



Supporting Online Material for

**Polarized Light Cues Underlie Compass Calibration in Migratory
Songbirds**

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Materials and Methods

Study site and experimental species

The study was carried out at Kanaryarmiut Field Station (N 61° 21' 41", W 165° 07' 43") in the Yukon Delta National Wildlife Refuge, Alaska, USA, during autumn migration 2005 (Aug 11–Sept 21). Savannah sparrows, *Passerculus sandwichensis*, of the nominate subspecies *sandwichensis* are abundant breeding birds in the vicinity of the field station. This subspecies winters in coastal southwestern British Columbia to western, central California (S1), thus is expected to depart from the Yukon Delta in southeasterly migratory directions.

We caught the birds in the vicinity of the field station: juvenile birds Aug 5–19, adult birds Aug 31–Sept 7. The first juveniles captured were most likely offspring from the local breeding population, while juveniles caught later in the season, as well as all adult birds, were migrants on passage. We kept the birds indoors in metal holding cages (30 cm x 40 cm x 40 cm), 3–4 individuals per cage. The windows of the holding room were covered with milky paper, so that the birds could not see celestial cues (sun, skylight polarization patterns, stars), but were exposed to the natural photoperiod. The birds were fed with seeds (millet, nyjer, sun flower), mealworms, fish pellets and water with vitamins *ad libitum*. We started to test their orientation when they had reached a fat score of at least 3 [according to the visual fat classification scale by (S2)].

Orientation experiments

All orientation experiments were carried out in Emlen funnels (S3) covered with milky Plexiglas sheets and lined with type-writer correction paper (Tipp-Ex, Bic Inc.) in a 3 m x 6 m enclosed shelter (Hansen Weatherport Inc.). During testing the birds had access to magnetic cues only, and therefore all directions are given relative to magnetic North=0° [geomagnetic field values at the study site: total intensity ~54400 nT, inclination 72.2°, declination 13.2°; World Magnetic Model 2005 (S4), 1 Sept 2005]. The experiments lasted for 60 min, between 70 min (one exception: 170 min) before and 85 min after sunset, which is the time of day that nocturnal migrants like Savannah sparrows normally initiate migration [e.g., (S5)]. During this time the birds left scratch marks on the Tipp-Ex paper lining the Emlen funnels. The directional tendency in the distribution of scratch marks was analyzed by a visual estimation method (see below). The birds were first tested for their initial response, and individuals that oriented to the southeast ±90° (Figs. 1B, S1B) were then exposed to an experimental condition. The distribution of initial responses was not tested for statistical significance; rather each bird's initial response was used as the reference direction to determine if exposure to the cue conflict resulted in a consistent shift in direction relative to that bird's initial response.

Experimental exposures

Experimental exposures were carried out at sunrise (SR) and sunset (SS). At these times of day the band of maximum polarization (BMP), located 90° from the sun, passes directly through the zenith and, along with the e-vector of polarization, is aligned vertically on the horizon (S6, S7). A variety of studies have shown that celestial cues at SS and SR are important orientation cues for migratory birds [e.g., (S5, S8-16)], especially from the region of sky close to the horizon (S17-19).

Sunrise and sunset exposures to rotated polarization axis with view of horizon: We created an artificial polarized light pattern to simulate the natural polarization pattern at SR and SS, vertically aligned on the horizon. Birds were placed in a holding cage consisting of a cardboard box with a window (14 cm high x 14 cm long) covered with netting on each of the four sides. The holding cage was placed in the center of a larger cardboard box with windows on each side that were covered on the outside with a pseudo-depolarizing filter [2 sheets of Hostaphan polyethylene terephthalate, Hoechst AG, Germany, aligned at an angle of 45° relative to each other, resulting in a depolarization of 90% with a 10–15% reduction of light intensity between 400 and 800 nm; cf. (S20)] and on the inside with a polarizing filter (linear polarizer #POA1, 3Dlens Corporation, Taiwan, transmittance 38%, polarizing efficiency 99.98% at wavelengths ~400–700 nm). By first depolarizing the light, before it passed through the polarizing filters, we avoided any effects on light intensity caused by an interaction of the polarizing filters with the natural skylight

polarization when clear sky was visible during exposures (S21). The polarizing filters in two windows separated by 180° were aligned so that the e-vector of polarization was vertical, while the polarizing filters in the two windows on the perpendicular axis were aligned so that the e-vector of polarization was horizontal. Confining the birds in a smaller holding cage inside the box holding the polarization filters reduced the effects of parallax on the alignment of the artificial polarization axis. The birds had only limited access to overhead polarized light cues (overhead sky was blocked within approximately ±30° of the zenith). All exposures took place between 30 min before to 30 min after SR or SS, in an open area about 100 m to the North of the field station. The two vertically aligned polarizing filters were aligned on an axis that coincided with the azimuth of SR or SS, so that the birds perceived the BMP rotated by ±90° relative to the natural condition (Fig. 1C). Weather conditions varied considerably during the times that different individuals were exposed to cue conflicts [cloud cover: 25-100%; disk of sun visible to 8 individuals during SR exposure (open symbols in Fig. 1)].

SS exposures to rotated polarization axis without view of horizon: A small sample of birds (N=6) previously exposed to a ±90° shifted artificial polarization pattern with view of the horizon was exposed to a further ±90° shift in the artificial polarization pattern at SS in a polarizing box that blocked the view of the horizon. This second polarizing box was used to simulate the polarization cues available to the birds exposed to cue conflicts in Emlen funnels (S3), i.e. access to the overhead band of maximum polarization, but not at the horizon. This box had a single round polarizing filter (diameter 31 cm) on top, but no openings at the sides, allowing the birds to see a ~105° view of sky around the zenith when sitting on one of the perches at the bottom of the box to ~140° when jumping or flying up towards the ceiling of the box (Fig. S1C). In conventional funnel experiments, views of skies are limited to between 90° and 160° centered around the zenith [for review see (S19)]. Exposures lasted from 30 min before to 30 min after SS. The box was placed ca. 16-25 m from the closest building of the research station, which was not visible to the birds.

Exposure procedures

Before the exposures, the birds were placed into the exposure boxes in the bird holding room and covered with black clothing during the transportation to the exposure site to prevent any opportunities for recalibration during transport. Following exposures, experiments were carried out in the same way as the initial experiments. Birds exposed to an experimental condition during SS were tested during the following evening. Between exposure and testing, the birds were not allowed to see any outdoor cues, so that they could not recalibrate any of their compasses. Birds not meeting the activity and concentration requirements (see below) were tested until they showed a valid direction, for a maximum of 4 times. Once a bird was tested under an experimental condition it was set outside for several hours around SS and/or SR to normalize the calibration of its compasses under natural conditions. When the sky was clear and SS/SR visible, these birds were exposed to the natural polarization pattern in an open box with one window on each side. When the sky was completely overcast, with no natural polarization visible, the same polarization box was used as in the experiments, but aligned along the natural polarization axis. After normalization, each bird was tested until it oriented to the southeast ±90° (see above). A few birds were given additional normalization exposures. All experiments were carried out with permission of the U.S. Fish & Wildlife Service (permit # 05-YDNWR-02).

Data evaluation

To determine the directional response of individual birds, we used the visual method of estimating the median of the distribution of scratches (S22, S23), which has been shown to be highly repeatable and in close agreement with the method of counting scratches in different sectors across the Tipp-Ex paper (S24). Scoring of the data was carried out by an observer who was blind to the experimental condition and position of the North mark (used to align the Tipp-Ex paper). When the distribution of scratches was axial, the end of the axis with the larger number of scratches was taken as the bird's directional choice. Each paper was given a score for activity (0: 0-50 scratches, 1: 50-150, 2: 150-500, 3: 500-2000, 4: >2000) and concentration [the repeatability with which the median direction could be estimated; score 0: >45°, 1: 20°-45°, 2: 10°-20°, 3: 5°-10°, 4: 0°-5°; for comparison see (S23)]. We used a very conservative approach similar to that of Mouritsen (S23), excluding birds with activity =0, concentration ≤1 or total score (activity + concentration) <3, thus including only birds that showed well-directed distribution of scratches on the Tipp-Ex paper.

Statistical analysis

We used standard circular statistics to test whether the distribution of responses obtained from each group of birds was non-randomly distributed [Rayleigh test; (S25)] and the method of doubling the angles to test whether a group was axially distributed ($r_{\text{axial}} > r_{\text{unimodal}}$). Since we were interested in the response of individual birds to the exposure, we calculated the deviation between each individual's direction chosen before (initial direction) and after the exposure. We used the 95% confidence intervals around the mean responses to examine whether the observed distributions were consistent with the expected shifts (Figs. 1E and S1E). Comparisons between groups were carried out with the Watson U^2 test (S25).

After experimental treatments, we expected a radially symmetrical axial distribution of responses from birds for the following two reasons: (1) Previous studies have found that both hand-raised and wild-caught Savannah sparrows show axial orientation when tested repeatedly [(S26-28), but see (S29, S30)]; they usually show unimodal orientation during a given night of testing, but often switch direction from one test to the next (S27). (2) The polarization filters used to artificially shift the polarization pattern of the sky produced an axially symmetric polarization pattern. Since the sun was not visible to a majority of birds during exposure to the rotated polarization pattern (solid symbols in Figs. 1 and S1), it could not be used to resolve the ambiguity between the two ends of the polarization axis.

Calculation of predicted shifts in response to SR and SS exposures

For experiments in which birds were exposed to the cue conflict with a view of the horizon, we tested two alternative hypotheses regarding the expected response to the rotated polarization axes at SR and SS: calibration of the magnetic compass with respect to the alignment of the natural polarization pattern (1) only at SS (as proposed by S31), or (2) only at SR. We calculated the predicted shifts as the difference between the alignment of the artificial BMP during the cue-conflict exposure and the alignment of the natural BMP at SR or SS. The way the experiments were designed, the predicted shift was 90° if the birds had calibrated the magnetic compass relative to the BMP at the same time of day as the cue-conflict exposure (see above). Due to the difference in alignment of the BMP at SR and SS at the time of year the experiments were carried out, however, the predicted shift differed from 90° if birds exposed to the cue conflict at SR had calibrated the magnetic compass with respect to the natural polarization pattern at SS, or if birds exposed to the cue conflict at SS had calibrated the magnetic compass with respect to the natural polarization pattern at SR. Because the expected shifts differed somewhat for individual birds depending on the date of exposure, the mean predicted shift was calculated by vector addition [see above; (S25)] and shown in Fig. 1 as large triangles outside the circle (predicted shift relative to SR and SS in purple and orange, respectively).

Results and Discussion

Response to $\pm 90^\circ$ shifted polarization pattern at SR or SS with view of horizon

Juvenile versus adult birds: Both juvenile and adult birds were tested after exposure to the rotated polarization pattern at SR (Table S1). There was no difference between the age groups in the responses before exposure (Watson U^2 : $U^2=0.06$, $P>0.5$; Fig. 1B), after exposure ($U^2=0.11$, $P>0.2$; Fig. 1D) or in the deviations between their responses before and after exposure ($U^2=0.12$, $P>0.1$; Fig. 1E). This result supports previous findings indicating that recalibration of the magnetic compass occurs repeatedly throughout a bird's lifetime, i.e., is not restricted to its first migration (S32), and also that recalibration occurs in both juvenile and adult birds during migration (S33).

Seasonal effects: Our experimental birds included both birds presumed to be from the local breeding population in the area of the research station, captured in the early part of the field season, and migrating individuals en route, captured later in the season. We divided the sample into three groups: group 1: captured Aug 5–9, birds # 7-25, all juveniles; group 2: captured Aug 12–19, birds # 26-49, all juveniles; group 3: captured Aug 31–Sept 7, birds # 50-67, all adults (Table S1). We found no statistical difference between these groups in their response to either the SR or SS exposure (Watson U^2 : SR: $P>0.2$ in all comparisons; SS: $P>0.5$). Thus, birds captured early in the migration season, possibly from the local population, did not respond differently from birds captured later.

Successive exposures to cue conflicts at SR and SS: A subset of 15 Savannah sparrows experienced the cue-conflict exposure at both times of day, first at SR and then at SS, or vice versa (cf. Table S1). In between the two experimental exposures, the birds were exposed outdoors to the natural alignment of the BMP in the ambient magnetic field to normalize the calibration of the magnetic compass (see Exposure procedures; above). Their response to the exposure was not statistically different from birds tested only under one of the exposure conditions (SR: $U^2=0.03$, $P>0.5$; SS: $U^2=0.03$, $P>0.5$; Table S1). Thus, recalibration of the magnetic compass takes place repeatedly, and individual birds use information from both SR and SS cues for recalibration of the magnetic compass.

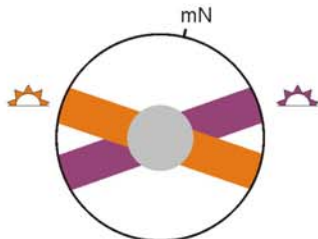
Response to $\pm 90^\circ$ shifted polarization pattern without view of horizon

In an earlier review, we concluded that the contradictory pattern of findings reported in the extensive cue-conflict literature could be explained by polarized light cues from the region of sky near the horizon playing a critical role in recalibration of the magnetic compass (S19), as suggested by earlier work on homing pigeons (S34). We tested this hypothesis by exposing a small sample of birds ($N=6$) previously exposed to a $\pm 90^\circ$ shifted artificial polarization pattern (Fig. S1A and B) to a further $\pm 90^\circ$ shift in the alignment of the artificial polarization pattern at SS. This time, the view of the horizon was blocked (polarization box with a single polarizing filter on top), simulating the polarization cues perceived by birds tested in orientation funnels (see Methods). The e-vector of the artificial polarization pattern was aligned perpendicularly to the sun's position (i.e., coinciding with the natural alignment of the BMP). If the birds recalibrate the magnetic compass with respect to the overhead polarized light pattern, they should shift their directions of orientation by $\pm 90^\circ$ to coincide with the axis of their initial responses in Fig. 1B. Instead they continued orienting in a magnetic direction that was indistinguishable from their response prior to exposure to the overhead polarization pattern (deviations between the directional choices of each individual bird before and after the exposure: $\alpha=352^\circ$, $r=0.86$, $P=0.006$, $N=6$; Fig. S1D and E, Table S2). Thus, in contrast to exposure to rotated polarized light cues from the region of sky near the horizon (Fig. 1), exposure to a rotated polarization pattern in the overhead sky had no effect on the calibration of the sparrows' magnetic compass.

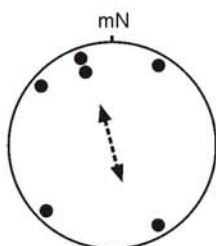
Compass information derived from averaging SR/SS vs. using SR only or SS only

The three-dimensional diagrams in Figures S2A and B show the relationship of the BMPs to sun position at SR and SS at the onset of fall migration. Because the solar ephemeris (i.e., the path of the sun across the sky) generally intersects the horizon at angles substantially different from 90° , the point at which the sun disappears from view will depend both on horizon height and on the elevation of the observer above the ground. In contrast, at true SR and SS, and only at these two times of day, the BMP passes overhead through the zenith and, along with the e-vector of polarization, is vertically aligned on the horizon. Consequently, directional information derived from the alignments of the BMP at these two times of day is unaffected by horizon height and, potentially, independent of the circadian clock. At the Spring and Fall equinoxes, the intersections of the BMP with the horizon at SR and SS coincide with the North-South meridian (not shown), so polarized light cues could be used as a reference at either time of day without introducing a systematic error in the calibration of other compass systems. At other times of year, the intersections of the BMP with the horizon are displaced by symmetrical amounts on either side of the North-South meridian [Figs. S2A-D; and see (S34)]. Consequently, averaging the alignments of the BMP at SR and SS can be used to derive the North-South meridian (Fig. S2E; and see below).

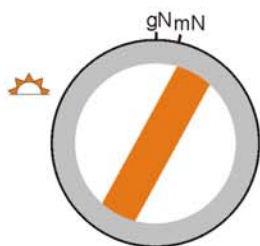
A Pre-experimental experience



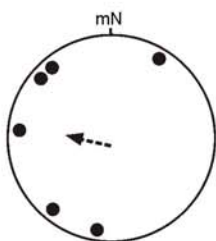
B Initial magnetic orientation before exposure (indoor experiments)



C Outdoor exposure



D Magnetic orientation after exposure (indoor experiments)



E Deviations from each individual's initial response

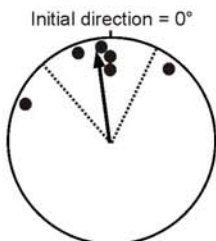


Fig. S1. Experimental setup and magnetic orientation of Savannah sparrows exposed to conflicting information between the magnetic and polarized light cues at SS, without view of the horizon. (**A, C**) 360° view of sky from horizon to zenith (middle of circle) as experienced by birds under natural and experimental conditions (mN=magnetic North, gN=geographic North). Purple and orange suns and bars give the mean positions of the sun and band of maximum polarization at SR and SS, respectively; gray zones indicate areas of sky not visible to the birds during exposure. (**A**) Relationship between celestial cues and geomagnetic field experienced prior to the experiment, i.e. artificial polarization pattern shifted $\pm 90^\circ$ relative to the natural condition at SR or SS (Fig. 1C). (**C**) Outdoor exposure to an artificial polarization pattern aligned parallel to the natural condition for one hour around SS. (**B, D**) Magnetic orientation of birds tested indoors with access to magnetic cues only. Each dot gives the direction of an individual bird relative to mN=0°. (**B**) Magnetic orientation of birds selected for exposure (data from Fig. 1D). (**D**) Magnetic orientation after exposure. (**E**) Deviations from each individual's response before exposure (initial direction of each individual set to 0°). [Note: distribution shows repeatability of the responses of individual birds when there is no recalibration of the magnetic compass]. Circular diagrams: arrows give the mean direction of the group of birds, length of the arrow (measure of concentration) is drawn relative to the radius of the circle=1, solid/dashed arrows indicate samples significantly/not significantly directed according to the Rayleigh test (S_{25}), dotted lines give the 95% confidence interval for significantly directed samples. For statistics see Table S2.

The horizon is likely to play an important role in averaging the alignments of the BMP at SR and SS, because an individual songbird must retain the alignment of the polarization pattern at (e.g.) SS for subsequent comparison with the alignment at SR. Transferring the alignment of the BMP to surrounding landmarks at SS would enable the bird to average the SS alignment of the BMP with that at SR, provided it remained at the same location (e.g., at the same perch or roost overnight). For example, prior to the onset of migration, a songbird could view the polarized light pattern at SS and then again the following morning at SR from its overnight roost. Similarly, during migration a bird could land at a stopover site where it can view the SR polarization pattern in the morning and then the SS polarization pattern the following evening before resuming flight on another leg of its migration. Once the relative alignments of the BMP at SR and SS are determined, polarized light cues can be used at either time alone to derive the proposed geographic reference system. Periodic updating, requiring access to polarized light information at both SR and SS, would be necessary to correct for “errors” resulting from seasonal and latitudinal changes in the alignment of the BMP (see below).

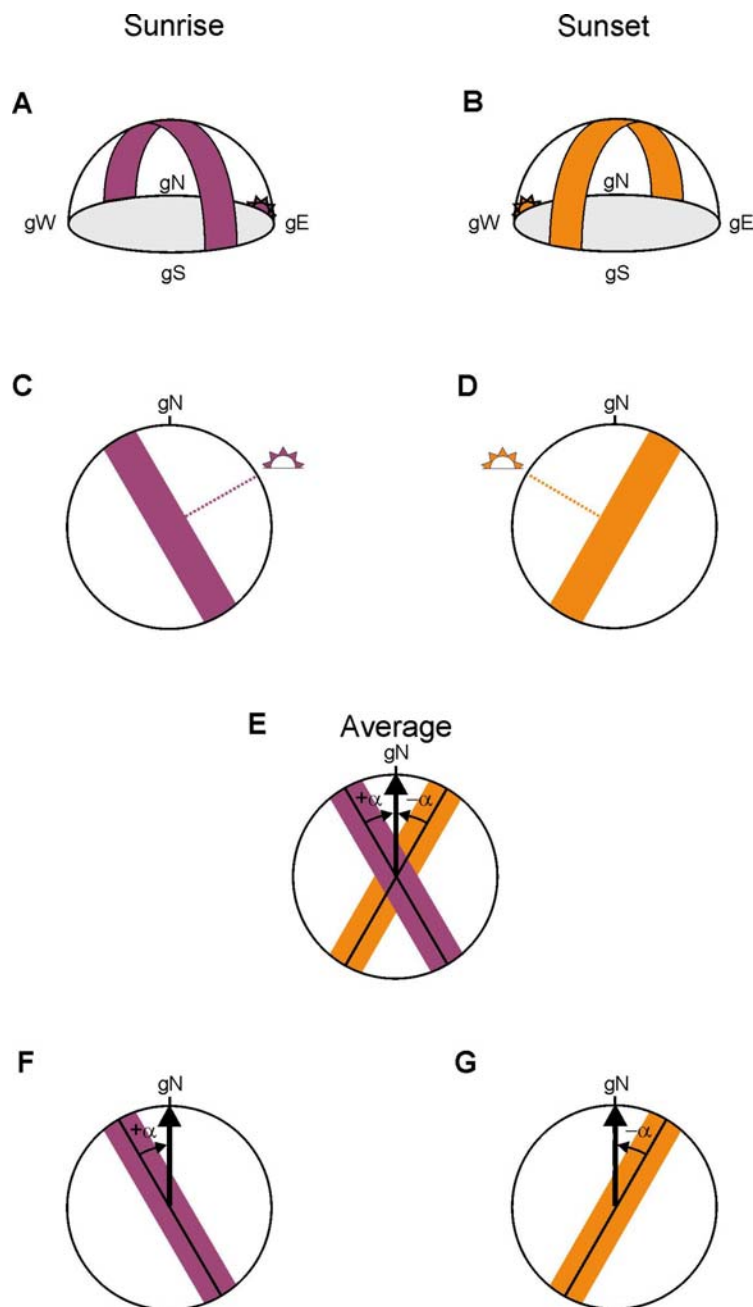


Fig. S2. Use of the polarized light patterns at SR and SS to derive a geographic reference system (after S34). Alignment of the band of maximum polarization (BMP) at (A) SR and (B) SS. At both SR and SS the BMP and e-vector of polarization pass through the zenith and are vertically aligned on the horizon $\pm 90^\circ$ from the sun. Except at the Spring and Fall equinoxes, the intersections of the BMP with the horizon at SR and SS deviate symmetrically on either side of the North-South meridian, with the magnitude of these deviations varying with latitude and time of year. (C, D) Two-dimensional representations of BMP at SR and SS. (E) Since the deviations of the BMP from the North-South meridian at SR and SS are equal in magnitude, but opposite in direction, averaging the alignments of the BMP at these two times of day from the same location can be used to derive the North-South meridian (S34). Once the relative alignments of the BMPs at SR and SS have been learned, the alignment at SR (F) or SS (G) can be used alone to derive the reference direction (e.g., gN) for calibrating other compass systems. However, periodic updating of the calibration reference, requiring access to polarized light cues at both SR and SS, would be necessary to correct for seasonal and latitudinal changes in the alignments of the BMP (see Fig. S3).

Due to the axial symmetry of the polarization patterns at both SR and SS, an independent source of directional information would have to be used to distinguish between the two ends of the polarization axis and, therefore, between geographic North and South, e.g., the position of the sun and/or the radiance or spectral distribution of skylight at SR and SS. Both the distributions of Savannah sparrows exposed to the rotated polarization pattern at SR under partial cloud cover (i.e., when sun and patches of clear sky were visible; 84° -- 264° , $r=0.67$, $N=8$, $P=0.02$; Fig. 1E, open symbols) and the bearings of birds exposed to the rotated polarization pattern at SR without the sun visible were bimodally distributed (86° -- 268° , $r=0.49$, $N=22$, $P=0.004$; Fig. 1E, solid symbols). However, consistent with one or more of the above mentioned possibilities, the bearings of the sparrows exposed to the rotated polarization pattern at SR under partial cloud cover also showed a significant unimodal distribution along the 90° rotated axis (95° , $r=0.64$, $N=8$, $P=0.03$; Fig. 1E, open symbols).

Some songbird species exhibit changes in orientation direction during migration that may be adaptive either because they approximate orthodromic routes, which are the shortest distances between different locations on the Earth's surface (*S35-40*), or avoid energetically costly or dangerous topographic features such as open water or mountain ranges (*S41-43*). If the proposed reference system derived from SR/SS polarized light cues is correct, such programmed changes in migratory direction are likely to be secondary strategies. Such strategies may include an endogenous time program (*S44-46*) or a response to exogenous factors along the migration route (*S47*). Also, in migrants that fly long distances without stopovers, so there is no opportunity to recalibrate their compass systems or reset their internal clock, the failure to reset the internal clock during longitudinal movements can result in migration along orthodromic sun compass routes (*S36*). [Note: In the latter case, the shift in migratory direction results from a lag in updating the time-compensation mechanism for the sun compass, not an error in the directional calibration of the sun compass (*S36*, *S48*).] A gradually curving migratory path will not occur if birds use both SR and SS polarization patterns as independent calibration references, i.e., using one or the other opportunistically as sky conditions permit. During fall migration in the northern hemisphere the cumulative effects of changes in time and latitude will cause the alignments of the BMP at SR and SS to diverge in opposite directions from their alignments at the onset of migration (Fig. S3); the magnitudes of these effects vary with time of year and latitude.

Therefore, use of both SR and SS polarization patterns to calibrate other compass systems would be highly non-adaptive unless the information from these two times of day is averaged. Use of SR and SS polarization patterns as independent calibration references (i.e., not averaging) will result in a highly irregular and unpredictable migratory path, depending on weather conditions, and will substantially increase the distance covered and energy consumed during migration (Fig. S3). We predict, therefore, that any species that uses horizon polarized light cues at both SR and SS to calibrate other compass systems, and whose migratory path differs appreciably from a constant geographic compass heading, will be found to have a geographically fixed calibration reference obtained by averaging SR and SS polarization cues, and a secondary adaptation (see previous discussion) that causes the migratory path to depart from the constant geographic compass heading.

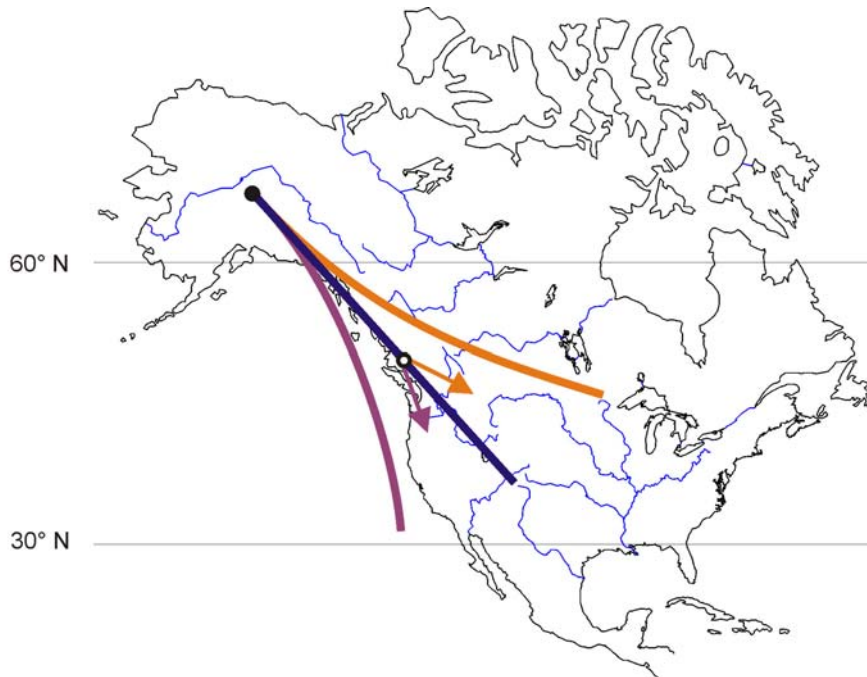


Fig. S3. Hypothetical migratory path followed by a Savannah sparrow with a starting point (solid circle) at the approximate center of breeding distribution of the subspecies *P. s. sandwichensis* in Alaska ($64^{\circ} 50' \text{ N } 147^{\circ} 50' \text{ W}$), a departure date of Aug 20, and an initial heading of 140° , using one of three mechanisms to calibrate its other compass systems. Blue line: the route followed by a bird using a compass calibration reference obtained by averaging the alignments of horizon polarization patterns at SR and SS, i.e., the proposed geographic reference system. Purple line: the route followed by a bird using the horizon polarization pattern at SR only as a calibration reference. Orange line: the route followed by a bird using the horizon polarization pattern at SS only as a calibration reference. The hypothetical paths were determined assuming that the compass systems were recalibrated daily, with a travel rate of 100 km/day for 40 days; changes in the alignments of the BMP at SR and SS were calculated for each new date and location, and then used to calculate the new migratory direction. Between each step the birds were assumed to follow rhumb lines, i.e. constant geographic routes, which in this example did not significantly differ from a constant magnetic compass course. The open circle on the blue path shows the directional response of a bird that was exposed to total overcast during the first three weeks of migration (forcing it to rely on its initial compass calibration during the initial phase of migration), but then experienced a brief period of clear skies allowing it to recalibrate the magnetic compass at SR (purple arrow) or at SS (orange arrow). Note that the cumulative effects of changes in date and latitude will produce a clockwise or counter-clockwise deviation in directional heading if the bird's compass systems are calibrated with respect to the polarized light pattern at SR or at SS, respectively. Consequently, use of the polarization patterns at SR and SS as independent calibration references (updating calibration of the bird's compass systems as sky conditions permit), would produce a 'zigzagging' path. On average, the birds would exhibit the same directional heading as the direct geographic compass path, but would travel a greater distance (requiring a greater expenditure of time and energy) to reach the wintering site. In contrast, averaging the alignments of the BMP at SR and SS, as sky conditions permit, would eliminate seasonal and latitudinal variation and enable the birds to correct for this variation when forced to rely on either SR or SS cues alone to update the calibrations of their other compass systems (see Fig. S2). The map is drawn according to a Mercator projection, where straight lines represent constant geographic courses, but distances are exaggerated at northern latitudes.

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Table S1. Magnetic orientation of Savannah sparrows exposed to a $\pm 90^\circ$ shifted polarization pattern in the local magnetic field at sunrise and/or sunset with full view of the surrounding horizon (data from Fig. 1). For each bird (age: juv=juvenile, ad=adult), the directions of orientation of the initial response and the response after exposure are given relative to magnetic North (mN)= 0° . Deviations from each individual's control response before and after exposure are given relative to the direction before exposure (control= 0°). For birds tested under both experimental conditions, "1" or "2" indicates whether the SS or SR experiment was carried out first (Order). For each group the number of individual birds (N), mean direction or axis relative to mN (α), $\pm 95\%$ confidence interval (CI), mean vector length (r) and level of significance (P) according to the Rayleigh test (S25) are given at the bottom. For illustration see Fig. 1.

Bird	Age	Polarization pattern shifted $\pm 90^\circ$ at sunrise					Polarization pattern shifted $\pm 90^\circ$ at sunset				
		Initial response ($^\circ$ mN)	Response after exposure ($^\circ$ mN)	Deviation from initial response ($^\circ$)	Order	Sun	Initial response ($^\circ$ mN)	Response after exposure ($^\circ$ mN)	Deviation from initial response ($^\circ$)	Order	Sun
7	juv	120	190	70		Y					
8	juv						185	150	325		N
10	juv	100	35	295	2	N	175	270	95	1	N
13	juv						165	280	115		N
14	juv						140	225	85		N
17	juv						50	330	280		N
18	juv	85	185	100		N					
19	juv	220	100	240	2	N	--	--	--	1	N
20	juv	165	260	95	2	N	130	90	320	1	N
21	juv	210	270	60	2	N	150	240	90	1	N
22	juv	200	285	85	2	N	75	130	55	1	N
23	juv						170	55	245		N
28	juv	190	255	65	1	Y	80	340	260	2	N
29	juv	145	180	35		N					
30	juv	110	120	10	1	N	120	30	270	2	N
31	juv	140	230	90	1	N	150	30	240	2	N
32	juv	140	240	100	1	Y	135	195	60	2	N
33	juv	190	100	270		N					
34	juv	165	330	165	1	Y	110	340	230	2	N
35	juv	85	160	75	1	Y	60	170	110	2	N
37	juv	85	330	245	1	N	170	280	110	2	N
40	juv	45	135	90		Y					
42	juv	120	45	285	1	N	95	10	275	2	N
43	juv						190	285	95		N
44	juv	45	145	100		Y					
45	juv	115	200	85		N					
46	juv	55	145	90	1	N	55	10	315	2	N
49	juv	70	330	260	1	Y	55	180	125	2	N
52	ad	215	320	105		N					
58	ad	80	220	140		N					
60	ad	150	45	255		N					
62	ad	60	30	330		N					
64	ad	150	60	270		N					
65	ad	75	120	45		N					
66	ad	160	310	150		N					
67	ad	90	325	235		N					
N		30	30	30			20	20	20		
$\alpha \pm 95\%$ CI			138 $^\circ$ --318 $^\circ$	85 $^\circ$ --265 $^\circ \pm 12^\circ$				309 $^\circ$	94 $^\circ$ --274 $^\circ \pm 14^\circ$		
R		N/A	0.08	0.54			N/A	0.17	0.58		
P			0.82	<0.001				0.59	<0.001		

Table S2. Magnetic orientation of Savannah sparrows exposed to a $\pm 90^\circ$ shifted artificial polarization pattern in the local magnetic field at SS without access to visual cues near the horizon (data from Fig. S1). For explanations see Tab. S1, for illustration see Fig. S1.

Polarization pattern shifted $\pm 90^\circ$ at sunset without view of horizon				
Bird	Age	Initial response ($^\circ$ mN)	Response after exposure ($^\circ$ mN)	Deviation from initial response ($^\circ$)
8	juv	150	190	40
14	juv	225	220	355
28	juv	340	275	295
30	juv	30	30	0
34	juv	340	320	340
66	ad	310	310	0
N		6	6	6
$\alpha \pm 95\%$ CI		166 $^\circ$ --346 $^\circ$	283 $^\circ$	352 $^\circ \pm 33^\circ$
r		0.45	0.47	0.86
P		0.32	0.29	0.006