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Moon orientation in adult and young sandhoppers

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Abstract In the last 30 years, lunar orientation has received little attention from students of animal orientation. Even in *Talitrus saltator*, the first animals in which the lunar compass was demonstrated, research did not continue. Our studies have demonstrated that: 1) chronometrically compensated lunar orientation is independent of the earth's magnetic field (an ever present non-chronometric orientation reference); 2) lunar orientation is independent of the lunar shape; and 3) the lunar compass is also used by young animals born in the laboratory (without experience in nature).

Key words Sandhoppers · *Talitrus saltator* · Moon orientation · Compass orientation · Magnetic field

Abbreviations *AZm* lunar azimuth · *LD* light–dark · *Yl* landward direction

Introduction

It is known that sandhoppers orient themselves along the Y-(sea-land)-axis of the beach on the basis of astronomical compass references (sun and moon). They assume the seaward direction when dehydrated for a few minutes and the landward direction when released in the seawater. Since its discovery in talitrids (Papi and Pardi 1953), lunar orientation has often been a controversial subject (see Wallraff 1981); indeed, it has been accepted only with scepticism. There are at least four reasons for this: 1) the difficulty – at least in theory – to use the moon as an orienting factor on account of the significant variations of the moon during the lunar month (e.g. the moon is not always present, it never rises at the same time, it never has the same shape); 2) the fact that the use of the moon as an orientation cue is certainly not widespread in the animal kingdom: it was suspected for the first time by Santschi (1923) but has subsequently been demonstrated in very few animals (see Wallraff 1981) and with in-depth investigations only in sandhoppers (Papi and Pardi 1953, 1959, 1963; Papi 1960; Enright 1961, 1972); 3) the results of poorly conducted experiments (Craig 1971); 4) the fact that some authors – rather unusually – remain unconvinced even by their own results on the grounds that "the moon compass hypothesis" is simply "esthetically unattractive" (Enright 1972).

Therefore, we considered it of interest to resume the study of lunar orientation in sandhoppers by assessing its existence, in a possibly definitive manner, even after the horizontal component of the natural magnetic field has been cancelled: the earth's magnetic field (an ever-present non-chronometric reference) could also be used as an orienting factor (Arendse 1978; Ugolini and Pardi 1992) at least under certain conditions, and the possible ability of young inexpert sandhoppers (i.e. those born in the laboratory) to use the moon as a chronometric orienting factor.

Materials and methods

We utilised adult individuals of *Talitrus saltator* collected at Albegna (southern Tuscany, Italy), with a landward Y-axis direction = 88°. In the laboratory, the animals were kept under artificial light in containers with wet sand and dried fish food. The duration and phase of the light–dark (LD) cycle corresponded to the natural photoperiod and the temperature was subject to natural variations. In the experiments conducted on the young (15–30 days old), we used only individuals born in the laboratory, and thus lacking experience in the wild, from mothers collected at least 1 month previously. The animals were prevented from seeing the sky until the moment of their release.

The tests were carried out on a hill about 20 km from Florence in spring-summer 1988 and 1997–1998.

For the experiments, we used an apparatus similar to the one employed by Pardi and Papi (1952) but slightly modified (Ugolini and Macchi 1988). The sandhoppers were released in a transparent

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Plexiglas bowl (diameter 15 cm) with 1–2 cm of seawater. The bowl was placed horizontally on a transparent plate so that the sandhoppers could be observed from below. The bowl and plate were mounted on a tripod and surrounded by a white Plexiglas screen that blocked vision of the surrounding landscape. The screen was about 1–3 cm higher than the water level.

Groups of about ten individuals were released in the seawater inside the bowl. Release in seawater was preferred over dry release (i.e. in an empty bowl), adopted in all the previous experiments, because the necessary partial dehydration of the individuals is often difficult to achieve and maintain in nocturnal experiments, with a consequent decrease or absence of the motivation to assume the ecologically efficient direction (sea–land axis of the home beach). The release in water thus allowed us to test the individuals at ambient temperature without having to resort to heating and reducing the relative humidity of the air in the bowl (Papi and Pardi 1953).

A single direction for each individual was determined about 2 min after the introduction of the animals to the bowl. The directions were measured from freeze-frame images recorded with a video camera placed under the bowl. Illumination was provided by an electric torch with an infrared filter (830 nm) placed about 2 m from the bowl. In this manner, we avoided any possible interference from zenithal illumination and/or from photographic flashes. Indeed, the use of flash, adopted by Papi and Pardi (1953, 1959, 1963), has been subject to criticism (Enright 1972) in view of its possible influence on the behaviour of the sandhoppers in the bowl.

All the releases were performed under a full moon: moon phase from 75% to 99%, azimuth from 95° to 232°, height from 16° to 47°.

Analysis of the data was carried out with the methods of circular and linear statistics (Batschelet 1981; Zar 1984). For each distribution, we calculated the length of the mean resultant vector and the mean angle. The *V*-test was applied to assess whether the distribution differed from uniformity ($P < 0.05$ at least).

Moon orientation in adults

Adults were collected under the new moon and tested under the subsequent full moon, with the natural magnetic field present or cancelled, at various hours of the night.

In this case, the device was equipped with a pair of Helmholtz coils (diameter 64 cm, distance 35 cm); the axis passing through the centres of the two coils was horizontal to, and ideally lying in, the plane of the experimental individuals. This allowed us to cancel the horizontal component of the natural magnetic field (Ugolini and Pardi 1992).

In order to be sufficiently certain that the results could be attributed to the use of the moon as an orienting factor and not to possible reference points offered by the experimental apparatus, we also performed releases of sandhoppers under an artificial moon (cf. Papi and Pardi 1953), with the true moon screened. The false moon, consisting of an electric torch provided with a diffuser to reduce its brightness, was placed 2–3 m from the bowl and was projected onto the individuals from a fixed azimuth (= 328°) independently of the hour of the night. The light intensity of the false moon was 0.8 μW cm^{-2}. These tests were carried out in the absence of the horizontal component of the earth's magnetic field.

Some releases were also performed during the moon's exit from the shadow of the lunar eclipse on 16 September 1997. This allowed us to test for possible influences of the shape of the moon on sandhopper orientation. In fact, the lunar shape has been considered a possible source of information for sandhoppers about the phase of the moon (see Hoffmann 1964).

Moon orientation in inexpert young

Young inexpert (laboratory-born) animals were released with and without the natural magnetic field at various hours of the night.

To test whether the inexperienced young really use the moon as an orienting cue, we carried out the classical mirror experiment of Santschi (1911): the image of the moon reflected in a mirror was projected onto the individuals from a predetermined azimuth, the true moon being screened.

We also carried out tests with inexpert young during the lunar eclipse of 16 September 1997.

Results

Figure 1A shows that when the moon is covered by a wooden screen the individuals are randomly distributed.

With full vision of the lunar disc, not only are the adult sandhoppers able to compensate for the moon's azimuthal variation even after being prevented from

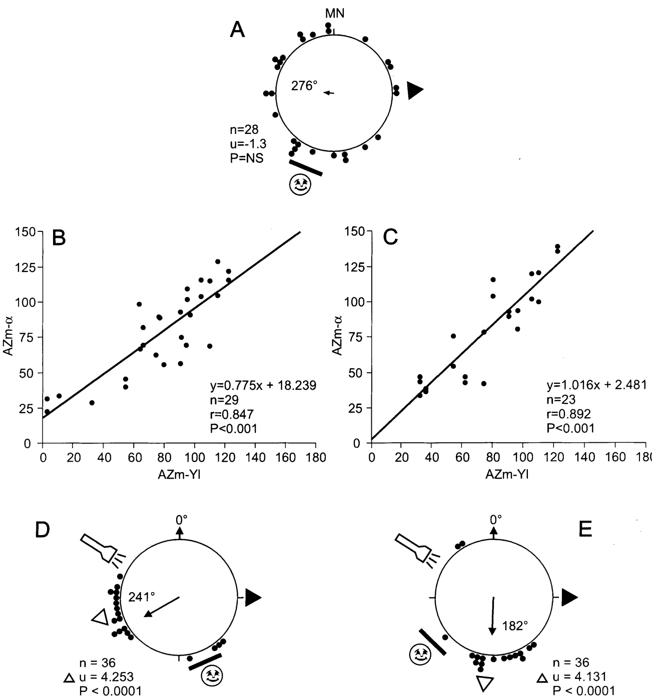

Fig. 1A–E Adult releases. **A** Releases made under the screened moon. **B**, **C** Relation between the absolute values of the lunar azimuth – expected direction (AZm–Yl) and lunar azimuth – mean angle (AZm–α) differences. **D**, **E** Individuals tested under the artificial moon (light of an electric torch) projected from 328° at two different hours of the same night in the absence of the magnetic field (North = 0°). In this test, the true moon was covered with a screen. In **B** and **C** each dot corresponds to a release of ten individuals. In the case of perfect positive phototaxis (non-chronometric), the AZm-α difference is constant and = 0 (hence coincident with the abscissa). **B** Controls tested under the moon and with the natural magnetic field. **C** Experimentals tested under the moon without the horizontal component of the magnetic field. Each figure shows the regression line with its equation, the sample size *n*, the correlation coefficient *r*, and the probability level *P*. In **B** the mean vector length is between 0.303 and 0.966; in **C** between 0.419 and 0.966. In **A**, **D** and **E** the symbol of the moon corresponds to the lunar azimuth at the moment of the release. *MN* magnetic North, the *arrow* inside each distribution represents the mean vector (length varies between 0 and 1 = radius of the circle); *dots*, sandhopper directions (each dot represents one individual). The *black triangle* outside the distributions represents the direction of the home beach, the *white triangle* represents the new expected direction. Sample size, *n*, *V*-test value *u*, with the probability level *P*, are also given seeing the sky (nocturnal and diurnal) for 9–17 days before the test (Fig. 1B), but such ability does not rely on reference to the earth's magnetic field (Fig. 1C): the comparison between correlation coefficients does not reach significance ($\chi^2 = 1.392$, $df = 1$, $P =$ NS, Chi-square test), nor does the comparison of the regression lines, for slope ($t = 1.631$, $df = 48$, $P =$ NS, Student's t) or elevation ($t = 0.603$, $df = 49$, $P =$ NS, Student's t).

The distributions in Fig. 1D, E demonstrate that even in the absence of the magnetic cue the orientation of the sandhoppers under the light of the electric torch continues to be largely along the expected directions independently of the hour of night (in this case, since the azimuth of the orienting factor is constant, the individuals change the angle with respect to the orienting cue).

The tests with the young inexpert sandhoppers (Fig. 2) show that they are able to assume the correct landward direction with any lunar azimuth despite never having been exposed to a natural sky (nocturnal or diurnal) and independently of the presence of the earth's magnetic field. The comparison between correlation coefficients does not reach significance ($\chi^2 = 0.806$, $df = 1$, $P =$ NS, Chi-square test). The regression lines do not differ significantly either in slope ($t = 0.020$, $df = 42$, $P =$ NS, Student's t) or in elevation ($t = 0.284$, $df = 43$, $P =$ NS, Student's t) (Fig. 2A, B).

The mirror experiment (Fig. 2C) demonstrates that in these tests the moon is the orienting cue used by the young inexpert sandhoppers: the young exposed to the reflected image of the moon deviate to an angle corresponding in amplitude and direction to the expected one (expected = 316°, observed = 341°).

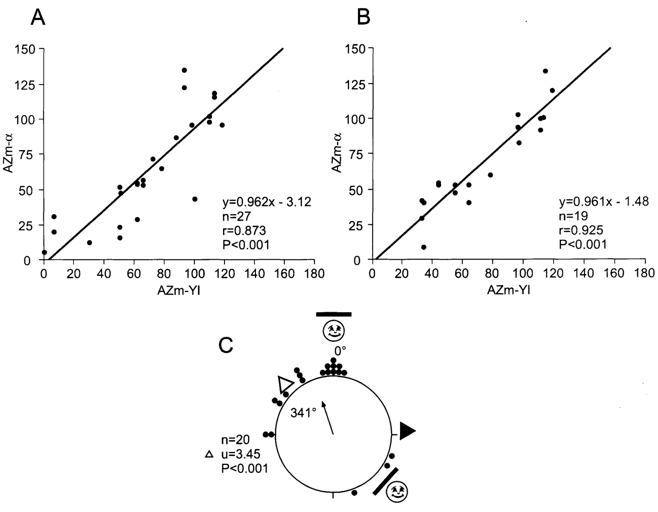

Fig. 2A–C Releases with inexpert young. **A**, **B** Relation between the absolute values of the lunar azimuth – expected direction (AZm–Yl) and lunar azimuth – mean angle (AZm–α) differences. **A** Controls tested under the natural moon with the magnetic field. **B** Experimentals tested under the natural moon without the horizontal component of the magnetic field. In **A** the mean vector length is between 0.361 and 0.931; in **B** between 0.258 and 0.732. **C** Releases were carried out without the horizontal component of the magnetic field and the lunar azimuth was deviated by 132° with a mirror while the true moon was covered with a screen. See Fig. 1 for further details

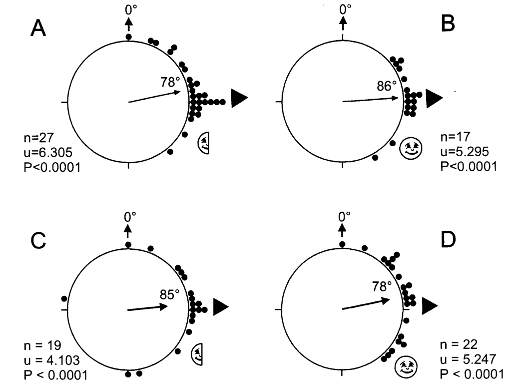

Fig. 3A–D Eclipse releases. **A**, **B** Adult releases; **C**, **D** inexpert young releases. **A**, **C** Half moon visible; **B**, **D** releases performed just after the end of the eclipse. For further explanation see Fig. 1

In the releases during the eclipse, both the adult and young individuals tested in the absence of the magnetic field during the moon's exit from the shadow of the eclipse, with the illuminated portion of the moon corresponding to a half moon in the decrescent phase (Fig. 3A, C), exhibited no relevant variation in their orientation with respect to the test carried out at the end of the eclipse (Fig. 3B, D) (adults, $U^2_{27,17} = 0.025$, $P =$ NS; young, $U^2_{19,22} = 0.074$, $P =$ NS, Watson U^2 two-sample test).

Discussion

First of all, we wish to emphasise that our results for the adult sandhoppers fully confirm and reinforce the results of Papi and Pardi (1953, 1959, 1963) on the existence of lunar orientation in *T. saltator*. This is in spite of the new techniques of observation and the different conditions of release (in water rather than onto a dry surface) with respect to the procedures adopted previously (Papi and Pardi 1953, 1959, 1963; Papi 1960). In fact, *T. saltator* exhibits the ability to use the moon as a chronometrically compensated orienting factor.

The influence of possible dishomogeneities of the experimental apparatus on the sandhopper orientation is shown to be non-existent both by the experiment with the electric torch with fixed azimuth, in which the mean direction of the individuals varied in the expected manner with time, and by the experiment with the mirror, in which the animals deviated by a number of degrees that largely corresponded to that expected. Nevertheless, the presence of a possible non-chronometric compass-orienting factor, such as the earth's magnetic field, might explain the constancy of mean direction exhibited by the animals, independently of the azimuthal variations of the moon. The natural magnetic field is a widely used factor of animal orientation (Wiltschko and Wiltschko 1995), which though had never been considered in experiments on lunar orientation. Our experiments allow us to say that the chronometrically compensated lunar orientation of *T. saltator* does not depend on the earth's magnetic field.

It is also appropriate to emphasise that our results were obtained with animals captured during the new moon and held in the laboratory under artificial illumination for about 13–15 days. Hence, it is difficult to hypothesise that continuous vision of the moon is necessary for the functioning of the lunar compass. Moreover, the sandhoppers maintained the correct orientation independently of the shape of the lunar disc; thus, the form of the moon does not provide information about the time of the synodic cycle. This fully confirms the findings of Papi and Pardi (1953) when they projected a quarter moon onto the sandhoppers with a mirror. Moreover, we can also hypothesise that the 250–300 ommatidia of the *T. saltator* eye are not sufficient for discrimination between the first and last quarters of the lunar cycle.

Obviously our investigations do not, for the moment, aim to resolve the difficulty of utilisation of the moon as an orienting factor from the point of view of chronometric compensation. However, the good orientation obtained with the inexpert young sandhoppers (with the natural magnetic field both present and cancelled) and the deflection obtained with the mirror experiment demonstrate that the young born in the laboratory are able to use the moon as an orientation cue. Therefore, despite the well-known theoretical difficulties of utilisation of the moon as a compass-orienting factor, we can hypothesise the presence of an innate component of the sandhopper's chronometric use of the moon, similar to what has been found for solar orientation (Pardi et al. 1958; Pardi 1960).

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