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RESEARCH ARTICLE

Evidence for a southward autumn migration of nocturnal noctuid moths in central Europe

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ABSTRACT

Insect migrations are spectacular natural events and resemble a remarkable relocation of biomass between two locations in space. Unlike the well-known migrations of daytime flying butterflies, such as the painted lady (*Vanessa cardui*) or the monarch butterfly (*Danaus plexippus*), much less widely known are the migrations of nocturnal moths. These migrations – typically involving billions of moths from different taxa – have recently attracted considerable scientific attention. Nocturnal moth migrations have traditionally been investigated by light trapping and by observations in the wild, but in recent times a considerable improvement in our understanding of this phenomenon has come from studying insect orientation behaviour, using vertical-looking radar. In order to establish a new model organism to study compass mechanisms in migratory moths, we tethered each of two species of central European Noctuid moths in a flight simulator to study their flight bearings: the red underwing (*Catocala nupta*) and the large yellow underwing (*Noctua pronuba*). Both species had significantly oriented flight bearings under an unobscured view of the clear night sky and in the Earth's natural magnetic field. Red underwings oriented south-southeast, while large yellow underwings oriented southwest, both suggesting a southerly autumn migration towards the Mediterranean. Interestingly, large yellow underwings became disoriented on humid (foggy) nights while red underwings remained oriented. We found no evidence in either species for a time-independent sky compass mechanism as previously suggested for the large yellow underwing.

KEY WORDS: Insect migration, Moth migration, Noctuid, Orientation, Navigation, Compass sense

INTRODUCTION

The suggested evolutionary benefits of energetically costly insect migrations (Rankin and Burchsted, 1992) are manifold and a matter of healthy debate (e.g. Holland et al., 2006; Chapman et al., 2015). The following benefits are usually mentioned in the literature: (1) an increase in the production of offspring via exploitation of 'green waves' of vegetation, (2) the avoidance of severe weather conditions during winter time and (3) the avoidance of predators and/or parasites (Chapman et al., 2015). Moth migrations additionally incur a considerable agricultural (Porter et al., 1991; Drake and

Gatehouse, 1995) as well as ecological (Green, 2011; Hu et al., 2016) impact. In the case of the migration of the Australian bogong moth (*Agrotis infusa*), the resulting transportation of biomass from the Australian plains into the Snowy Mountains provides a remarkable inflow of nutrients and energy into this harsh alpine environment, a vital resource for the survival of many animal (and presumably even plant) species (Green, 2011; Warrant et al., 2016). Similar to what is known from birds (e.g. Hein et al., 2011; Richardson, 1990), the migratory behaviour of flying insects is influenced by weather conditions, and this has been documented in a wide variety of species (e.g. Drake, 1994; Brattström et al., 2008).

In European moths, the most convincing evidence for truly migratory behaviour, involving southward autumn migrations and northward spring migrations, comes from the British Isles (Sothibandhu and Baker, 1979; Baker and Mather, 1982; Chapman et al., 2010). In continental Europe, nocturnal species like the silver Y moth (*Autographa gamma*) and the dark sword-grass (*Agrotis ipsilon*) are mentioned as 'very definite migrants', with seasonally dependent migratory directions (Williams, 1958). However, apart from this, little is known about the migratory behaviours of continental moth species.

The external compass cues used as orientation references by nocturnally migrating insects have also been little explored, although recent work suggests that the Australian bogong moth relies on the Earth's magnetic field and visual landmarks during migration (Dreyer et al., 2018). In contrast, much more is known about the compass cues used by insects migrating during the day – these migrants rely primarily on celestial cues, such as the sun and the celestial polarization pattern, for orientation (e.g. Reppert et al., 2016; Homberg, 2015).

Since seasonally dependent migratory orientations in the large yellow underwing (LYU) have previously been described by Chapman and colleagues (2010), with a northward orientation in spring and a southward orientation in autumn, we hypothesized that noctuid moths captured during autumn in central Europe would show a similar pattern, with a southbound migration towards the Mediterranean. In this study, we tethered moths within a computer-interfaced flight simulator (Mouritsen and Frost, 2002) to investigate the autumn migratory orientations of two species of noctuid moths: the large yellow underwing [*Noctua pronuba* (Linnaeus 1758)] and the red underwing [RU; *Catocala nupta* (Linnaeus 1767)]. We also tested which weather conditions were associated with directional migration behaviour. These studies were performed at a migratory nodal point in central Europe (Fig. 1A): the Lake Neusiedl Biological Station, located within the Austrian National Park Seewinkel near the eastern foothills of the European alps. The wetlands and saline lakes of this area provide an important stopover location for many migratory bird species and also act as an important refuge for waterfowl. Nightly light trapping of insects also revealed a similar richness in migratory moth species.

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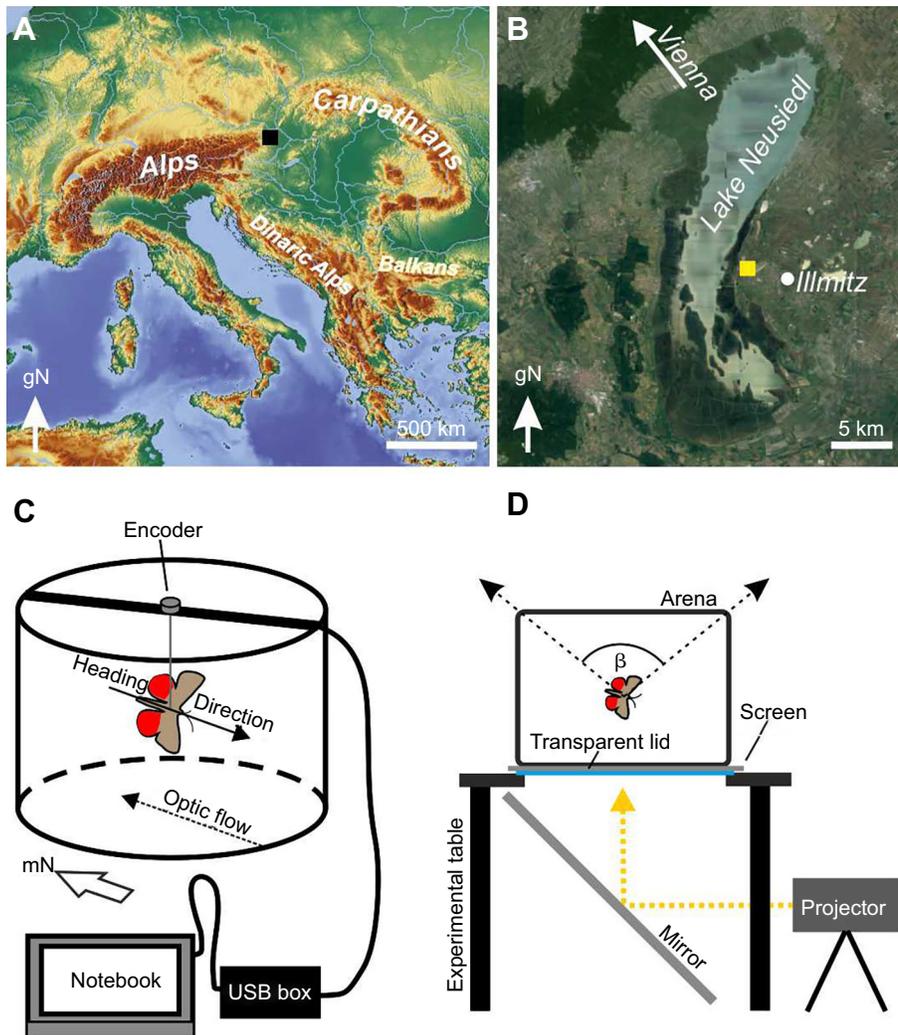


Fig. 1. The experimental site and flight arena. (A,B) The National Park Neusiedler See is located between the Eastern foothills of the Alps and the northwest foothills of the Carpathians (black and yellow squares in A and B, respectively, mark the location of the Biological field station). Towards the southeast, the landscape widens into the Hungarian plain. This specific geographical location represents a migratory hotspot for smaller migratory animals that generally tend to avoid flying directly across high mountain crests. Map in A adapted from maps-for-free.com (2017); map in B adapted from Google Maps (2017). (C) While a moth performs orientation behaviour in the arena, the direction of the optic flow (dashed arrow) is automatically and instantaneously updated, and always flows in a direction opposite to the heading direction (continuous arrow). The encoder reads the heading direction of the tethered moth and the corresponding timestamp. The USB box serves as the interface between the encoder and the connected notebook. (D) Schematic diagram of the apparatus used in this study. The dorsal viewing angle (dashed arrows, β) of the sky was restricted to ~ 100 deg. The arena rested on a translucent Plexiglass table top. The optic flow (in closed loop) was projected onto the bottom of the screen via a 45 deg mirror. mN, magnetic North; gN, geographic North.

MATERIALS AND METHODS

Preparations and rearing conditions

All moths tested in the present study were caught using light traps at the Biological Field Station Lake Neusiedl during the first half of September 2016. The captured moths were transferred into plastic containers before they were stored in a sheltered place to recover from the stress induced by capture and were fed with 10% honey solution. The moths were usually tested 1–3 days after being captured. Prior to tethering, the moths were immobilized in a refrigerator for up to 5 min. The scales on a moth's pterothorax were removed using a micro-vacuum pump. Afterwards, a vertical tungsten stalk was glued to the dorsal thorax using contact cement while the moth was restrained by a weighted plastic mesh. Moths were then placed individually in clear plastic containers and provided with a clear view of the sky prior to their experimental night. Since insulating scales were removed from the pterothorax, and the glued Tungsten stalk could potentially cool the animal down, we placed the animals in a box warmed by hot water bottles wrapped in towels prior to the experiments. All animals were released one day after they were tested.

Flight simulators

Ferromagnetic-free, modified Mouritsen-Frost flight simulators (Mouritsen and Frost, 2002) were used to continuously record the heading directions of moths (see Fig. 1). The circular arena had a

diameter of 50 cm and a height of 36 cm; the inner wall of the arena was covered with black cloth.

A moth equipped with a vertical tungsten stalk was connected with a piece of thin rubber tubing (inner diameter slightly smaller than diameter of the stalk for a tight fit; length: 1.5 cm) to a vertical tungsten rod (diameter: 0.5 mm; length: 15.3 cm) that was attached very tightly via a very small grub screw to the centre of an optical encoder (US Digital, Vancouver, WA, USA) located at the centre of the upper opening of the arena (Fig. 1C). This optical encoder continuously recorded the rotations of the tungsten rod assembly (between 0 deg and 360 deg). Thus, the attached moths were able to flap their wings and freely choose any flight direction around the yaw axis while their heading directions were recorded relative to magnetic North with a sampling rate of 5 Hz and a horizontal resolution of 3 deg. Using a projector (BENQ, GP3), a mirror and neutral density filters, a very dim moving image (a bitmap satellite image of the local region from Google Earth) was back-projected onto a tracing paper screen (mounted on a clear Perspex sheet below the arena; a hole with the same diameter of the arena was cut into the table top). Custom-written software controlling the movement direction of the image was coupled to the encoder system (USB4; US Digital, Vancouver, WA) via a feedback loop. The resultant ventral flow-field image, which was always moving 180 deg relative to the moth's heading (i.e. from head to tail), created the visual sensation of a ventral ground moving below the moth during

forward flight, irrespective of which direction the moth flew in, because it was continuously updated by the moth's own flight direction. The average luminance of the optic flow was 6.7×10^{-4} cd m⁻².

Experimental procedure

Moths were tethered and allowed to fly for several minutes. In order to allow a meaningful comparison of the recorded tracks, only the first 10 min were used for the analysis (except LYU #13, which flew for 7.3 min). Since the RU moths were caught less frequently (~1–2 per catch), we were only able to record the orientation choices of 14 individuals. 11 out of 14 individuals flew over the full recording time of 10 min, 1 individual flew 20 min, and 2 individuals stopped flying after 7.7 and 9.5 min, respectively. The 36 LYU moths (usually ~5–15 were caught per night) flew for at least 10 min within the arena. In the case when a moth continuously spiralled after it was attached to the encoder (putatively due to a skewed stalk), or showed a faltering flight behaviour (characterized by several stops in a row), the experiment on this individual was immediately aborted and the data excluded from the analysis.

Experimental conditions

While the moths were performing in the flight simulator, they experienced a clear view of the sky (viewing angle ~120 deg; see Fig. 1C,D). A paddock close to the biological field station was chosen as an experimental site (47°46'05.0"N, 16°46'05.5"E). Great care was taken to position the arena at a sufficient distance from putative landmarks (e.g. trees) to remove the possibility that moths might see them. The simulator arena was placed on a wooden table that was given a different orientation each experimental night. The arena itself was rotated by about 90 deg after each individual experiment. A small aluminium camping table and two plastic chairs were deployed about ~5 m from the arena. Three sides of the experimental table were covered with black cloth to prevent stray light (reflected by the mirror beneath the table) from influencing the behaviour of the tested moths. One side remained uncovered to allow the projection of optic flow onto the mirror (see above). All artificial light sources were either covered with several layers of duct tape or with red filter film. Headlamps equipped with red LEDs were used while the moths were being handled. The experiments were conducted on seven nights between 1 and 13 September 2016. All experiments were conducted after sunset, under more or less clear and windless conditions, and under a natural magnetic field. The moon's disc was not visible to the moths in any of the experiments.

Weather conditions

The first three experimental nights were dry and clear. Owing to slight showers on 4 September, the experiments were put on hold. On the following two nights, the weather changed dramatically with a temperature drop from 29.4°C on the 4th to 21.7°C and 16.3°C (maximum temperatures), respectively, on the 5th and 6th, which was accompanied by moderate winds (17 and 20 km h⁻¹) and rain. Considerable changes in humidity were noticed on the experimental nights that followed the rainy nights, probably due to the resulting soggy of the surrounding fields (see below).

The experimental nights of the 7, 8, 9 and 12 September were quite foggy and moisture condensed noticeably on our gear. The atmospheric pressure and the air temperature varied between the first three experimental days and the last four, but the most notable difference was related to the relative humidity and the dew-point spread (difference between the air temperature and the dew-point).

Changes in the dew-point spread give some indication of whether fog will gather or not; the lower the spread, the higher the chance of fog formation. Fog and high humidity were observed on the last four experimental nights, but not on the first three. All meteorological measurements derive from meteorological stations in Andau and Eisenstadt (Burgenland, Austria, both around 20 km from the experimental site), which are available online (kachelmannwetter.com; wetter.com) and from personal notes taken during the experiments.

Statistical analysis

Data analysis was conducted using custom-written MATLAB scripts (2010b, 2013a; MathWorks Inc.) and Oriana (4.01; Kovach Computing Services). Since the analysis of the orientation choice of each individual moth gives a mean vector in which the *r*-value encodes the magnitude of the mean vector (the longer the mean vector the higher is the concentration of the data around the mean direction), and the direction of the vector is the mean direction, we were able to conduct second order statistics, using the non-parametric Moore's modified Rayleigh test (MMR; Moore, 1980; Zar, 1999). This test involves different weightings of the respective mean angles according to their respective *r*-values. The test assumes the null hypothesis that the means of the tested sample are uniformly distributed around the circle. A low *P*-value (conventionally *P*<0.05) indicates that the data are distributed non-uniformly and that the tested sample had a preferred migratory direction. The length of the black arrow encodes the *R** value (score of MMR test). The dashed circles in the circular diagrams encode the critical *R** values needed for statistical significance: inner circle, *P*<0.05; middle circle, *P*<0.01; outer circle, *P*<0.001. A black arrow crossing a respective dashed circle indicates statistical significance at the corresponding level.

In order to compare the orientations of the respective groups, we used the non-parametric Mardia–Watson–Wheeler Test (MWW; Fisher, 1993; Mardia and Jupp, 2000; Batschelet, 1981) and a Moore's paired test (MPT; Zar, 1999), to test against the null hypothesis of identical distributions. All orientation choices with *r*<0.2 were not included in the evaluation since we assumed that the moths were performing behaviours other than migratory behaviour (see Nesbit et al., 2009), the stalk was not attached correctly to the moth's thorax, or the stalking process had interfered with flight performance by introducing wing beat asymmetries (due for example to glue spill).

Celestial cues

To compare our results with those of Soththibandhu and Baker (1979), we used the open-source program Stellarium (version 0.14.0) to reproduce the stellar constellations of the night sky over Manchester in 1977 (Soththibandhu and Baker, 1979) and Illmitz during September 2016 at 22:30 and 01:30 h local time, according to the experimental time mentioned in fig. 9 of Soththibandhu and Baker (1979). To avoid confusion, we want to stress that this procedure was simply part of a *post hoc* data analysis: Stellarium was not used during the actual experiments. Unfortunately, Soththibandhu and Baker (1979) did not provide precise dates and locations for their experiments (on moths tested under moonless starlit conditions), apart from the fact that the experiments took place in July 1977 in Manchester or Bristol. We thus chose Manchester on 15 July 1977 for the reconstruction of the night sky. On a clear night in Manchester, the bright star Arcturus – a possible orientation cue – would have been visible throughout July. The orientation angle of each moth was compared with the time the

experiments were conducted using linear regression (see Results), performed using the Data statistics Toolbox in MATLAB (2010b). Because the ‘starting angle’ of the visible celestial bodies at the beginning of each experimental night changed only marginally between the experimental nights (1–13 September 2016), and because their rate of rotation is constant, the slight angular differences between the experimental nights included for the data recorded in Illmitz (2016) are negligible versus the respective time of the night.

RESULTS

Red underwings

The RUs oriented SSE when provided with a clear view of the starry sky and while experiencing the natural magnetic field of the Earth (Fig. 2A). According to the MMR test, the tested sample was statistically significantly directed towards 146 deg (α) relative to magnetic North (95% CI: 119 deg, 169 deg; $r=0.49$; $R^*=1.71$; $P<0.001$; $n=12$). Since most of the RUs ($n=8$) were tested during four foggy nights, the orientation of this species was evidently not

affected by the weather conditions prevalent on these final four experimental nights.

Large yellow underwings

The preferred mean direction of all tested LYUs (Fig. 2B) was significantly towards SSW (α : 201 deg; 95% CI: 141 deg, 257 deg; $r=0.173$; $R^*=1.1$; $P<0.05$; $n=36$). We also analysed the orientation choices of the LYU against the presence or absence of fog. A lower dew-point spread (Fig. 3) is associated with an increased presence of water particles in the air, and an increasing chance of fog. From experimental day 4 onwards, the dew-point spread dropped severely, indicating a much higher likelihood of fog during our experiments (and fog was noticed).

Interestingly, as a group, LYUs were significantly oriented on clear nights (Fig. 2C; α : 205 deg; 95% CI: 167 deg, 260 deg; $r=0.275$; $P<0.01$; $n=19$), but not in a groupwise analysis on foggy nights (Fig. 2D; α : 160 deg; $r=0.083$; $R^*=0.381$; $0.5<P<0.9$; $n=17$). In addition, the mean directions of the RUs and the LYUs were significantly different (MWW, $P=0.007$, $W=9.826$; MPT, $P<0.01$, $R'=1.291$; all LYU data used for comparison), suggesting that these two species have different migratory directions (Fig. 2A,B).

In order to test for a time-dependent ‘shift’ of the heading directions of moths over the course of a night, as detected by Sothibandhu and Baker (1979), we plotted the heading directions of moths as a function of the experimental time (see Fig. 4; slopes for RU: -1.29 ; LYU in Illmitz: -14.8 ; LYU in Manchester: 16.95). The implications of this analysis for migratory headings measured in our moths will be explored in the Discussion.

DISCUSSION

Orientation in red underwings

RUs have previously been reported to be ‘occasional vagrants’ (Skinner, 2009), and have been considered to merely expand their distribution by invading other territories during favourable seasons (Fox et al., 2011). In Austria, members of the genus *Catocala* are thought to regularly invade the country from the Mediterranean region (Malicky et al., 2000). We found that when tested in a flight simulator during September in southeast Austria, RUs demonstrated a highly concentrated SSE orientation. A plausible explanation for this behaviour could be that RUs perform a roughly southbound autumn migration towards the Mediterranean (possibly to as yet unknown wintering grounds), and that we recorded the orientation choices of individuals that were undertaking this migration. According to this hypothesis, one could assume that we caught individuals that had flown into the National Park from locations to the north or northwest of the study site. This assumption is supported by a quantitative sampling of RUs until late August in Lower Bavaria using bait traps (Sturm, 2002) – released RUs were never subsequently re-captured, strongly suggesting a lack of site fidelity. Thus, we conclude that the directedness of our tested RUs does not simply reflect dispersal movements (i.e. expansion of the species distribution) but rather a regular autumn southbound migration from central Europe towards regions of the Mediterranean (e.g. Greece; Kailidis, 1964). Judging from the recorded mean direction of the tested RUs, one might draw the conclusion that their conspecifics in the wild would fly farther into the Balkans.

Orientation in large yellow underwings

The LYU is listed as a migratory moth species in Central Europe and migratory movements have been reported in regions spanning from Britain to the Caucasus (Chapman et al., 2010; Poltawski, 1982). The southwesterly orientation of the LYUs recorded in Illmitz

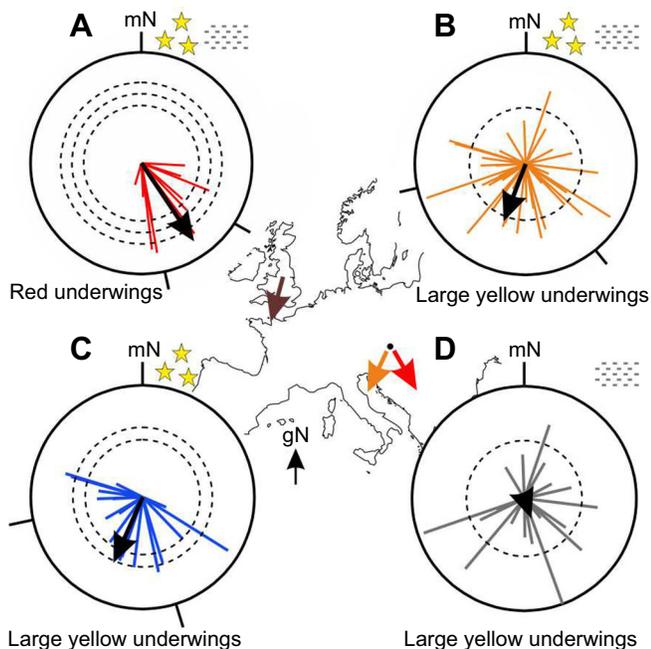


Fig. 2. The orientation of red underwings and large yellow underwings relative to magnetic North plotted as circular diagrams. The virtual flight of each moth is represented by a single vector (radial lines) whose mean direction is represented by the vector's direction. The lengths of the black arrows within the circular diagrams represent the R^* value according to the Moore's modified Rayleigh test, and this value reveals the direction and directedness of the moths as a group. The radii of the dashed circles indicate the significance levels required for directional significance (MMR Test; from inner to outer dashed circle: $P<0.05$, $P<0.01$, $P<0.001$). The black lines either side of the arrows show the 95% confidence interval. While the red underwings were well-oriented in clear and foggy weather (A, data from all experimental nights pooled, $P<0.001$), the large yellow underwings (B, data from all experimental nights pooled, $P<0.05$) were oriented on clear nights (C, $P<0.01$). On foggy nights they were not oriented as a group (D). The black dot on the map of continental Europe in the centre of the figure marks the approximate location of the experimental site, and the red and orange arrows encode the mean directions of the two moth species relative to geographic North. The mean orientation direction for Austrian large yellow underwings matches the seasonally appropriate orientation for the same species in southern Britain (brown arrow, after Chapman et al., 2010). Stars represent clear nights; small dashes indicate foggy nights.

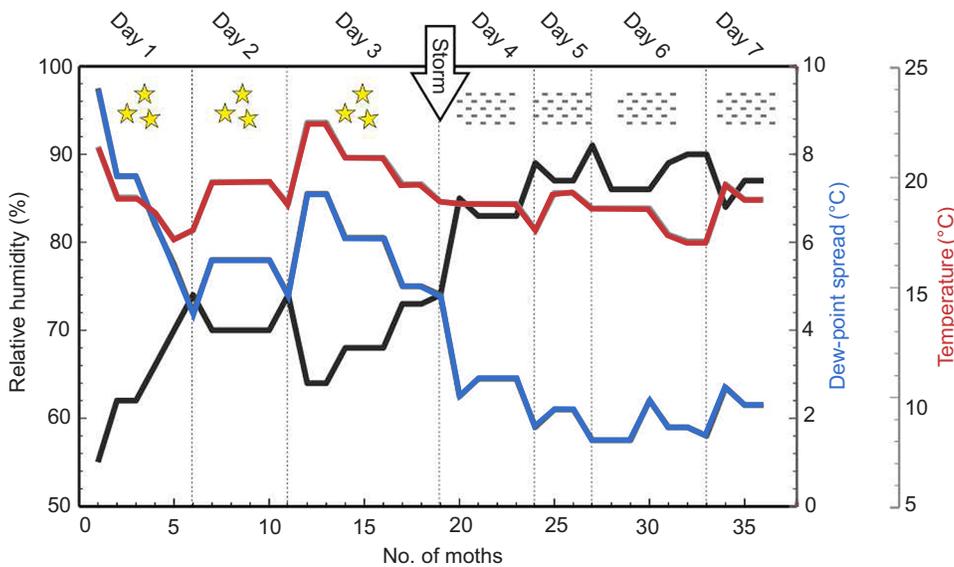


Fig. 3. The putative effect of humidity and dew point on moth orientation. The development of the relative humidity (black line), the dew-point spread (blue line) and the temperature (red line) over the course of the experimental period. Note that the dew-point spread decreases to less than 3°C on day 4. A dew-point spread of less than 3°C is an indicator for fog. On days 1–3, the orientation data depicted in Fig. 2C were recorded, while days 4–7 correspond to the data in Fig. 2D. Stars indicate clear night; small dashes indicate foggy night (fog was indeed observed on nights 4–7).

seems to be seasonally appropriate and in agreement with the data obtained in southern Britain (see brown arrow in Fig. 2; Chapman et al., 2010). This suggests that LYUs, similarly to RUs, perform an annual roughly southbound autumn migration passing through Illmitz and heading towards their wintering grounds situated to the south. The exact wintering locations are, however, still unknown.

Putative influence of the weather on orientation behaviour

The influence of wind and temperature on the behaviour of insects is described extensively in the literature (e.g. Pedgley et al., 1990; Taylor and Carter, 1961; Gregg et al., 1994; Marchand and McNeil, 2000; Chapman et al., 2016). The ‘minimum temperature threshold’ required for migratory flights in two medium-sized noctuid moth species (wingspan: 30–40 mm) was estimated to be ~8°C (Taylor and Carter, 1961). We conducted our experiments on practically windless nights, with air temperatures distinctly above 8°C, so if the weather influenced our experiments at all, wind and air temperature are presumably only of secondary importance for the interpretation of our data.

RUs have been reported at bait traps even in bad weather conditions (ongoing rain, strong winds and temperatures less than 15°C; Sturm, 2002). This observation is in line with our results indicating that the orientation of RUs was evidently not influenced by the weather. In contrast, we noticed that a subsample of LYUs tested under foggy conditions was less oriented (in fact random, Fig. 2B) compared with a subsample that was highly significantly oriented under clear conditions. Both species readily flew in the arena, so the motivation to fly was not necessarily reduced because of the weather conditions. However, in the case of the LYUs, the motivation to perform oriented migratory flights in the arena might have been reduced as a result of unfavourable weather conditions (e.g. lack of favourable initiation factors for migration).

The presence of fog might technically explain this phenomenon since the lower visibility could negatively influence the moth’s ability to orient during migration by obscuring a clear view of the sky. However, we never observed the arena veiled in thick fog. As mentioned earlier, the most dramatic meteorological factors that accompanied foggy nights are changes in the relative humidity and the dew-point spread. However, in a quantitative long-term study in northern New South Wales (Australia), a positive correlation was observed between the local relative humidity and the numbers of

migratory moths caught (Gregg et al., 1994). This implies that these particular Australian moth species preferred higher humidity while in flight. Unfortunately, that study did not provide any data on the presence or absence of fog.

Factors that could explain the reduced directedness of LYUs compared with RUs on foggy nights might include an unfavourable presence of water particles in the air, a slightly reduced outside temperature and/or a smaller body size. If water particles in the air moistened the bodies of LYUs, this could have led to an overall critical decrease in their body temperature and a reduced motivation to migrate.

Moreover, in our experiments, the moths were tethered to a tungsten rod and performed flight behaviour within an arena that was located ~1 m above the ground. Thus, the conditions under which our tested moths performed were quite different to those experienced by their conspecifics in the wild. Besides the obvious limitations that were introduced by our experimental setup, we also wish to stress a less obvious factor that might have influenced the results: we removed the dorsal scales of the pterothorax in order to glue the tungsten shaft to the animal. The ‘coats of hair’ of moths are an ‘excellent insulator’ (Church, 1960) and in noctuids, an intact layer of scales contributes to maintaining up to 90% of the insect’s internal temperature while in flight (Church, 1960). If during our experiments the tungsten shaft collected water particles due to the fog, and was fanned by the air current generated by the beating wings of the moth (with a damaged layer of scales), this could have decreased the overall body temperature due to evaporative chill, thereby reducing the motivation of the moth to migrate. The smaller LYU may have been much more susceptible to such a situation than the considerably larger RU. Another putative explanation would be that a humid evening simply resembles an unfavourable trigger for a migratory flight for the smaller LYU.

Putative compass cues and compass mechanisms

During the daytime, insects can rely on obvious and reliable celestial cues such as the position of the sun’s disc and the polarization pattern of the sky to navigate (e.g. Wehner and Müller, 2006; Homberg, 2015), whereas compass cues that are utilized for long-distance movements at night are still comparatively little studied. Two obvious directional cues at night are stars (Foster et al., 2018) and/or the Earth’s magnetic field (e.g. Baker and Mather,

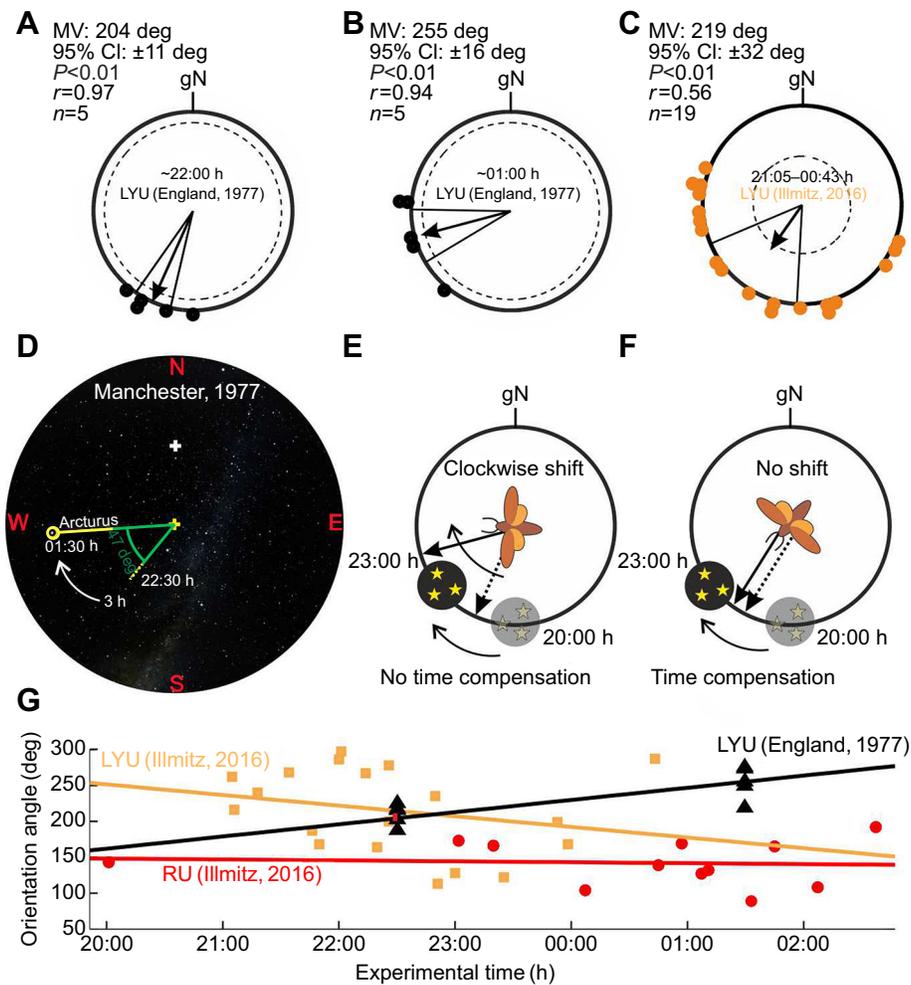


Fig. 4. Clockwise shift in orientation of large yellow underwings, as shown in Sothibandhu and Baker (1979), revisited. (A,B) The mean orientation angles listed in table V in Sothibandhu and Baker (1979) were plotted relative to geographic North (gN) as circular diagrams. The data were pooled according to the experimental time. In A, animals were tested at $\sim 22:00$ h. In B, the same animals were tested at $\sim 01:00$ h. (C) Orientation data for LYUs recorded in Austria in 2016 (data from Fig. 2C) but plotted as in 4A,B to allow comparison. Black arrows in A–C,E,F represent mean vector (MV). Solid lines flanking the arrows are 95% confidence intervals (CI). Dashed circles show level of significance for $P < 0.01$ according to the Rayleigh test. (D) A Stellarium reconstruction of the starry sky over Manchester on 15 July 1977 at 01:30 h with special focus on the position of the star Arcturus over the course of this night. Solid yellow line in D shows position of a particular star at 01:30 h. Dashed yellow line is the position of a particular star at 22:30 h. Yellow cross is celestial zenith, as seen by a virtual spectator looking vertically upwards into the sky. White cross is the star Polaris, the centre of celestial rotation. Green angles show angular difference in the apparent movement of a particular star over the course of 3 h relative to the position of a virtual spectator looking vertically upwards into the sky. (E) Scheme demonstrating a non-time-compensated star compass mechanism. A non-time-compensated star compass mechanism was suggested for the orientation behaviour of some LYUs in Sothibandhu and Baker (1979) and is discussed in the main text. (F) Scheme demonstrating the putative orientation behaviour of a moth as would be expected if a time-compensated star compass mechanism was operating. Solid radial arrows in E and F show putative heading direction of a moth at 23:00 h. Stippled radial arrows in E and F are putative heading direction of moth at 20:00 h. Faded/dark sky symbols in E and F show position of putative navigational cues at 20:00 h and 23:00 h. (G) The orientation angles of moths plotted as a function of the experimental time. Linear regressions of the particular datasets of angles are represented by the orange, black and red lines. Note that neither the orange (LYUs) nor the red regression lines (RUs; both species tested in Illmitz, Austria, 2016) has a positive slope like that of the black regression line (LYUs tested in England, 1977).

1982; Guerra and Reppert, 2015; Dreyer et al., 2018). Indeed, birds that migrate at night are capable of using both of these cues for compass orientation (Emlen, 1967; Wiltchko and Wiltchko, 1972). In addition, the light conditions are about 1 million times dimmer at night and this might have an impact on the cue hierarchy used during migration (el Jundi et al., 2015). Taken together, using the sky at night might require a more dynamic compass system that is different to the one in diurnally orienting insects.

A moth fixed in our flight arena would have experienced an apparent movement (due to the rotation of the Earth) of celestial bodies from east to west at a constant rate, depending on their distance to Polaris. In figure 9 and table V of Sothibandhu and

Baker (1979), the authors presented orientation directions of LYUs (see Fig. 4A,B) recorded on a moonless starry night in England (using a different orientation apparatus) and suggested that this species is likely to use celestial cue(s) for compass orientation. Since all tested individuals showed a shift in orientation of about 16 deg per hour from east to west between $\sim 22:00$ h and $\sim 01:00$ h local time, and coupled to the fact that both samples were significantly directed, the authors concluded that all individuals used the azimuth positions of particular stars or star groups to orient (Sothibandhu and Baker, 1979). The stars or star groups could be (estimated from the shift of 16 deg per hour) located at a position about 95 deg away from the pole star (Sothibandhu and Baker, 1979). The star

constellation Boötes, featuring Arcturus (α boo) as the most radiant star of the northern hemisphere (Kaler, 2002), roughly fulfils these requirements, and should have been visible with the naked eye during the experiments conducted in England in 1977 (see Fig. 4D). During our experiments in 2016, at the more southerly experimental location in Illmitz, one constellation would have been clearly visible as well: Aquila featuring Altair as its brightest element. Regardless of which celestial body (or bodies), the tested individuals in Sothibandhu and Baker (1979) might have used (if any), they clearly exposed a positive ~ 50 deg shift of the group's mean vector. If the hypothesis that stellar orientation drove this shift was correct, then the English moths obviously did not show any form of time compensation (see Fig. 4F), as has been found, for example, in the Monarch butterfly (Mouritsen and Frost, 2002). Without the ability to time compensate for the rotation of celestial bodies, moths would continuously hold a constant angle relative to celestial cues, meaning that their flight trajectories would change direction over time (i.e. become curved: Fig. 4E). If Sothibandhu's and Baker's hypothesis is correct, and their moths indeed relied on stars for orientation, then they must have used a non-time-compensated celestial compass [as discussed in Nesbit et al. (2009) for the day-active painted lady, *Vanessa cardui*]. This would imply that these moths would not have had the ability to maintain a constant migratory bearing over the course of a night. This is indeed what their data suggest (black solid line in Fig. 4G).

If the LYUs tested in our flight simulators used a particular celestial cue as a reference point, such as the star constellation Aquila or the Milky Way (or some other constellation of stars), and like the English moths, did not apparently compensate for the celestial rotation, their bearing should systematically change over the course of an evening. However, our data did not find any indication of such a systematic change in the bearing of LYUs (orange solid line in Fig. 4G), nor indeed in the bearing of RUs (red solid line in Fig. 4G). A couple of explanations for this difference in our results from those of Sothibandhu and Baker might be as follows (not an exhaustive list): (1) the Austrian moths did not use the stars as a compass cue, or (2) they did use the stars as a compass cue but were capable of time compensation. An obvious major difference between our experiments and those described in Sothibandhu and Baker (1979) is that we did not test our animals twice at different times of the night and this, together with the degree of 'noise' in our data, may have masked any systematic change in bearing in the Austrian moths.

Another potential directional cue is the geomagnetic field, and a magnetic compass sense was reported in the large yellow underwing by Baker and colleagues (1982). Additionally, the day-flying Monarch butterfly has been suggested to use a light-driven inclination compass (Guerra et al., 2014, but see Mouritsen and Frost, 2002 and Stalleicken et al., 2005). Moreover, a magnetic compass sense has very recently been implicated in the highly directed migration of the nocturnal Australian Bogong moth (Dreyer et al., 2018). Since we used a ferromagnetic-free experimental apparatus, it is at least plausible that our moths used the undisturbed magnetic field of the Earth for navigation.

In conclusion, we believe that our study provides an ideal baseline for further investigations of compass mechanisms used for nocturnal orientation during long-distance moth migration. The two European moth species we have investigated – the red underwing and the large yellow underwing – are ideal for studying the navigational mechanisms that steer nocturnal insect migrations and for investigating the roles of navigational cues proposed in earlier studies, but using different techniques. Follow-up studies using

computerized three-dimensional Helmholtz coil systems (in order to manipulate the experimental magnetic field within the arena) and top projections of the starry sky are already planned.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: D.D., B.J.F., E.J.W.; Methodology: D.D., B.e.J., C.S., L.C.; Software: D.D.; Validation: D.D., B.e.J., D.K., B.J.F., E.J.W.; Formal analysis: D.D., B.e.J., D.K., B.J.F., E.J.W.; Investigation: D.D., B.e.J., D.K., C.S., L.C., T.Z.; Resources: T.Z., E.J.W.; Writing - original draft: D.D., E.J.W.; Writing - review & editing: D.D., B.e.J., D.K., B.J.F., T.Z., E.J.W.; Visualization: D.D.; Supervision: D.D., E.J.W.; Project administration: D.D., T.Z.; Funding acquisition: E.J.W.

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