New cue-conflict experiments suggest a leading role of visual cues in the migratory orientation of Pied Flycatchers *Ficedula hypoleuca*

Dimitri Giunchi[1,*], Lorenzo Vanni[1], N. Emilio Baldaccini[1], Fernando Spina[2] & Francesca Biondi[1]

[1]Department of Biology, Pisa University, Via Volta 6, 56126 Pisa, Italy
[2]Istituto Superiore per la Protezione e la Ricerca Ambientale (ISPRA), Sede Amministrativa ex-INFS, Via Ca' Fornacetta 9, 40064 Ozzano Emilia, Bologna, Italy

*Author for correspondence (dgiunchi@biologia.unipi.it)

Abstract

Migratory birds use both geomagnetic and celestial cues to select and maintain their seasonally appropriate migratory direction. The integration of the different compass cues is still poorly understood. Previous cue-conflict experiments suggested that Pied Flycatchers *Ficedula hypoleuca* did not recalibrate their magnetic compass against the polarization pattern at twilight, but the available evidence are problematic given the high variability of birds directional preferences. We performed a new set of cue-conflict experiments where (1) we modified the protocol in order to try to reduce scatter of data and (2) we integrated the results of two experimental approaches, i.e. orientation cages and releases of radio-tagged birds. Pied Flycatchers were tested in Emlen funnels without access to celestial cues before and after being exposed to conflicting visual and geomagnetic information. After the second test birds were equipped with radio-transmitters and followed until the vanishing of radio signal. Contrary to previous experiments, our data showed a general dominance of celestial cues: polarized light sun related pattern in captive birds tested without access to stars and stellar dominance in free flying birds released under starry sky at night-time. These results underline the importance of experimental protocols when testing ways in which birds integrate their compass systems.

Keywords Cue-conflict experiments - Light polarization - Migratory orientation - Orientation cage - Magnetic compass

Introduction

A remarkable number of studies indicates that birds rely on several compass mechanisms, based on celestial (sun, starry sky and polarized skylight pattern) and magnetic cues, to select and maintain the seasonally appropriate migratory direction (see Wiltschko and Wiltschko 2003 and references therein). The integration of the different orientation cues is however poorly understood, even though the relationships among different compasses have been investigated in last 40 years (reviews in Wiltschko and Wiltschko 1999; Muheim et al. 2006a; Liu and Chernetsov 2012). Cue-conflict experiments performed during the migratory period have provided indeed quite contradictory results both when considering caged (e.g. Muheim et al. 2006b, 2007, 2009; Wiltschko et al. 2008a, 2008b) and free-flying birds (Cochran et al. 2004; Chernetsov et al. 2011; Schmaljohann et al. 2013). These data cast some doubts on the existence of a universally shared calibration reference system among migrants, however the number of tested species is still too low to draw any firm conclusion.

Previous experiments suggested that Pied Flycatchers *Ficedula hypoleuca* did not recalibrate their magnetic compass against the polarization pattern at twilight when tested in orientation cages during spring migration (Gaggini et al. 2010). Despite being confirmed under different experimental protocols, these results are problematic due to the high variability recorded in birds directional preferences, suggesting a possible influence of stress-induced responses (Liu and Chernetsov 2012).

The aim of this paper is twofold:

1) To repeat the above mentioned cue-conflict experiments by modifying the experimental protocol in order to reduce the scatter of directions observed in our data possibly due to stress in freshly caught birds (see Methods).

2) To evaluate the effect of the cue-conflict treatment on the same birds tested both in orientation cages and while free-flying equipped with radio tags. Such comparison is rare in literature (see Schmaljohann et al., 2013), yet it can provide useful indications on the hierarchical relationships among the different compass systems used by birds when different cues are available. Indeed, migratory restlessness is clearly not identical to bird flight because environment in captivity is very simplified and orientation activity might be heavily affected by stress. Cage experiments, however, allow a strict control of the cues available to experimental subjects. On the other hand, radiotracking of free-flying birds is a more natural experimental set-up, as subjects have full access to several stimuli, though birds directional preference might be affected by cues (e.g. visual landmarks) not directly related to the compass systems under test.

Methods

Experimental birds and housing

Experiments were carried out at Ventotene Island (40°48' N, 13°25' E). Pied Flycatchers were mist-netted during spring migration (mid April – mid May 2011). The amount of visible subcutaneous fat deposits were scored according to Kaiser (1993). The median fat class of experimental subjects was 3 (interquartile range: 2-3, n=70). Birds were held in captivity indoor for a maximum of 9 days inside individual cardboard cages, being fed with *ad libitum* mealworms larvae (*Tenebrio molitor*) and water, and allowed access to natural light (natural photoperiod), but not to direct view of celestial cues. During captivity birds tested before and after the cue-conflict treatment (see below) showed median fat score increases of 1.5 (interquartile range: 0.5-1.5, n=33). Pied Flycatchers were tested for orientation after two full days of captivity, since we had previously observed that birds which had longer time to get accustomed to captivity showed less variable and more seasonally appropriate directional preferences (Gaggini et al. 2010).

Cage experiments

Orientation experiments were carried out between 20 min before and 70 min after civil sunset and lasted 40 min. They were performed in modified Emlen funnels (Emlen and Emlen 1966; top external diameter =350 mm; inner height =210 mm), made of non-magnetic material and lined with pressure-sensitive recording paper (130 g/m^2, Barbé, Pavia, Italy). The funnels were covered with loose-meshed plastic netting (10 × 10 mm) fixed to a cylindrical, 60 mm-high plastic ring. To prevent birds from seeing any celestial cues, funnels were covered with 3-mm-thick milky Plexiglas lids (Sandberg et al. 1988). Individuals not clearly oriented or inactive were tested again on subsequent evenings (possibly consecutive depending on weather conditions) for a maximum of three times. According to ringing data, spring migratory directions of Pied Flycatchers crossing the Mediterranean are quite variable, even though all birds ringed in Italy during spring migration were recovered in the northern semicircle (−90°,+90°) during the breeding season (Spina and Volponi 2008). For this reason, we loosely expected that in the first test (pre-cue-conflict test, hereafter *pre-CC* test) birds oriented northward. For cue-conflict test, we selected only birds which did not lose weight in captivity and oriented in the northern semicircle because our previous study showed that individuals selected by these criteria tend to show more consistent orientation results and are more likely to be in migratory state (N-birds in Gaggini et al. 2010). Therefore, only Pied Flycatchers oriented in a seasonally appropriate direction (−90°,+90°) in this test were exposed to the cue-conflict before being tested again (post-cue-conflict test, hereafter *post-CC* test). In this way we tried to exclude birds which did not react well to the captivity and possibly took longer to become accustomed to experimental manipulations (see Gaggini et al. 2010 for further details). This approach is not new to orientation literature (see e.g. Muheim et al. 2006a) and no available data indicate that it can substantially affect the outcome of the experiments as birds were sub-sampled before any experimental treatment.

The conflict between magnetic and celestial cues was obtained by manipulating the polarization pattern by means of wooden boxes (900×900×500 mm) provided with four square windows (500×500 mm) in the centre of each side (these boxes were the same used in previous experiments, but their dimensions were wrongly reported in Gaggini et al. 2010). A 0.8 mm-thick polarizing filter (cellulose triacetate, Intercast Europe, Parma, Italy) were placed inside each window and covered on the outside by a pseudo-depolarizing filter made by two sheets of 180 µm-thick polyester aligned at an angle of 45° relative to each other (Metalloy Italiana, Vicenza, Italy), which prevented the reduction of skylight intensity caused by the filtering action of the polarizing filter. Polarizing filters from two opposite windows were aligned so that the *e*-vector was vertical, while in the other two windows the filter axis was horizontal. During the cue-conflict treatment (hereafter: *CC*) the vertical filters were aligned with the solar azimuth and thus caged birds perceived the Band of Maximum Polarization (hereafter: *BMP*) shifted by ±90° relative to natural conditions. Because under natural conditions *BMP* crosses the horizon at azimuths ±90° relative to the position of setting sun, during *CC* we had our artificial *BMP* aligned along the line crossing the horizon at an average of 291° and 111° (see Fig 1). Each box was covered by a 3-mm milky Plexiglas sheet. During *CC*, four birds were kept in a small cage subdivided into four rooms (200×200×200 mm) with netting on each side, and placed in the centre of the box; birds can see each other, but they did not show any sign of aggressiveness, as also testified by preliminary trials. *CC* was performed in an open area, where birds had a broad view of the horizon through the polarizing filters except for a few bushes and a small building towards S–SE. 33 northward-oriented birds were exposed to *CC* for two consecutive sunsets in order to reinforce the effect of the treatment. This protocol was different to the "Repeated exposure to shifted *BMP*" protocol of Gaggini et al. (2010) as in latter case birds were exposed for three consecutive sunrises to cue-conflict and for two consecutive sunsets to natural conditions. *CC* was carried out between 20 min before and 70 min after civil sunset and lasted 40 min.

After *CC*, birds were returned to their indoor cages and prevented from seeing any visual cue. Magnetic orientation of treated birds was tested again the following day (*post-CC* test) in the same manner as *pre-CC* test.

We could not include a control group of birds tested after being exposed to natural conditions (vertical filters aligned with natural *BMP*) due to logistic restrictions on the number of birds which we could held in captivity at the same time.

Data analyses of Emlen funnel data

Following Cherry and Able (1986) and Mouritsen (1998), birds' orientation were visually and independently assessed to the nearest 5° by three persons blind to the experimental conditions. Birds which left more than 30 scratches in both tests were considered active. Only tests for which at least two out of three readings did not differ by more than 30° were considered oriented and thus included in the analysis. Individual orientations were calculated by averaging visual estimations. The mean group directions were calculated based on individual mean directions so that each data points represents one bird (Batschelet 1981). When the mean vector length resulting from doubling the angles was larger than the unimodal vector length, we used a mean axis of orientation as the basis of the analysis, because axial bimodality in *post-CC* test is a reasonable expectation if tested birds recalibrated their magnetic compass with respect to *BMP*, i.e. to one cue possessing an axial character (Able and Able 1990; Muheim et al. 2006b; see also Gaggini et al. 2010). The 95% confidence intervals of mean group directions were calculated by means of bootstrap methods with 5,000 resampled data sets (Adams and Anthony 1996). Randomness was tested with the Rayleigh test (Batschelet 1981). Birds' orientation in the two tests was compared by analyzing the distribution of the angular differences of individual directional preferences (*post-CC* test – *pre-CC* test) and using the nonparametric paired-sample test by Moore (1980). All statistical analyses were performed with the package *circular* 0.4-7 (Agostinelli and Lund 2013) of the software R 3.0.1 (R Core Team 2013).

Radiotracking

Immediately after *post-CC* test, n=26 active and oriented birds were equipped with small radio-transmitters (0.40 g Ag337 Pip Tags from Biotrack Co., UK) glued on their back and released, in order to track their vanishing bearings (*Van*). This technique has minimal or no effects on bird's behaviour (Barron et al. 2010), yet the temporary attachment increased the likelihood of tag loss especially when birds did not depart immediately from the island but spent longer on the spot, likely preening their feathers, as suggested by the strongly irregular radio signal intensities recorded. Tags were not deployed before Emlen funnel tests: 1) not to affect birds behaviour within the orientation cages; 2) not to introduce noise in the data due to the antennas touching the funnel walls lined with pressure-sensitive recording paper; 3) to reduce the likelihood of tags getting loosely glued as birds were moving in the funnel. Transmitters were on average 3.6% of the birds weight (range: 3.2-4.0%), hence complies within the recommended 5% of body weight (Barron et al. 2010; Schmaljohann et al. 2013). Magnetic disturbance due to radio tags was 14.5±44.6 SD nT (measurements performed by A. De Santis, INGV, Rome, data not reported) and was assumed negligible with respect to the strength of the local geomagnetic field (total intensity=46 μT; De Santis et al. 2003). Preliminary tests performed in the study area indicated that the inshore detection range of radio tags was >2 km (maximum distance between the release site and the sea: 1.6 km); we did not test the actual range during the experiment but several studies reported values >8 km for analogous devices (e.g. Schmaljohann et al., 2011; Chernetsov et al 2011). Birds were singly released after nautical twilight, i.e. when sun elevation was lower than 12°, in calm wind (Beaufort scale <1), under starry clear sky condition (cloud cover <3/8) and at a distance of less than 50 m from the *CC* site. Movements of released birds were tracked by two operators with AVM LA-12 receivers (AVM Instrument Company, Champaign, IL, USA) and hand-held four-element Yagi antennas (Televilt, Stockholm, Sweden) until signal loss. We used the strongest signal method to determine the position of birds and compass aligned with the antenna axis to register the vanishing bearings. After each vanishing we searched the island in order to confirm the bird's departure. As Ventotene Island is quite small (1.54 km^2), we are confident that birds included in the *Van* group had actually left during the night of their release. Due to logistic constraints we stopped tracking five hours after release; unfortunately several birds (*n*=18) lost their tags before departure or did not leave within this time interval. This forced us not to consider a control group of radio-tagged birds not exposed to the cue-conflict, as the number of available tags was limited.

Data analyses of tracking data

The mean vector of *Van* birds (*n*=8) was calculated from individual vanishing bearings using standard circular statistics (Batschelet 1981). The 95% confidence intervals of mean group direction were calculated by means of bootstrap methods with 5,000 resampled data sets (Adams and Anthony 1996). Randomness was tested with the Rayleigh test (Batschelet 1981).

Birds directional preferences were compared with Emlen funnel results by analyzing the distribution of the angular differences of individual headings (*Van – pre-CC* test*, Van – post-CC* test) and using the nonparametric paired-sample test by Moore (1980). All statistical analyses were performed with the package circular 0.4-7 (Agostinelli and Lund 2013) of the software R 3.0.1 (R Core Team 2013).

Expectations

The predicted orientation responses are summarized in Figure 1. If magnetic cues dominate over celestial cues, as suggested in Gaggini et al. (2010), we expected no differences between *pre-CC*, *post-CC* and *Van* test (Fig 1a,c). On the contrary, if *BMP* calibrates magnetic compass, we expected a significant ±90° shift of directional preferences in *post-CC* test with respect to *pre-CC* test

(Cochran et al. 2004; Muheim et al. 2006b; Fig. 1b). We predicted the following outcomes for free flying birds (*Van* test):

1. If celestial cues (the star pattern) dominate over magnetic cues, *Van* distribution should not differ from *pre-CC* test (Fig. 1d).
2. If *BMP* calibrates magnetic compass and stars are ignored, then we would expect ±90° shift of *Van* with respect to *pre-CC* test i.e. results similar to *post-CC* test when birds showing ±90° shift (Fig. 1e).
3. If *BMP* calibrates magnetic compass but free flying birds take both stars and magnetic cues into consideration then we would expect not complete ±90° shift but rather a compromise between the conflicting information from magnetic compass and true information from stars (see e.g. Wiltschko and Wiltschko 1999). In this case, it is hard to predict exact outcome but if we assume 50/50 weighing on star and magnetic cues then we would expect roughly ±45° shift in orientation relative to *pre-CC* (Fig. 1f).

Results

Cage experiments

In *pre-CC* test, we tested a total of 70 Pied Flycatchers (*pre-CC* test). 10 birds were inactive or disoriented in three consecutive trials and were thus excluded from the analysis. The directional preferences of the remaining 60 birds were not randomly distributed (α=345°, r=0.32, P=0.002, Rayleigh test; Fig. 2a). 19 southward-oriented [−90°,+90°] plus 8 northward-oriented (−90°,+90°) birds, which lost weight during the period of *pre-CC* test thus suggesting that they did not react well to captivity, were not considered for the rest of the experimental protocol.

The distributions of directional preferences of 33 Pied Flycatchers before (*pre-CC* test) and after (*post-CC* test) two consecutive *CC* between magnetic and visual information at sunset are reported in Figure 2a,b. The two distributions were significantly different according to the Moore Test (r_m=1.69, P<0.001). The angular differences between *post-CC* test and *pre-CC* test were bimodally distributed and the modal directions were not different from the expected ±90° if birds recalibrate their magnetic compass on *BMP* (Fig. 2c).

Radiotracking

Released Pied Flycatchers included in the *Van* group (n=8) spent on average 79±71.2 SD min in nearby bushes and then took off and flew away with a firmly set direction, consistently vanishing toward NNE (Fig. 3a; see also supplemental material) without performing any obvious explorative flight. Their headings distribution was not different from *pre-CC* test (r_m=0.66, P>0.05, Moore

Test; Fig. 3b), while it was significantly different from *post-CC* test (r_m=1.34, P<0.01, Moore Test; Fig. 3c). The 95% confidence intervals of the mean of the angular differences between *Van* and *post-CC* test (α=—49°) did not include either 0° or 90° (Fig. 3c).

Discussion

The results obtained with orientation cages are clearly in contrast to Gaggini et al. (2010). Indeed, while in previous tests northward-oriented birds (N-birds) apparently did not recalibrate their magnetic compass after various cue-conflict treatments, in this experiments Pied Flycatchers seem to use celestial cues, in particular *BMP*, as the calibration reference for migratory orientation, as observed for other species of passerines (see Muheim 2011 and references therein). These different outcomes might suggest some flexibility of the orientation strategies of these birds, which could make use of different compass cues depending on their availability and/or reliability. Despite being confirmed under different experimental protocols, the results by Gaggini et al. (2010) are however not completely convincing mainly because of the high variability of birds' directional preferences, which could be due to stress (see also Liu and Chernetsov 2012). For this reason, while we can not completely exclude the above-mentioned explanation, it seems more straightforward to ascribe the inconsistency of the results to the differences between the two experimental protocols (see Methods). In particular, in these last tests birds had more chance to become accustomed to captivity and to the experimental treatment, both because of the length of the experimental protocol (birds were tested for orientation after two full days of captivity) and for having been exposed to the same conflict twice. These conditions could have reduced the stress-induced responses (i.e. disorientation or "nonsense orientation"; see e.g. Muheim and Jenni 1999; Muheim et al. 1999; Marchetti and Zehtindjiev 2009) thus increasing the number of birds oriented in a seasonally appropriate direction, and also reinforced the effect of the cue-conflict treatment. In this regard it should be noted that in Gaggini et al. (2010) a group of birds actually was repeatedly exposed to the cue-conflict at sunrise, but the same birds were also exposed to natural conditions at sunset, in order to test whether they could integrate the information derived from the intersection of *BMP* and the horizon at sunrise and sunset (see Muheim et al. 2006a for further details). The treatment of this group of birds was thus not comparable to the one reported here, as in this last case the experimental birds were repeatedly confronted to the same cue-conflict conditions.

Vanishing bearings of radio-tagged Pied Flycatchers did not show the shift observed in *post-CC* test and their orientation did not differ from that recorded in *pre-CC* test. Since logistic problems forced us not to consider a control group of tagged birds not exposed to the cue-conflict (see Methods for further details), the interpretation of this result rests on the critical assumption that the orientation recorded in experimental funnels is comparable to that observed in free flying birds. As discussed by Mouritsen and Larsen (1998), the relatively few studies which tested this assumption are not completely in agreement, probably because part of the cage experiments was performed under clear sky around sunset and birds orientation could have been affected by positive phototaxis towards the setting sun (see e.g. Able 1990; Sandberg and Moore 1996). When this possible side effect was controlled by testing the same subjects well after sunset under starry sky, the correlation between funnel orientation and vanishing bearing after release was rather strong (Mouritsen 1998; Mouritsen and Larsen 1998). Our funnel experiments were performed at sunset, while birds were released in the dark when the stars were the only celestial cue. However, cage experiments were done under simulated overcast conditions obtained by placing a panel of opaque, diffusing Plexiglas on the top of the funnel, thus preventing the birds from seeing any celestial cues. In this conditions, any effect of phototaxis on birds orientation was rather unlikely. We thus expected a good correlation between funnel and release tests if birds would rely on unshifted compass cues to orient toward a seasonally appropriate migratory direction. This was what we obtained by comparing *pre-CC* and *Van* test. However, while *pre-CC* subjects were not manipulated, *Van* birds had been exposed to a cue-conflict between geomagnetic and visual cues (shifted *BMP*) before the release. The interpretation of the *Van* test thus depends on the outcome of *post-CC* test. Indeed the lack of difference observed in birds' directional preferences before (*pre-CC* test) and after (*Van*) the cue-conflict treatment resembles the results obtained by Chernetsov et al. (2011) and Schmaljohann et al. (2013), who did not record any effect of the cue-conflict. The orientation of *Post-CC* birds, which could not see stars contrary to *Van* birds, however indicates that the magnetic compass was recalibrated. This suggests that, contrary to Cochran et al. (2004), birds ignored the magnetic information immediately after release, only relying on their unshifted star compass. This result supports prediction 1 (Fig. 1d, see Methods for further details). Interestingly, since the mean angular difference between *post-CC* and *Van* test was significantly different from 90°, it could be also speculated that Pied Flycatchers did not disregard the magnetic compass completely, making a sort of compromise between unshifted visual information and shifted magnetic cues (see Wiltschko and Wiltschko 1999 for a review). This hypothesis can not be tested with the presented data given the lack of the above-mentioned control group. Indeed, it can not be excluded that the view of lights on the coast influenced the take-off directions of Pied Flycatchers and eastward shifted their course, given that Ventotene Island is located only ~50 km westward with respect to the mainland. It is important to notice that up to now all cue-conflict studies on free flying European bird species did not support a calibration of the magnetic compass on twilight cues when stars are available (Chernetsov et al. 2011; Schmaljohann et al. 2013; present study), whereas all North American species tested so far showed a calibration of the magnetic compass by *BMP* (Cochran et al. 2004). It should be noted, however, that the experimental protocols of the above-mentioned studies were slightly different. Indeed, in Cochran et al. (2004) and in Chernetsov et al. (2011) birds could see the stars during the last part of the cue-conflict treatment, as (1) the exposure lasted till the end of nautical twilight and (2) birds had an unobstructed view of the sky since the conflict was obtained by shifting the magnetic field using a coil system. On the contrary, in Schmaljohann et al. (2013) and in the present study, stars were not directly involved in the conflict because (1) the cue-conflict treatment was obtained by shifting the *BMP* near the horizon and (2) birds had only a reduced access to overhead cues. Future studies should test whether the different outcomes obtained in these experiments can be ascribed to regional patterns, specific feature of tested groups of songbirds or to methodological issues.

Even though the variability of our cage experiments still suggests caution in the evaluation of our results, the presented data underline the importance of the experimental protocol in cue-conflict experiments on wild migratory birds. Indeed, on the one hand, the modification of the experimental procedures, aimed at reducing the possible stress from captivity while reinforcing the effect of the treatment, completely changed the outcome of previous tests (Gaggini et al. 2010). On the other hand, the integration of different approaches provided further elements for a more complete description of the way Pied Flycatchers make use of the available orientation cues, even though further tests are needed to better clarify the use of visual and magnetic information at release. This combination of orientation cage and radio tracking experiments seems a promising approach to shed light upon the variety of compass systems' hierarchy in different migratory birds and to understand the different ways of weighing and integrating available cues, which may be explained by regional availability of used orientation cues, ecology and/or evolutionary history of given species (see also Chernetsov et al. 2011).

Acknowledgments

We would like to thank all the people who helped us in the field (P. O'Shaughnessy, E. Scordamaglia and all ringers working in the ringing station of Ventotene Island) and A. De Santis for the measurements of the magnetic disturbance due to radio tags. The comments of two anonymous reviewers greatly improved an earlier draft of the manuscript. This work complies with the current Italian laws on animal welfare. This study was supported by the Italian Ministero dell'Istruzione, dell'Università e della Ricerca (MIUR – Prin 2008). Results from the "Progetto Piccole Isole" ISPRA: paper no. .

References

Able KP (1990) Comparison of vanishing bearings, orientation directions and ringing recoveries of spring migrant white-throated sparrows, *Zonotrichia albicollis*. J Orn 131:317–323

Able KP, Able MA (1990) Ontogeny of migratory orientation in the Savannah sparrow, *Passerculus sandwichensis*: calibration of the magnetic compass. Anim Behav 39:905–913

Adams DC, Anthony CD (1996) Using randomization techniques to analyse behavioural data. Anim Behav 51:733–738

Agostinelli C, Lund U (2013) R package 'circular': Circular Statistics (version 0.4-7). https://r-forge.r-project.org/projects/circular/

Barron DG, Brawn JD, Weatherhead PJ (2010) Meta-analysis of transmitter effects on avian behaviour and ecology. Methods Ecol Evol 1:180–187

Batschelet E (1981) Circular statistics in biology. Academic Press, New York

Chernetsov N, Kishkinev D, Kosarev V, Bolshakov CV (2011) Not all songbirds calibrate their magnetic compass from twilight cues: a telemetry study. J Exp Biol 214:2540–2543

Cherry JD, Able KP (1986) An alternative method for the analysis of Emlen funnel data. Auk 103:225–227

Cochran WW, Mouritsen H, Wikelski M (2004) Migrating songbirds recalibrate their magnetic compass daily from twilight cues. Science 304:405–408

Emlen ST, Emlen JT (1966) A technique for recording migratory orientation of captive birds. The Auk 83:361–367

Gaggini V, Baldaccini N, Spina F, Giunchi D (2010) Orientation of the Pied Flycatcher *Ficedula hypoleuca*: cue-conflict experiments during spring migration. Behav Ecol Sociobiol 64:1333–1342

Kaiser A (1993) A new multi-category classification of subcutaneous fat deposits on songbirds. J Field Ornithol 64:246–355

Liu X, Chernetsov N (2012) Avian orientation: multi-cue integration and calibration of compass systems. Chinese Birds 3:1–8

Marchetti C, Zehtindjiev P (2009) Migratory orientation of sedge warblers (*Acrocephalus schoenobaenus*) in relation to eating and exploratory behaviour. Behav Processes 82:293–300

Moore BR (1980) A modification of the Rayleigh test for vector data. Biometrika 67:175–180

Mouritsen H (1998) Redstarts, *Phoenicurus phoenicurus*, can orient in a true-zero magnetic field. Anim Behav 55:1311-1324

Mouritsen H, Larsen ON (1998) Migrating young Pied Flycatchers *Ficedula hypoleuca* do not compensate for geographical displacements. J Exp Biol 201:2927–2934

Muheim R, Jenni L, Weindler P (1999) The orientation behaviour of chaffinches, *Fringilla coelebs*, caught during active migra- tory flight, in relation to the sun. Ethology 105:97–110

Muheim R, Jenni, L (1999) Nocturnal orientation of robins, *Erithacus rubecula*: birds caught during migratory flight are disoriented. Acta Ethol 2:43–50

Muheim R (2011) Behavioural and physiological mechanisms of polarized light sensitivity in birds. Philos Trans R Soc B Biol Sci 366:763–771

Muheim R, Akesson S, Phillips J (2007) Magnetic compass of migratory Savannah sparrows is calibrated by skylight polarization at sunrise and sunset. J Ornithol 148:S485–S494

Muheim R, Moore FR, Phillips JB (2006a) Calibration of magnetic and celestial compass cues in migratory birds - a review of cue-conflict experiments. J Exp Biol 209:2–17

Muheim R, Phillips JB, Åkesson S (2006b) Polarized light cues underlie compass calibration in migratory songbirds. Science 313:837–839

Muheim R, Phillips JB, Deutschlander ME (2009) White-throated sparrows calibrate their magnetic compass by polarized light cues during both autumn and spring migration. J Exp Biol 212:3466–3472

R Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. http://www.R-project.org/

Sandberg R, Moore FR (1996) Migratory orientation of red- eyed vireos, *Vireo olivaceus*, in relation to energetic condition and ecological context. Behav Ecol Sociobiol 39:1–10

Sandberg R, Pettersson J, Alerstam T (1988) Shifted magnetic fields lead to deflected and axial orientation of migrating robins, *Erithacus rubecula*, at sunset. Anim Behav 36:877–887

De Santis A, Gaya-Piqué L, Dominici G, Meloni A, Torta JM, Tozzi R (2003) ITalian Geomagnetic Reference Field (ITGRF): update for 2000 and secular variation model up to 2005 by autoregressive forecasting. Ann. Geophys. 46:491-500

Schmaljohann H, Becker PJJ, Karaardic H, Liechti F, Neaf-Daenzer B, Grande C (2011) Nocturnal exploratory flights, departure time and direction in a migratory songbird. J. Ornithol 152:439–452

Schmaljohann H, Rautenberg T, Muheim R, Naef-Daenzer D, Bairlein F (2013) Response of a free-flying songbird to an experimental shift of the light polarization pattern around sunset. J Exp Biol. 216:1381-1387

Spina F, Volponi S (2008) Atlante della Migrazione degli Uccelli in Italia. 2. Passeriformi. Ministero dell'Ambiente e della Tutela del Territorio e del Mare, Istituto Superiore per la Protezione e la Ricerca Ambientale (ISPRA), Roma

Wiltschko R, Munro U, Ford H, Wiltschko W (2008a) Response to the comments by R. Muheim, S. Akesson, and JB Phillips to our paper “Contradictory results on the role of polarized light in compass calibration in migratory songbirds". J Ornithol 149:663–664

Wiltschko R, Munro U, Ford H, Wiltschko W (2008b) Contradictory results on the role of polarized light in compass calibration in migratory songbirds. J Ornithol 149:607–614

Wiltschko R, Wiltschko W (1999) Celestial and magnetic cues in experimental conflict. In: Adams NJ, Slotow RH (eds) Proc 22 Int Ornithol Congr, Durban. BirdLife, South Africa, Johannesburg, pp 988–1004

Wiltschko R, Wiltschko W (2003) Mechanisms of orientation and navigation in migratory birds. In: Berthold P, Gwinner E, Sonnenschein E (eds) Avian Migration. Springer, Berlin, pp 433-456

Legends to figures

Fig. 1. Scheme of the expectations of the experiments. See Methods for further details.

Fig. 2. Results of the orientation-cage experiments. a) Orientation of all Pied Flycatchers in *pre-CC* test (*n*=60). Gray dots represent birds not included in further analyses (19 southward-oriented plus *8* individuals which lost weight after *pre-CC* test). Dots represent 33 birds exposed for two consecutive sunsets to conflicting information between natural magnetic field and ±90° shifted band of maximum polarization; 8 birds included in the *Van* group are reported in black. b) Birds' directional preferences after cue-conflict treatment (*post-CC* test). c) Distribution of the differences between individual directional preferences recorded in the two tests *(post-CC* test − *pre-CC* test). The mean vector (α) of each distribution is represented by an arrow, whose length (r) is drawn relative to the radius of the circle =1. Double arrows indicate axially distributed samples; dotted lines are 95% confidence interval. Probability according to the Rayleigh test.

Fig. 3. Orientation of released radio-tagged Pied Flycatchers (*n*=8) followed until the loss of the radio signal (*Van* group, a). b-c Distributions of the differences between *Van* and *pre-CC* test (b) or *post-CC* test (c). See Figure 2 for other details.