroundings and the sky near the horizon should be crucial for successful provisional and full magnetic compass recalibration. Therefore, if birds are tested in Emlen funnels that block the view of the horizon, they should not show recalibration of the magnetic compass (Sjöberg and Muheim, 2016). However, in many experiments in free-flying birds (Chernetsov et al., 2011; Schmaljohann et al., 2013), the birds

had access to the surroundings and the sky (plus the BMP) during cue-conflict treatment and still did not show any sign of calibration. We also did not find any relationship between a view of the sky near the horizon and provisional compass calibration. Additionally, according to Pakhomov and Chernetsov (2020), the BMP might become broader and blurrier when moving towards the horizon at sunset and sunrise, so it could be difficult to use the BMP as a calibration cue in the way proposed by the authors of this theory.

Another suggestion was that compass calibration in migratory birds is a two-phase process: birds calibrate their magnetic compass by solar cues (maybe the polarized light), after which the information from the newly calibrated magnetic compass is used to calibrate a stellar compass system. If cue-conflict treatment in experimental studies takes place before stars become visible in the sky (Giunchi et al., 2015; Sjöberg and Muheim, 2016) or if polarizing filters are used to create a cue conflict between the magnetic field and solar cues (the relationship between the magnetic field and the stars remains unchanged in this condition; Schmaljohann et al., 2013), birds will not have an opportunity to calibrate their stellar compass by the magnetic field and will not change their orientation in circular cages or after release with radio tags. However, firstly, birds were oriented in the seasonally appropriate population-specific direction after cue-conflict treatment in our experiments even if they could see the stars during the exposure to the cue-conflict between the magnetic field and celestial cues (we turned off the magnetic coils after nautical twilight). Secondly, there is no direct evidence that all songbird migrants use the stellar compass but not the magnetic one during nocturnal flights (Chernetsov, 2015).

A third suggestion was that if the birds are kept in an outdoor aviary with a full view of the natural celestial cues prior to the cue-conflict exposures in the same area (e.g. Wiltschko et al., 2008; Chernetsov et al., 2011), they may have already fully calibrated their compasses before the first experiments and will not pay attention to any cue-conflict during the tests (Muheim et al., 2008). This suggestion cannot explain the results obtained by us in three different songbird migrants in the present study because we kept our birds in a windowless indoor aviary after capture, so our birds could not see any orientation cues (the sun and polarized light, and the stars) and/or local landmarks before and between tests and thus had no opportunity to calibrate their compasses before the cue-conflict exposure.

Finally, we would like to discuss a situation that could take place (theoretically) in our experiments. As mentioned above, according to the extended theory, compass calibration in birds is a two-step process: calibration of the magnetic compass by solar cues and calibration of the stellar compass by information from the newly calibrated magnetic compass. Owing to our experimental design, there is no opportunity to determine with certainty what compass system (magnetic or stellar) is usually used by our model species to determine and maintain migratory direction during a nocturnal flight. To investigate that, the birds should be tested in two different conditions after the cue-conflict phase: in the natural magnetic field without stars and in the vertical magnetic field with access to stars. However, in previous studies, it has been shown that two of our model species (garden warbler and European robin) prefer certain compass systems in direct cue conflict during orientation tests (Wiltschko and Wiltschko, 1975a,b). The authors of these studies tested birds in the 120 deg CW rotated magnetic field with a view of the clear natural night sky, and found that garden warblers changed their orientation according to the rotation of the magnetic field, in spite of contradicting information from the stars. European robins, in contrast, did not respond (in early tests) to a deflection of

magnetic north and used their stellar compass as the main compass system. This difference between two species could be explained by the fact that long-distance migrants cannot use the same stars in breeding and wintering sites and thus should rely on a more constant cue source as the geomagnetic field. Therefore, if birds in our experiments calibrate their magnetic compass by the sun or polarized light at sunset but then do not transfer information from the magnetic compass to the stellar compass system and use the last one without calibration due to any reason, we would be unable to detect any difference between the cue-conflict and control groups. However, in the current project, we studied the whole compass calibration two-step process (without dividing it into two separate steps) and did not investigate each step separately from each other, as this was beyond the scope of the present study. The results of our experiments indicate that access to the sky near the horizon and stars during cue-conflict treatments cannot affect the 'to calibrate or not to calibrate' process, at least in two long-distance migrants.

Conclusions

In conclusion, we suggest that the interrelationship between compass systems among various species of migratory birds during migration is a very complicated phenomenon in the wild, and even though it does seem to be a part of human nature trying to find a simple unified answer for everything, in this case, it seems to be a 'wild goose chase'. All attempts to create a theory that can explain all the contradictory results of all cue-conflict experiments have failed so far. We suggest that the simple reason is that no uniform solution exists. A more probable explanation of the variable results obtained by different authors in different avian species on different continents is that each species and even different populations within one species may 'choose' their calibration strategy differently, and that such choices might even change during a migratory journey depending on the variable ecological contexts.

Author contributions
Conceptualization: A. Pakhomov; Methodology: A. Pakhomov; Software: F.C.; Validation: A. Pakhomov; Formal analysis: A. Pakhomov, A. Prokshina, F.C.; Investigation: A. Pakhomov, A. Prokshina; Resources: A. Pakhomov, H.M.; Data curation: A. Pakhomov, A. Prokshina; Writing - original draft: A. Pakhomov, N.C.; Writing - review & editing: A. Pakhomov, H.M., N.C.; Visualization: A. Pakhomov, F.C.; Supervision: A. Pakhomov, N.C.; Project administration: A. Pakhomov; Funding acquisition: A. Pakhomov, H.M., N.C.

Data availability
Python code for the Monte-Carlo simulation is stored on GitHub: https://github.com/pythoctopus/Circular/tree/master

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A

B

Fig. S1. A – A photo of our magnetic coils on the experimental site. B – A drone photo of our experimental site (1) and the main staton of building (2); mN (magnetic North) is on the top of the photo. Tested birds had a clear view of local surroundings and the Courish lagoon (North, West and East) and a restricted view of southern landmarks (due to trees in local park nearby).
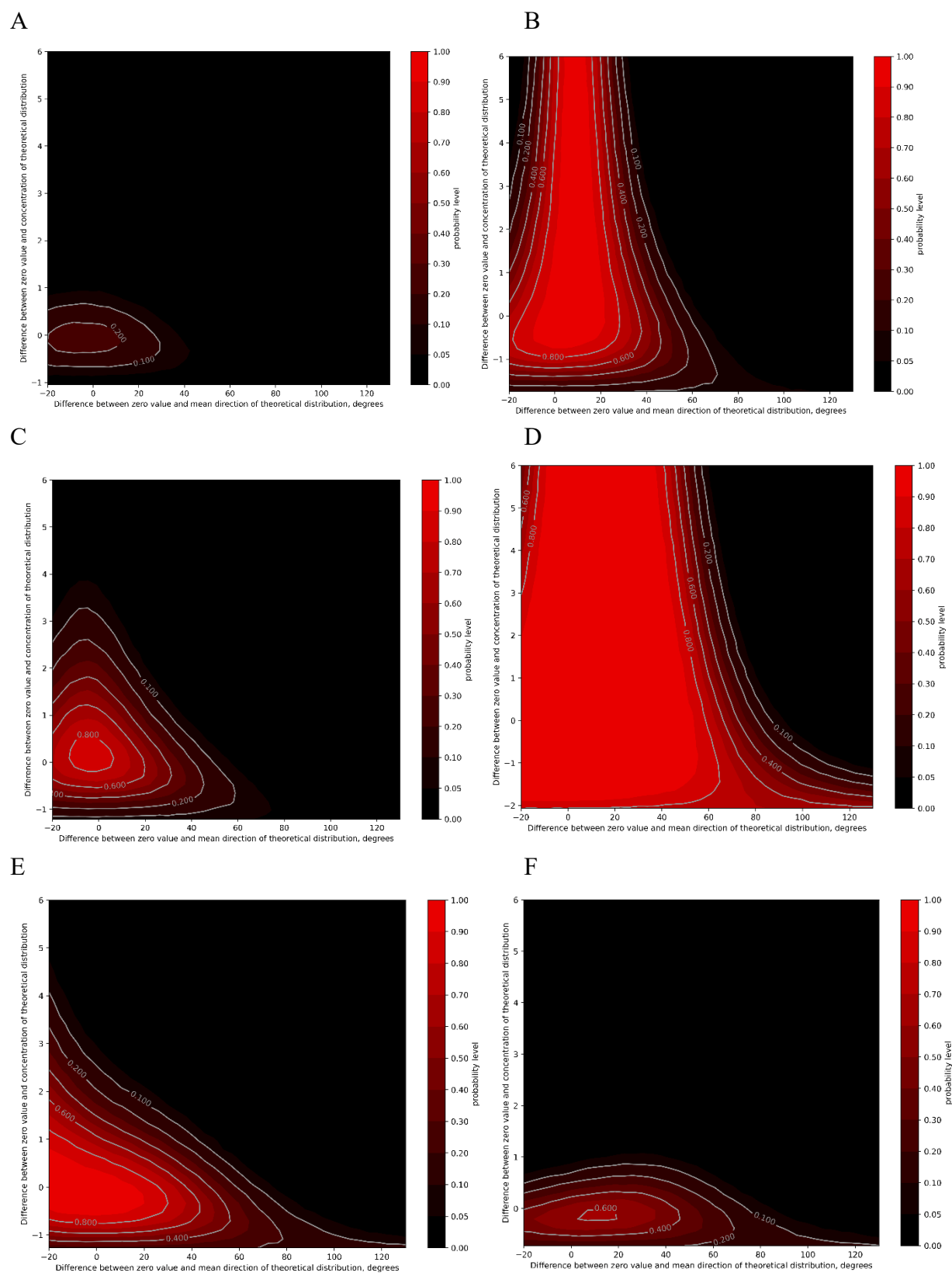
Fig. S2. **Heatmaps estimating second type error probability.**

Fig. S3. A photo of our CalOri setup.

1 - the upper part, for cue-conflitc phase.
2 - the lower part, for orientation tests (a modified Emlen funnel).

Table S1. Raw data of all orientation tests.

Orientation of garden warblers in the control condition and after cue-conflict during autumn migration 2019-2020.

№	control					cue-conflict				
	№ bird	results of each trial			Mean	№ bird	results of each trial			Mean
1	726*	168°	ns		168°	624	249°	na		249°
2	749*	113°	214°		164°	726*	31°	130°	235°	128°
3	721*	ns	257°		257°	749*	110°	112°	245°	140°
4	40	na	231°		231°	721*	215°	na		215°
5	988*	180°	na		180°	615	89°	164°		127°
6	182	208°	na		208°	988*	100°	233°		167°
7	68*	na	304°		304°	666	295°	312°		304°
8	748	228°	45°	117°	116°	68*	341°	322°		337°
9	601*	220°	191°		206°	042	ns	177°		177°
10	38	146°	271°		209°	601*	ns	233°		223°
11	771*	230°	na		230°	242	262°	na		262°
12	54	134°	45°	147°	113°	771*	280°	na		280°
13	697	75°	7°		41°	619	na	216°		216°
14	63	315°	170°		242°	653	na	94°		94°
15	917	148°	129°		139°	786	na	313°		313°
16	823	na	149°		149°	204	308°	na		308°
17	844	110°	325°	276°	313°	963	68°	262°		281°
18	970	na	257°		257°	608	na	250°		345°
19	209	290°	250°		270°	149	275°	na		250°
20	219	191°	ns		191°	786	261°	na		275°
21	240	na	247°		247°	963	na	264°		264°
22	295	236°	ns		236°	149	ns	290°		290°
23	318	248°	na		248°	204	na	217°		217°
24	626	260°	na		260°	210	ns	294°		294°
25	799	ns	187°		187°	242	na	na		
26	17	ns	ns			786	ns	na		
27	977	na	ns			210	na	na		

Orientation of garden warblers in the control condition and after cue-conflict during spring migration 2018.

№	control					cue-conflict				
	№ bird	results of each trial			Mean	№ bird	results of each trial			Mean
1	249	na	151°		**151°**	36	10°	na		**10°**
2	329	ns	151°		**151°**	193	26°	na		**26°**
3	253	308°	ns		**308°**	142	336°	ns		**336°**
4	255	38°	39°		**38°**	334	ns	138°		**138°**
5	269	125°	310°	330°	**335°**	474	na	3°		**3°**
6	265	18°	na		**18°**	606	20°	75°		**47°**
7	289	230°	1°		**296°**	534	109°	233°		**171°**
8	292	355°	148°		**72°**	542	307°	ns		**307°**
9	333	7°	37°		**22°**	477	103°	74°		**89°**
10	332	149°	ns		**149°**	500	37°	na		**37°**
11	361	305°	na		**305°**	602	34°	152°		**98°**
12	392	95°	ns		**95°**	452	82°	ns		**82°**
13	373	30°	ns		**30°**	580	254°	358°		**304°**
14	372	na	153°		**153°**	435	54°	343°	86°	**43°**
15	195	ns	342°		**342**	469	53°	77°		**65°**
16	201	91°	na		**91°**	743	90°	163°		**126°**
17	188	354°	na		**354°**	474	ns	54°		**54°**
18	26	341°	na		**341°**	606	ns	353°		**353°**
19	145	36°	ns		**36°**	556	330°	ns		**330°**
20	36	na	146°		**146°**	534	na	ns		
21	110	ns	105°		**105°**	331	na	ns		
22	958	na	51°		**51°**					
23	93	na	330°		**330°**					

Orientation of pied flycatchers in the control condition and after cue-conflict during autumn migration 2019.

№	control					cue-conflict				
	№ bird	results of each trial			Mean	№ bird	results of each trial			Mean
1	197	ns	252°		252°	439	188°	ns		188°
2	191	55°	na	105°	80°	279	61°	273°		348°
3	299	244°	ns		244°	277	199°	ns		199°
4	241	212°	ns		212°	323	ns	139°		139°
5	235	236°	na		236°	200	ns	268°		268°
6	234	263°	na		263°	238	69°	189°		129°
7	196	na	167°		167°	234	na	118°		118°
8	424	306°	na		306°	164	na	294°		294°
9	438	130°	na		130°	123	253°	ns		253°
10	425	ns	213°		213°	147	139°	na		139°
11	117	na	273°		273°	687	249°	na		249°
12	443	300°	ns		300°	166	255°	na		255°
13	258	167°	na		167°	631	178°	ns		178°
14	436	ns	330°		330°	218	124°	ns		124°
15	417	ns	251°		251°	266	na	304°		304°
16	619	ns	171°		171°	283	na	248°		248°
17	431	173°	na		173°	284	142°	na		142°
18	286	233°	na		233°	605	na	247°		247°
19	423	43°	254°		319°					
20	429	ns	ns							

Orientation of pied flycatchers in the control condition and after cue-conflict during spring migration 2019.

№	control				cue-conflict						
	№ bird	results of each trial		Mean	№ bird	results of each trial					Mean
1	159	357°	22°	10°	160	122°	60°				91°
2	147	293°	260°	277°	164	269°	315°	121°			282°
3	258	ns	345°	345°	123	338°	na				338°
4	218	18°	85°	51°	283	50°	67°				58°
5	266	340°	355°	348°	177	325°	283°				304°
6	284	335°	343°	339°	166	108°	271°	296°	69°	107°	77°
7	286	339°	344°	342°	283	ns	322°				322°
8	285	4°	341°	353°	162	69°	78°				74°
9	281	339°	na	339°	043	77°	ns				77°
10	279	136°	69°	103°	015	289°	29°				339°
11	934	359°	ns	359°	017	333°	na				333°
12	932	na	353°	353°	938	116°	34°				75°
13	939	ns	355°	355°	955	323°	na				323°
14	949	298°	na	298°	001	ns	46°				46°
15	954	na	69°	69°	933	183°	153°				168°
16	955	ns	113°	113°	934	72°	ns				72°
17	957	ns	318°	318°	945	ns	344°				344°
18	000	2°	na	2°	947	na	na	86°			86°
19	009	280°	ns	280°	032	325°	ns				325°
20	011	61°	ns	61°	948	11°	ns				11°
21	013	271°	83°	357°	953	62°	na				62°
22	034	na	46°	46°							

Orientation of European robins in the control condition and after cue-conflict during autumn migration 2018.

№	control				cue-conflict			
	№ bird	results of each trial		Mean	№ bird	results of each trial		Mean
1	319	336°	340°	**338°**	143	274°	na	**274°**
2	838	325°	256°	**282°**	839	ns	249°	**249°**
3	815	141°	na	**141°**	820	262°	300°	**281°**
4	817	**187°**	161°	**174°**	799	245°	ns	**245°**
5	786	237°	212°	**224°**	850	226°	240°	**233°**
6	793	na	222°	**222°**	869	na	235°	**235°**
7	863	ns	306°	**306°**	936	300°	ns	**300°**
8	873	na	180°	**180°**	900	251°	ns	**251°**
9	894	217°	na	**217°**	830	331°	271°	**291°**
10	874	na	196°	**196°**	876	186°	212°	**194°**
11	989	ns	138°	**138°**	954	na	284°	**284**
12	875	230°	253°	**242°**	955	na	279°	**279°**
13	837	35°	34°	**35°**	862	41°	ns	**41°**
14	844	141°	125°	**132°**	960	223°	297°	**255°**
15	836	210°	207°	**208°**	789	316°	282°	**299°**
16	562	210°	265°	240° **235°**	831	263°	297°	**281°**
17	950	270°	265°	230° **258°**	832	204°	214°	**209°**
18	142	230°	280°	**255°**	573	191°	259°	**225°**
19	951	210°	285°	**247°**	818	na	217°	**217°**
20	837	295°	na	**295°**	957	160°	160°	131° **146°**
21	903	ns	240°	**240°**	876	154°	182°	**168°**

Orientation of European robins in the control condition and after cue-conflict during spring migration 2019-2020.

№	control							cue-conflict						
	№ bird	results of each trial					Mean	№ bird	results of each trial					Mean
1	365	336°	356°				346°	350	267°	na				267°
2	342	293°	na				293°	398	355°	334°	270°	246°	236°	286°
3	362	332°	325°				324°	687	271°	ns				271°
4	370	310°	286	ns			298°	389	na	325°	73°			19°
5	394	na	323				323°	593	329°	324°	163°			311°
6	393	350°	na	na			350°	588	218°	ns				218°
7	414	333°	na				333°	591	na	232°				232°
8	361	327°	na				327°	387	ns	275°				275°
9	369	354°	331°				343°	359	228°	228°				228°
10	406	130°	353°	na			62°	399	13°	28°	49°	53°		36°
11	382	296°	302°	339°	37°		8°	353	131°	na				131°
12	10	na	317°				317°	407	116°	283°	311°	23°		342°
13	64	na	302°				302°	317	114°	182°				148°
14	852	ns	84°				84°	402	337°	283°				310°
15	995	283°	117°	30°			33°	63	223°	na				223°
16	994	348°	na				348°	54	ns	315°				315°
17	984	269°	na				269°	938	na	248°				248°
18	965	ns	325°				325°	18	ns	na	238°			238°
19	837	59°	82°				71°	58	ns	164°				164°
20	11	314°	325°				320°	842	318°	na				318°
21	896	115°	10°	356°			33°	970	327°	ns				327°
22	945	na	na					15	243°	na				243°
23	989	na	ns					55	327°	290°				309°
24								936	ns	122°				122°
25								937	253°	ns				253°
26								840	na	151°				151°
27								993	na	283°				283°

NA – not active, NS – not significant; red – 2019, blue – 2020; * tested in both control and cue-conflict conditions; magnetic North is 0°

Table S2. Orientation of garden warblers and pied flycatchers in the control condition and after cue-conflict treatments, without birds that showed opposite direction in two sequential tests (the difference between two directions was > 120°). α is the mean direction of birds in each condition, r is the length of a mean vector in each condition, p is the significance level according to the Rayleigh test, nthe is number of birds in each group, 95% CI is 95 % confidence interval; gN is 0°, MWW test – Mardia-Watson-Wheeler test.

Season	Species	Condition	α	r	p	n	95% CI	MWW test, W/p
Autumn	Garden warbler	control	219°	0.54	< 0.001	23	232° – 297°	4.663/0.1
		cue-conflict	265°	0.48	0.004	23	191° – 248°	
	Pied flycatcher	control	234°	0.54	0.004	18	202° – 266°	0.37/0.8
		cue-conflict	210°	0.49	0.01	17	174° – 247°	
Spring	Garden warbler	control	47°	0.39	0.04	21	4° – 90°	1.79/0.4
		cue-conflict	41°	0.6	0.001	18	13° – 69°	
	Pied flycatcher	control	6°	0.66	< 0.001	21	343° – 29°	4.56/0.1
		cue-conflict	31°	0.52	0.002	21	0° – 62°	