

RESEARCH ARTICLE

Ontogeny of the star compass in birds: pied flycatchers (*Ficedula hypoleuca*) can establish the star compass in spring

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ABSTRACT

The star compass of birds, like the sun compass, is not innate. To possess either of them, birds have to observe the rotating sky and determine its centre of rotation (in the case of the star compass) or the sun's movement (for the sun compass). Young birds are believed to learn how to use the star compass before their first migration, even though the evidence of this is lacking. Here, we tested whether hand-raised Pied flycatchers (*Ficedula hypoleuca*) that had not established the star compass prior to their first autumn migration can gain it later in their ontogeny, in spring. We also attempted to examine whether the observation of diurnal celestial cues (the sun and polarized light) prior to autumn migration would affect the process of star compass learning in spring. When tested in the vertical magnetic field under the natural starry sky, the group of birds that observed the stars in spring as the first celestial cues were able to choose the migratory direction. In contrast, the birds that had never seen the stars were not able to use the nightly celestial cues in the vertical magnetic field. However, birds that had seen the daytime celestial cues till autumn and the stars at spring were disoriented, although this might be due to the small sample size. Our data suggest the possibility that the star compass may be learned in spring and emphasize the necessity for further research into the interaction of celestial compasses.

KEY WORDS: Bird migration, Orientation, Compass systems, Celestial cues, Magnetic compass, Migratory birds, Emlen funnels

INTRODUCTION

Migratory birds can use three different compass systems to determine their migratory direction – the sun compass (Kramer, 1952; Schmidt-Koenig, 1990), the star compass (Emlen, 1967a,b; Pakhomov et al., 2017) and the magnetic compass (Wiltschko and Wiltschko, 1972; Hore and Mouritsen, 2016). Even though avian compass systems work independently of one another (Mouritsen, 1998), there exists a rather complex and species-specific hierarchy and interactions between them (Pakhomov and Chernetsov, 2020). It has been assumed that because of their common features, the star and the sun compasses form a single celestial compass system, which is independent of the magnetic compass (Alert et al., 2015b; Wiltschko and Wiltschko, 2015). The magnetic compass is innate and works around the clock (Mouritsen, 2013), while birds should

learn orientation according to the sun and the stars in ontogenesis, observing the rotation of celestial bodies and in some way processing this information (Emlen, 1970; Wiltschko et al., 1981). For the proper functioning of the solar and the star compasses, only vision is necessary, and for the magnetic compass, a separate magnetoreceptor system is needed (Mouritsen and Hore, 2012). Despite this, the validity of combining the solar and the star compasses into a single celestial compass system is not confirmed or refuted. An important point against this is the fact that the solar compass depends on the birds' sense of time (Schmidt-Koenig, 1990), whereas the star compass is time independent and can be used successfully even when the internal clock is out of sync with the external photoperiod (Mouritsen and Larsen, 2001; Pakhomov et al., 2017). Apart from theoretical considerations, the existence of a unified celestial compass could be shown experimentally if their common characteristics complement each other.

In order to develop the star compass, young birds should observe the rotating starry sky and detect the centre of its rotation (Emlen, 1970). However, the behavioural and physiological mechanisms behind this process remain unknown. After the establishment of a functional star compass, birds can orient in a species-specific direction even under a stationary planetarium sky (Emlen, 1967a,b; Mouritsen and Larsen, 2001).

Currently, the centre of rotation of the starry sky in the northern hemisphere coincides with the polar star, because of its position above the Earth's rotational axis. However, birds do not have innate knowledge of what the centre of rotation should look like – indigo bunting (*Passerina cyanea*) reared in the planetarium with the sky rotating around Betelgeuse interpreted it as the centre of rotation and consequently as the 'north' in orientation experiments (Emlen, 1970, 1975). Later, it was shown that garden warblers (*Sylvia borin*) could even find the centre of rotation of 16 artificial light dots, even without a dot in the centre (Wiltschko et al., 1987). It should be noted, however, that the exact duration of observation of the stars by birds remains unknown: in most studies, it is about 2 weeks or more (Emlen, 1970; Wiltschko et al., 1987; Able and Able, 1990; Prinz and Wiltschko, 1992; Michalik et al., 2014). There has only been one attempt to determine a minimum period of star observation, suggesting that 7 nights of observing the celestial rotation is not sufficient for young European robins (*Erithacus rubecula*) to establish the star compass, whereas 14 nights is enough (Michalik et al., 2014).

Since the first experiments of Emlen (1970, 1972), young birds have been thought to recognize the centre of rotation of the starry sky strictly before their first migration. There is only one piece of evidence of the relevance of the first year to the star compass learning, carried out in six birds (Emlen, 1972). Young indigo bunting trained to detect Betelgeuse as the sky rotational centre before their first autumn migration failed to retrain the star compass after being exposed to a normal rotated starry sky from spring prior to their second autumn migration. In contrast, implicit evidence that

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birds can learn how to use the star compass later in ontogeny, in spring, can be found in a study on compass calibration. European robins raised in the absence of celestial cues were unable to choose the autumn migratory direction in the normal magnetic field, but after being exposed to celestial cues from the end of winter, oriented significantly in their species-specific north-easterly spring migratory direction in the normal magnetic field (Alert et al., 2015b).

In the present study, we investigated whether birds are able to develop the star compass later than prior to their first autumn migration. We hand-raised pied flycatchers, *Ficedula hypoleuca* (Pallas 1764), without access to celestial cues till their first autumn migration with subsequent exposure of half of the group to the natural starry sky in spring. If flycatchers can learn how to use the star compass in spring, the group of birds exposed to the stars would be able to choose the migratory direction when tested in the vertical magnetic field, i.e. in the absence of magnetic compass information, with access only to stellar orientation cues. In contrast, birds that had not seen the stars would be disoriented.

Furthermore, we were interested in the role of daytime cues (the sun and polarized light) in the star compass learning process. Therefore, we hand-raised an additional group of flycatchers with exposure to the sun and polarized light, but not the nocturnal sky, in summer, and then exposed them to the starry sky in spring, with the group described above. If birds can learn how to use the star compass in spring, we expected to see orientation similar to that for the group that had seen the stars alone. However, if the birds with full access to celestial cues were oriented in the species-specific direction and the birds with access only to the stars were not, then the former had learned how to use the rotation (the sun movement) till autumn and later this facilitated the recognition of starry sky rotation for them. In this case, we would be able to conclude that the star compass and the sun compass are parts of one common celestial compass, based on the determination of celestial rotation.

MATERIALS AND METHODS

Experimental birds and site

In June 2019, a total of 58 pied flycatcher nestlings were removed from 17 nest boxes in the vicinity of Biological Station Rybachy on the Courish Spit (Kaliningrad Region, Russia; 55°09'N, 20°52'E). The eyes of all nestlings were still closed; thus, they had not seen any natural celestial cues. All birds were hand-raised indoors in a windowless aviary, the magnetic field was not disturbed and the photoperiod was identical to the local photoperiod. The birds were kept in individual cages (45×45×45 cm size). Water and food (mealworms, eggs, carrot, breadcrumbs and Padovan complete feed for insect-eating birds) were supplied *ad libitum*. The food was periodically enriched with greenery, beef heart, calcium gluconate and vitamins (Vinka). The aviary was equipped with a UV lamp (Cosmedico Cosmosun 36R, UVB/UVA 3.6%), which was plugged in every day for several hours. In order not to damage the birds' eyes, the lamp was situated higher than the level of the cages and the light from it was directed upwards to create scattered UV light. The aviary was also equipped with online infrared video cameras, so that we could register the nocturnal migratory restlessness of the birds.

On 8 July 2019, after the birds became self-sufficient, they were divided into two groups and subjected to the following conditions: group I, 'celestial cues-deprived group', birds ($N=44$) remained indoors in the local geomagnetic field without access to celestial cues; group II, 'stars-deprived group', birds ($N=14$) were transferred to the outdoor aviary, where they had full access either to the local geomagnetic field or to the day celestial cues (the sun and polarized light on sunrise and sunset). The birds of group II were kept in

individual net cages (45×45×45 cm). Every night after sunset until sunrise, we covered the cages with dense non-transparent fabric, to prevent the birds from seeing the stars. On 18 September 2019, because of cold weather and a hailstorm, this group of birds was transferred back to the indoor windowless aviary.

On 18 October 2019, the photoperiod in the aviary was changed from natural to fixed, 10.5 h:13.5 h light:dark. On 16 March 2020, the imitation of natural photoperiod of the Courish Spit was set again (earlier than flycatchers normally arrive to this area to stimulate the moult and the following nocturnal migratory restlessness of our experimental birds).

During the wintertime, the birds' diet was enriched with crickets 1–2 times per week and one common UV lamp was changed to several small ones (ReptiZoo, 2% and 5% UVB), which were switched on in front of the cages for 1–2 h, 2 times per week.

From the beginning of February 2020, the birds were observed 1–2 times per week and all mass changes and fat rate were recorded. The type of fat accumulation and the changes in mass suggested normal development of spring migratory disposition and were similar to the results of Helm et al. (2019). Spring nocturnal migratory restlessness started at the beginning of March 2020, and was widespread at the beginning of April 2020.

All animal procedures (the capture of the birds and simple, non-invasive, behavioural experiments) were approved by the appropriate authorities: Permit 24/2018-06 by Kaliningrad Regional Agency for Protection, Reproduction and Use of Animal World and Forests; and Permit 2017-12 by the specialized committee of the Scientific Council of the Zoological Institute, Russian Academy of Sciences.

Experimental conditions and setup

Each bird was tested for its migratory orientation in modified Emlen funnels made of aluminium (top diameter 300 mm, bottom diameter 100 mm, slope 45 deg with the top opening covered by netting). We put polyethylene coverings painted with a dried mixture of whitewash and glue inside funnels to record the birds' scratches in the direction they wanted to move.

The birds were tested in two different conditions: in the natural magnetic field without access to the stars (the NMF, indoors) and under the natural starry sky in the artificial vertical magnetic field (the VMF, outdoors) which had the same intensity as the NMF but provided no compass information.

Experiments in the NMF were held indoors, in a hut constructed especially for orientation experiments (see details in Bojarinova et al., 2020). The chamber for the test was screened, grounded and illuminated with dim green light. Light was produced by light-emitting diodes (LEDs) with a peak wavelength of 527 nm. Similar illumination in this hut was used in our previous study (Bojarinova et al., 2020). On the top of the Emlen funnels, we put opaque glass lids which let through enough scattered light for the birds to use the magnetic compass but completely obscured all environmental cues in the wooden house. Thus, the only orientation cue for the birds inside the funnels was the local geomagnetic field (intensity of $50,300\pm 50$ nT, 70.1 ± 0.2 deg inclination and $+5.5\pm 0.1$ deg declination, means \pm s.d.; Table S1).

Experiments in the VMF were held outdoors, under the natural starry sky in the artificial magnetic field, created by the double-wrapped, three-dimensional Merritt four-coil system (Kirschvink, 1992). The coil system was the same as that used in the previous studies of our group (Pakhomov et al., 2017, 2018). The parameters of the VMF were total intensity of $50,300\pm 50$ nT and inclination of 89.9 ± 0.1 deg (means \pm s.d.; Table S1); thus, while being tested, the birds were not able to use the magnetic compass. Additionally, the

nocturnal tests started after the beginning of the astronomical twilight so that information from neither the sun nor the polarized light patterns was available. Experiments in the VMF were carried out only on windless and moonless nights when >70% of the starry sky was visible; in most tests, the sky was 95–100% clear. Thus, the stars were the only orientation cue in the experiments in the VMF condition.

Exposure to the starry skies

In March 2020, group I, ‘celestial cues-deprived group’, was subdivided into two groups: group Ia, ‘celestial cues-deprived till spring group’, served as a control and remained indoors without access to celestial cues; and group Ib, ‘sun- and polarized light-deprived till spring group’, was exposed to the natural starry sky. All experimental birds from group II, ‘stars deprived group’, were exposed to the stars; thus, this group was renamed the ‘group with access to all celestial cues’. Thus, in spring we had three groups of birds: group Ia, ‘celestial cues-deprived till spring group’, birds ($N=23$) had not seen the sun, polarized light or stars; group Ib, ‘sun- and polarized light-deprived till spring group’, birds ($N=19$) had not seen the sun or polarized light, but had seen the movement of the natural stellar sky in spring; group II, ‘group with access to all celestial cues’, birds ($N=12$) had seen the sun and polarized light in the autumn, and had seen the movement of the natural stellar sky in spring.

The criteria for division of group I, ‘celestial cues-deprived group’, into two groups were the number of nestboxes (the siblings were assigned to different groups), mass, fat, moult stage and general body condition of birds at the moment of division. The researchers who divided birds into groups (G.U. and N.R.) exposed the birds to the stars on starry nights but did not take part in spring orientation tests. The researcher performing nocturnal tests (A.Z.) was not aware which bird was in which group, and so performed experiments double-blind.

Exposure of the experimental birds under the natural starry sky took place on the roof of the main building of the Biological Station Rybachy. The roof is oriented to the woodland on the beach of the Courish Lagoon; therefore, human-origin lights and noises are absent there.

The birds were transferred to the roof during astronomical twilight on clear starry nights (50% of stars, usually $\geq 90\%$), so that information from the sun polarized light patterns at sunrise or sunset was not available to them. The weather was checked every 30 min. After 2–3 nights of star observations, the birds were allowed to rest for at least one full day. The birds observed the starry sky from March 8 to April 9 2020 for 13 nights and several additional hours (Table 1). We counted it as ‘night’ if the birds observed the stars for at least 4 h.

For positioning the birds on the roof, we used two types of cages, located inside the wooden fence protecting them from sideways winds. The first type were made from net with a wooden framework (58×40×42 cm); the second ones were round plastic with a net top (34 cm height, 53 cm in diameter). In both types of cages, the perches were situated on different angles and levels relative to each other; thus, the birds could have full access to the starry sky. We put 3–4 birds in one cage.

Orientation tests and data analysis

Autumn orientation tests were performed from 14 August to 26 September 2019, spring experiments from 11 April to 28 April 2020.

Only birds that were in the migratory state were picked for experiments. The presence of cameras in the indoor aviary

Table 1. Duration of exposure to the starry sky

Date (dd.mm.yyyy)	Group Ib	Group II
08.03.2020	8 h	9 h
09.03.2020	30 min	1 h
12.03.2020	5 h	5 h
15.03.2020	6 h 30 min	4 h 45 min
16.03.2020	30 min	1 h 10 min
19.03.2020	7 h 50 min	7 h
23.03.2020	6 h 30 min	6 h 30 min
24.03.2020	6 h 5 min	6 h 50 min
25.03.2020	6 h 20 min	6 h 30 min
27.03.2020	6 h 55 min	6 h 45 min
28.03.2020	7 h 25 min	7 h 50 min
04.04.2020	6 h 25 min	6 h 55 min
06.04.2020	4 h 5 min	4 h 30 min
07.04.2020	5 h 55 min	5 h 55 min
08.04.2020	2 h 30 min	2 h 30 min
09.04.2020	5 h	5 h 45 min

Group Ib, ‘sun- and polarized light-deprived till spring group’, had not seen the sun or polarized light, but had seen the movement of the natural stellar sky in spring. Group II, ‘group with access to all celestial cues’, had seen the sun and polarized light in autumn, and had seen the movement of the natural stellar sky in spring.

(‘celestial cues-deprived group’) allowed us to select only birds that showed nocturnal migratory restlessness for experiments. In the outdoor aviary (‘stars-deprived group’), we could not register the nocturnal restlessness of the birds because of the fabric covering their cages every night; nevertheless, all birds had a fat score of 3–4 or higher when used for experiments (the intestine was invisible owing to fat coverage, according to the 0–8 scale; Kaiser, 1993). The birds from the indoor aviary were tested simultaneously with the birds from the outdoor aviary on most nights to minimize any potential effects that weather could have on the orientation results.

Experimental birds were transferred from their cages to the funnels in individual opaque textile bags. Each test lasted for 20–25 min, with a maximum of two tests with one bird per night. After 2–3 nights of tests, the birds had a rest for 1–3 days.

During the tests, the funnels were positioned so that the joint in the covering coincided with the direction of magnetic north, so we could analyse the preferred direction. Each bird’s mean direction from the distribution of scratches was determined by two or three independent researchers (one of them was not taking part in this project) using the simple visual estimation method (following Mouritsen and Larsen, 1998; Fig. S1). If both observers considered that the distribution of scratchers was random or if there was more than 30 deg between their two mean directions, the bird was considered disoriented in the given test. At the moment of counting, the researchers did not know which birds the data were from or what experimental condition (if there were two of them on one night) they were estimating.

For group direction analysis, we used only mean data of the birds from which we obtained two or more angle results in the orientation tests to avoid pseudoreplication. Thus, the sample size differed from the number of experimental birds.

Statistical tests were performed with ORIANA (Kovach Computing Services, v.4.0). The differences in mean orientation direction between experimental groups were tested by the non-parametric Mardia–Watson–Wheeler (MWW) test, because the r -values for our group mean vectors in many cases were <0.75 , so we could not use the more powerful parametric Watson–Williams

test. Additionally, we used the bootstrap technique (Fisher, 1995) to identify whether significantly oriented groups showed significantly more directed behaviour than non-statistically significantly oriented groups. According to this method, a random sample of orientation directions (n angles) was drawn with replacement from the sample of orientation directions present in the significantly oriented group. Based on these n orientation angles, the corresponding r -value was calculated, and this procedure was repeated 100,000 times. After that, the resulting 100,000 r -values are ranked in ascending order: the r -values at rank 2500 and 97,500, and at rank 500 and 99,500 define the 95% and 99% 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 oriented group is significantly more directed than the disoriented group with a significance of $P < 0.05$ and $P < 0.01$, respectively.

RESULTS

In autumn, all groups of pied flycatchers in the natural magnetic field without access to the stars (the NMF) were oriented in the southern direction (Fig. 1A,B). The mean direction of group I, 'celestial cues-deprived group', was 178 deg [$r = 0.42$, $N = 35$, $P = 0.002$, 95% confidence interval of the mean group direction (CI mean) 148–209 deg; Fig. 1A]. The mean direction of group II, 'stars-deprived group', was 210 deg ($r = 0.60$, $N = 14$, $P = 0.004$, 95% CI mean 178–243 deg; Fig. 1B). The two distributions were statistically indistinguishable (MWW test: $W = 2.68$, $P = 0.26$) and their 95% CIs overlapped broadly.

The mean autumn migratory direction calculated from the same-year recoveries of young Scandinavian pied flycatchers (Mouritsen and Larsen, 1998) was 212 deg ($N = 1138$, 95% CI mean 211–213 deg). According to data of ringing recoveries, it is known that

pied flycatchers from the eastern Baltic have a complex spatio-temporal migration programme: at the beginning of migration they adhere to the south-west, and afterwards to the south and south-east (Bolshakov et al., 2001). Pied flycatchers hand-raised on the Courish Spit and maintained with full access to celestial cues (Kishkinev et al., 2006), tested on 21–25 August 2005, oriented 278 deg ($r = 0.76$, $N = 6$, $P = 0.02$, 95% CI mean 234–323 deg), but those tested on 2–10 October 2005 oriented 151 deg ($r = 0.89$, $N = 7$, $P = 0.001$, 95% CI mean 125–178 deg). Thus, all pied flycatchers hand-raised in Rybachy in 2019 were significantly oriented in the seasonally appropriate population-specific direction.

The experimental birds from all groups tested in the vertical magnetic field, i.e. without access to the magnetic compass information, under the natural starry sky (the VMF), were active, but not significantly oriented (Fig. 1C,D). The mean direction of pied flycatchers from group I, 'celestial cues-deprived group', was 286 deg ($r = 0.1$, $N = 31$, $P = 0.74$; Fig. 1C), and from group II, 'stars-deprived group', was 131 deg ($r = 0.15$, $N = 14$, $P = 0.74$; Fig. 1D). The random orientation of birds from both groups in the VMF condition was significantly different from the oriented behaviour of these birds in the NMF condition (the bootstrapped 99.9% CI for the r -value of birds from group I, 'celestial cues-deprived group' and group II, 'stars-deprived group', was $0.19 < r < 0.75$ and $0.38 < r < 0.91$, respectively).

In spring, all groups of birds tested indoors in the NMF without the view of the stars were oriented in the northern direction (Fig. 2A–C). Pied flycatchers from group Ia, 'celestial cues-deprived till spring group', were oriented in the north-eastern direction (mean direction 47 deg; $r = 0.70$, $N = 16$, $P < 0.001$, 95% CI 23–70 deg; Fig. 2A). The birds from group Ib, 'sun- and polarized light-deprived till spring group', showed a similar orientation (mean direction 45 deg, $r = 0.50$, $N = 14$, $P = 0.018$, 95% CI 6–84 deg;

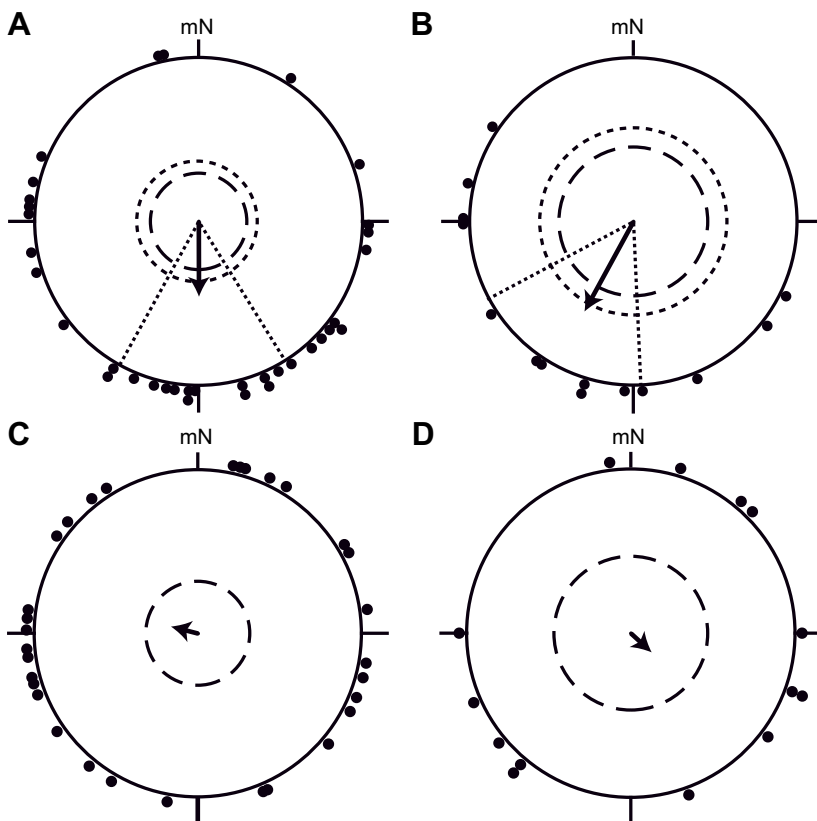


Fig. 1. Autumn orientation of hand-raised pied flycatchers.

(A) The direction of birds that were maintained indoors without access to natural celestial cues; group I, 'celestial cues-deprived group', in the natural magnetic field, without access to the stars (the NMF). $N = 35$, mean direction 178 deg, $r = 0.42$, $P = 0.002$, 95% confidence interval of the mean group direction (CI mean) 148–209 deg. (B) The direction of birds maintained outdoors with full access to the natural diurnal celestial cues, group II, 'stars-deprived group', in the NMF. $N = 14$, mean direction 210 deg, $r = 0.60$, $P = 0.004$, 95% CI mean 178–243 deg. (C) The direction of birds that were maintained indoors without access to natural celestial cues; group I, 'celestial cues-deprived group', in the vertical magnetic field, under the natural starry sky (the VMF). $N = 31$, mean direction 286 deg, $r = 0.1$, $P = 0.74$. (D) The direction of birds maintained outdoors with full access to the natural diurnal celestial cues, group II, 'stars-deprived group', in the VMF. $N = 14$, mean direction 131 deg, $r = 0.15$, $P = 0.74$. The dots at the periphery of the circle represent the mean heading of a single bird, the arrow represents the group orientation mean vector (circle radius represents a vector length $r = 1$); the radial lines indicate the 95% CI; the inner and outer dashed circles indicate the required length of r for significance levels of 5% and 1% according to the Rayleigh test, respectively. mN, magnetic north.

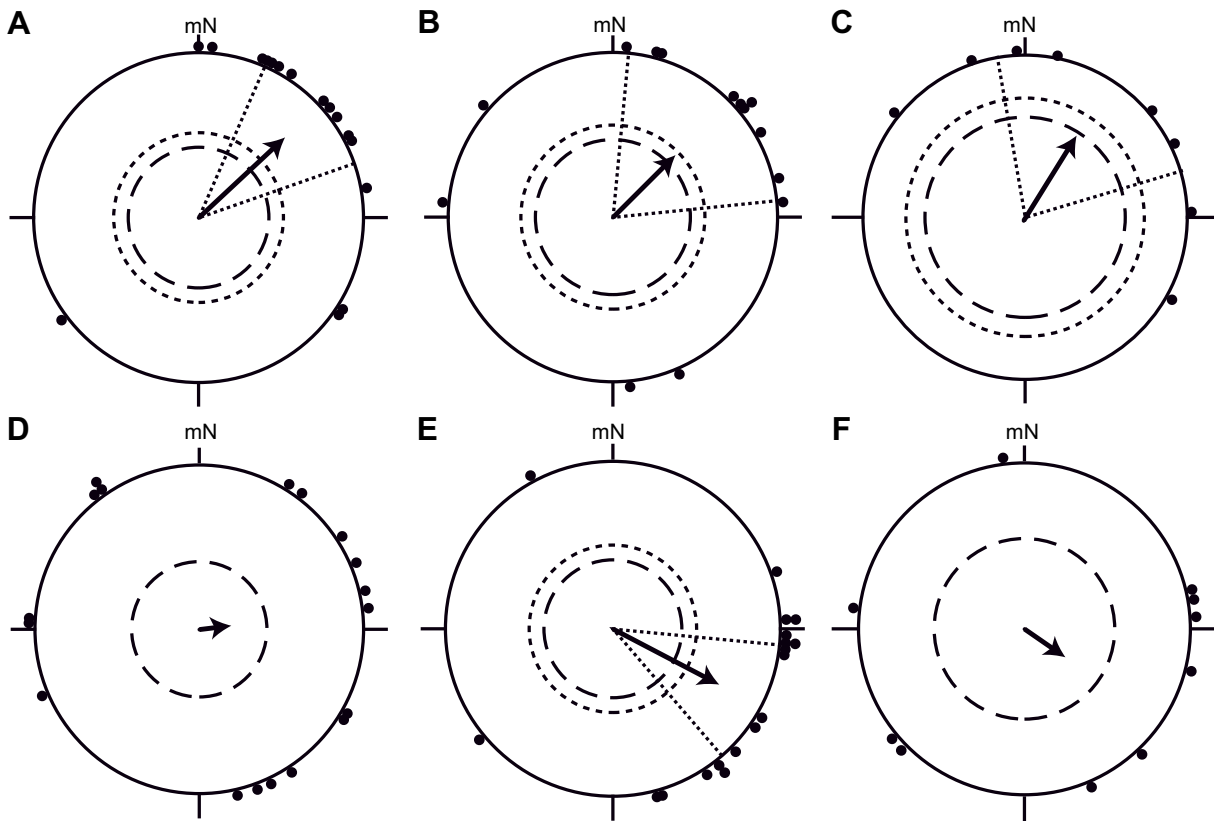


Fig. 2. Spring orientation of hand-raised pied flycatchers. (A) The direction of birds maintained indoors without access to natural celestial cues prior to spring experiments, group Ia, 'celestial cues-deprived till spring group', in the natural magnetic field without access to the stars (the NMF). $N=16$, mean orientation 47 deg, $r=0.70$, $P<0.001$, 95% CI 23–70 deg. (B) The direction of birds maintained indoors without access to any natural celestial cues till spring, then in spring had seen the stars for 13 nights, group Ib, 'sun- and polarized light-deprived till spring group', in the NMF. $N=14$, mean orientation 45 deg, $r=0.50$, $P=0.018$, 95% CI 6–84 deg. (C) The direction of birds maintained outdoors with full access to the natural day celestial cues till autumn and that had viewed the stars in spring, group II, 'group with access to all celestial cues', in the NMF. $N=8$, mean orientation 32 deg, $r=0.60$, $P=0.038$, 95% CI 350–73 deg. (D) The direction of birds maintained indoors without access to natural celestial cues prior to spring experiments, group Ia, 'celestial cues-deprived till spring group', in the vertical magnetic field, under natural starry sky (the VMF). $N=18$, mean orientation 84 deg, $r=0.2$, $P=0.52$. (E) The direction of birds that were maintained indoors without access to natural celestial cues till spring, and in spring had seen the stars for 13 nights, group Ib, 'sun- and polarized light-deprived till spring group', in the VMF. $N=18$, mean orientation 118 deg, $r=0.7$, $P=0.001$, 95% CI 96–139 deg. (F) The direction of birds that were maintained outdoors with full access to the natural day celestial cues till autumn and had viewed the stars in spring, group II, 'group with access to all celestial cues', in the VMF. $N=10$, mean orientation 124 deg, $r=0.29$, $P=0.44$. The dots at the periphery of the circle represent the mean heading of a single bird, the arrow represents the group orientation mean vector (circle radius represents a vector length $r=1$); the radial lines indicate the 95% CI; the inner and outer dashed circles indicate the required length of r for significance levels of 5% and 1% according to the Rayleigh test, respectively. mN, magnetic north.

Fig. 2B). Finally, the mean direction of birds from group II, 'group with access to all celestial cues', was 32 deg ($r=0.60$, $N=8$, $P=0.038$, 95% CI 350–73 deg; Fig. 2C). According to unpublished data of experiments performed at the Biological Station Rybachy by A.P., pied flycatchers caught on the Courish Spit during spring migration and tested in the NMF under the natural starry sky were oriented northwards (mean direction 358 deg, $r=0.70$, $N=15$, $P<0.001$, 95% CI 331–26 deg). Therefore, we conclude that the magnetic compass of hand-raised birds was working properly in spring, as 95% CI of data obtained from hand-raised flycatchers in the NMF without access to the stars overlapped with more natural data.

In the VMF under the natural starry sky, however, the results were different (Fig. 2D–F). Group Ia, 'celestial cues-deprived till spring group', was disoriented (mean direction 84 deg, $r=0.2$, $N=18$, $P=0.52$; Fig. 2D), as was group II, 'group with access to all celestial cues' (mean direction 124 deg, $r=0.29$, $N=10$, $P=0.44$; Fig. 2F). However, group Ib, 'sun- and polarized light-deprived till spring group', was highly significantly oriented to the east-southeast (mean direction 118 deg, $r=0.7$, $N=18$, $P<0.001$, 95% CI 96–139 deg; Fig. 2E). This distribution was statistically different from

the distribution of group Ia, 'celestial cues-deprived till spring group' (MWW test: $W=8.994$, $P=0.01$).

The results of the bootstrap analysis clearly indicate that the birds from all oriented groups showed significantly more directed behaviour than the birds from non-significantly oriented groups. (The bootstrapped 99.9% CI for the r -value of birds from group I, 'celestial cues-deprived till spring group' and group II, 'group with access to all celestial cues', in the VMF condition was $0.69<r<0.96$ and $0.37<r<0.94$, respectively; Fig. S2.)

DISCUSSION

Our results show that pied flycatchers, hand-raised in the absence of all celestial cues, as well as those hand-raised without access to the stars but able to see the natural movement of the diurnal sky, are able to orient in the species-specific direction in the normal magnetic field during either autumn or spring migration. The birds probably relied on their magnetic compass. In contrast to pied flycatchers, some other bird species require access to celestial cues for proper functioning of their innate magnetic compass, at least at some stages of their ontogeny (Able and Able, 1990; Alert et al., 2015b).

However, the data in the literature on pied flycatchers are rather contradictory. Birds reared in Germany without access to celestial cues could orient using magnetic information alone (Beck and Wiltschko, 1982; Bingman et al., 1985). In contrast, pied flycatchers raised under the same conditions, i.e. without access to celestial cues, in Latvia, behaved bimodally: they were oriented along the axis that included the seasonally appropriate migratory direction and the opposite one, but were not able to select the correct end of the axis (Weindler et al., 1995, 1998). If Latvian birds were allowed to see the rotating planetarium sky in summer, they were oriented unimodally, in the southerly autumn migratory direction. The authors stress the importance of viewing celestial cues for calibration of the magnetic compass in pied flycatchers (Weindler et al., 1998). They also suggest their experimental birds were not able to distinguish between their normal migratory direction and the opposite one because of the higher magnetic latitude of the Latvian site (56°55'N, 23°10'E; magnetic intensity 50,000 nT, with 73 deg inclination) in contrast to the German one (50°08'N, 08°40'E; intensity 46,000 nT with 66 deg inclination). Biological Station Rybachy is situated at higher latitude than the German site, but lower than the Latvian one (total intensity 50,118 nT, inclination 70.1 deg). Pied flycatchers in Rybachy could orient in the natural magnetic field without an established star compass, unlike their Latvian conspecifics. We have no clear explanation for this discrepancy.

In the VMF, under the starry sky in autumn, our experimental pied flycatchers were disoriented. In contrast, pied flycatchers were able to orient using only nightly celestial cues, in the VMF, even if they were hand-raised in this field (Bingman, 1984). Thus, in contrast to the ability to orient in the natural magnetic field, in our experimental birds that had not seen the starry night sky, the star compass was not established till autumn. The fact that the birds were disoriented even if they had the opportunity to observe diurnal celestial cues underscores that observing the sun moving and polarized light as the only celestial orientation cues is not sufficient for star compass learning. Nevertheless, if in spring this was the only group that could choose the migratory direction in the VMF after the starry sky observation, we would conclude that this group had adopted the principle of celestial rotation in autumn and in spring laid the knowledge of starry sky appearance on it. However, in spring in the VMF, this group was disoriented, which we find difficult to explain. One possible reason could be the small sample size (12 birds produced only 10 data points for analysis), but other issues cannot be ruled out.

We assume that the duration of observation of the stellar rotation during tests in the VMF was not sufficient for the establishment of a celestial compass in our experimental birds. In our study, during autumn migration, one bird could see the starry night sky for a maximum of 4 h (8 tests for 30 min each, but usually a bird took part in 4–6 tests), and in European robins, 1 and even 7 nights of continuous observation were not sufficient for star compass establishment (Michalik et al., 2014). Such long periods of time needed for star compass learning can probably be explained by the low speed of rotation: the birds should identify the centre of an extremely slowly rotating sky ($0.0042 \text{ deg s}^{-1}$), and currently there is no neurobiological evidence that birds can detect such low velocities (Mouritsen et al., 2016). To overcome this difficulty, the birds could theoretically use the ‘snapshot strategy’, i.e. comparing the current star pattern with a memorized snapshot of the pattern relative to fixed local landmarks from some prior time (Alert et al., 2015a), which would need rather advanced cognitive abilities and memory.

When tested in spring in the VMF under the starry night sky, birds that all had the ability to orient in the natural magnetic field showed varying results. The birds that had seen the nightly celestial cues in the beginning of spring were significantly oriented, whereas the birds from the control group, which were maintained without access to the celestial cues, were disoriented. We should acknowledge that orientation of that group was not in the seasonally appropriate direction, being more southerly than expected. One possibility is that in spring, the birds need more than 13 nights for proper learning of the compass based on nightly celestial cues or that the time window when they need to see these cues exceeds 1 month. Nevertheless, we conclude that our experimental birds developed the compass based on celestial cues after exposure to the starry night sky in spring. This is in line with the recent study by Alert et al. (2015b), where European robins could recalibrate their magnetic compass according to the natural celestial cues in spring if they had not established the star compass previously. Our results do not support the idea of the crucial role of time prior to the first migration for star compass development, as suggested earlier (Emlen, 1972). In Emlen’s (1972) study, indigo buntings had already established their star compass before the first migration in the planetarium with the celestial axis rotating around Betelgeuse and subsequently failed to relearn it when exposed under the natural starry sky for summer. Thus, the birds already had a functioning star compass and could not relearn it, whereas in our study, as well as in that of Alert et al. (2015b), the birds were naive, i.e. maintained without access to the nightly celestial cues till spring. However, it should be mentioned that Emlen’s (1972) experiment was conducted in six birds and should be replicated with a larger sample size.

The fact that at least some migratory birds can establish a compass based on nightly celestial cues later than prior to their first migration is of great evolutionary significance. As shown repeatedly, migratory birds need 14 nights or more for successful star compass establishment (Emlen, 1970; Able and Able, 1990; Michalik et al., 2014). Long-distance migrants have a relatively short time window available between fledging and the onset of migration, during which they have to establish their star compass. Moreover, it was also proposed that short-distance migratory birds might have a special time-limited sensitive period prior to their first migration for star compass learning (Michalik et al., 2014). If migratory birds do have a limited period for star compass establishment, adverse weather conditions during it could easily prevent the birds seeing the rotating sky to a sufficient extent. Another intriguing question is how Arctic breeders that initiate their autumn migration in the season when the nights are still not sufficiently dark to see the stars establish their star compass. If they use the stellar compass at all, they have to learn it en route. Compass systems of birds are redundant and at least in some species of birds the innate magnetic compass can work even if the compass based on celestial cues has not been established (Beck and Wiltschko, 1982; Bingman et al., 1985). All properly working compasses can be calibrated accurately by the birds, or birds can choose the most appropriate one for the ambient conditions (Pakhomov and Chemetsov, 2020). Our results suggest a plasticity of celestial compass learning, which means that if for some reason the birds have not established it before their first autumn migration they could learn it later, even in spring of the subsequent year.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

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

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Supplementary information

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