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Orientation at night: an innate moon compass in sandhoppers (Amphipoda: Talitridae)

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The supralittoral amphipod *Talitrus saltator* is well known for its capacity for astronomical orientation using the sun and moon as compasses. It has also been demonstrated that the sun compass is innate in this species. In our experiments, we released inexpert (naive) young born in the laboratory into a confined environment under the full moon and in the absence of the horizontal component of the magnetic field. They were allowed to see the natural sky and the moon only at the moment of release. The young individuals were obtained in the laboratory by crossing adult individuals from the same and different populations of sandhoppers. The young from intrapopulation crosses were well oriented towards the directions corresponding to those of their parents, whereas the young from interpopulation crosses were oriented in an intermediate direction. Therefore, our experiments demonstrate in the sandhopper *T. saltator* that sea–land moon orientation relies on an innate chronometrically compensated mechanism.

Keywords: sandhoppers; *Talitrus saltator*; orientation; innate moon compass

1. INTRODUCTION

The moon is not easy to use as a chronometrically compensated compass cue: during the lunar month, the moon is not always present, it never rises at the same time and does not have the same shape. Sandhoppers are the only invertebrates in which it has been definitely demonstrated that a chronometrically compensated moon compass coexists with a sun compass (Ugolini *et al.* 1999*a*,*b*). Moreover, in *Talitrus saltator* and *Talorchestia deshayesii* the capacity to use the sun to recover the damp belt of sand near the sea after displacements along the sea–land axis is innate (see Campan (1997) for a review). It is well known that sea–land axis orientation in sandhoppers is mainly based on astronomical compass cues (the sun and moon). They assume the seaward direction when dehydrated for a few minutes and the landward direction when released in sea water. Pardi and collaborators (Pardi *et al.* 1958; Pardi 1960; Pardi & Scapini 1983; Scapini *et al.* 1985) demonstrated that inexpert (naive) young (born in the laboratory) exposed to the sun and sky for the first time at the moment of release were able to assume the direction of the sea–land axis of the home beach of their parents. When tested under the sun, the young from crosses between individuals from differently oriented beaches show a direction intermediate between those of their parents' home beaches (Pardi & Scapini 1983).

Recently, it was shown that young sandhoppers are able to use the moon as an orienting cue independently of its shape (Ugolini *et al.* 1999*a*). Therefore, in *T. saltator*, sun compass orientation, its innate nature and the moon compass have been thoroughly investigated and demonstrated beyond any doubt (Papi & Pardi 1953, 1959, 1963; Pardi & Papi 1953; Papi 1960; Pardi 1960; Pardi & Scapini 1983; Scapini *et al.* 1985; Ugolini & Scapini 1988; Ugolini *et al.* 1999*a*,*b*).

The aim of the present research is to investigate the innate nature of the moon compass in *T. saltator.*

2. METHODS

Adults of *T. saltator* were collected on two beaches in Tuscany, Italy, with their sea–land axes 88° apart (Albegna, sea–land axis, 268°–88°; Feniglia, sea–land axis, 180°–0°). Adults were kept in the laboratory under an artificial light : dark cycle corresponding in phase and duration to the natural one. After one month in the laboratory, non-ovigerous females of both populations were allowed to mate with males of either the same or the other population. Young sandhoppers were kept in the laboratory in the same conditions as the adults. We tested only inexpert young sandhoppers (born in the laboratory and thus lacking experience in the wild), exposed for the first time to the natural sky at the moment of release, 15–30 days after hatching.

For the experiments, we used an apparatus similar to the one employed by Pardi & Papi (1953) but slightly modified (Ugolini & Macchi 1988; Ugolini 2001). The sandhoppers were released in a transparent Plexiglas bowl (18 cm diameter) with 1 cm depth of seawater. The bowl was placed horizontally on a transparent plate. 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 1–2 cm higher than the water level.

Groups of five or six individuals were released at different lunar azimuths. A single direction for each individual was determined 1–2 min after the introduction of the animals to the bowl. The directions were measured from freeze-frame images recorded by an infrared-sensitive videocamera placed below the bowl. Illumination was provided by an electric torch with an infrared filter (830 nm) placed *ca.* 2 m from the bowl. In this manner, any possible interference from zenithal illumination and/or photographic flashes was avoided.

To test the real use of the moon as an orientation cue by the inexperienced young, we did the classical mirror experiment of Santschi (1911): the image of the moon reflected in a mirror was projected onto the individuals from a pre-determined azimuth, the true moon being screened.

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All the releases were performed under a full moon (moon's phase from 86% to 99%). Even though the natural magnetic field does not seem to be used during moon orientation (Ugolini *et al.* 1999*a*,*b*), all the releases were done in the absence of the horizontal component of the natural magnetic field. For this purpose, the device was equipped with a pair of Helmholtz coils (64 cm diameter and 35 cm distance), the axis passing through the centres of the two coils that were 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. Tests were done in spring–summer 1998 and 1999, 30–40 km from Florence to minimize the effect of light pollution on the nocturnal sky.

The data were analysed by the methods of circular statistics (Batschelet 1981). For each distribution, we calculated the length of the mean resultant vector and the mean angle. The Rao test was applied to assess whether the distribution differed from uniformity ($p < 0.05$ at least). We determined the uni- or bimodality of the distributions by computer, by multiplying the data by an index that varied from one (unimodal) to four. If the length of the mean resultant vector increased when data were multiplied by an index of more than one, the distribution was considered non-unimodal and automatically open in the points of the greatest separation between the sandhoppers' directions. The mean angles of the groups of direction thus formed were calculated separately (see also Ugolini 2001).

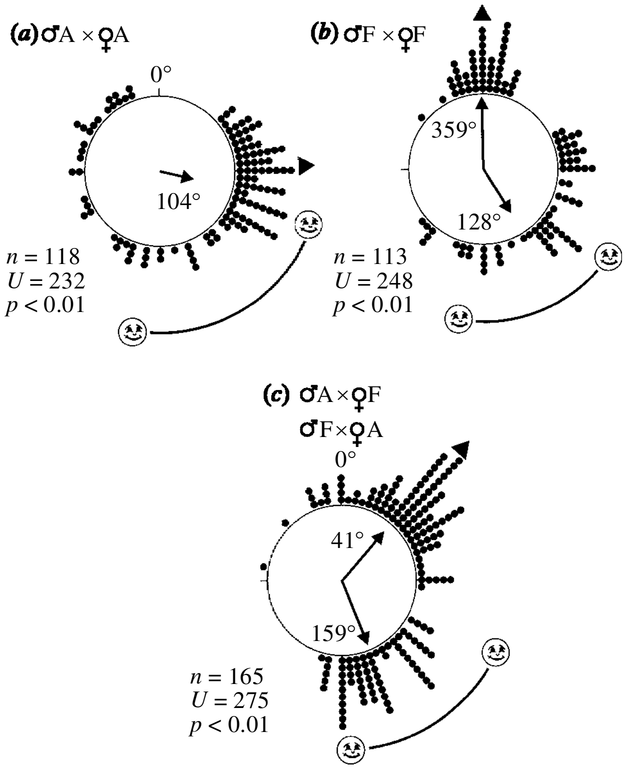

Figure 1. Lunar orientation in inexpert young sandhoppers. (*a*) The male Albegna × female Albegna (♂A × ♀A), and (*b*) male Feniglia × female Feniglia (♂F × ♀F), distributions represent the orientation of the young from intrapopulation crosses. (*c*) The male Albegna × female Feniglia and male Feniglia × female Albegna (♂A × ♀F and ♂F × ♀A) distributions represent the orientation of the young from interpopulation crosses. The arrows inside each distribution represent the mean vector (length varies between 0 and 1, radius of the circle); dots, individual directions; 0°, geographical North; black triangle, expected (landward) direction. The variation of the lunar azimuth is represented outside each distribution. *n*, sample size; *U*, Rao test value; *p*, probability level are also given.

3. RESULTS AND DISCUSSION

Figure 1 shows that the young sandhoppers from the intrapopulation crosses (male Albegna × female Albegna and male Feniglia × female Feniglia) were quite well directed towards the respective sea–land directions of their parents' home beaches. By contrast, the mean directions of a great number of young from the interpopulation crosses (59%; $n = 165$; figure 1) were intermediate between the sea–land directions of their parents' home beaches. In some distributions, there was a clear bimodal tendency due to photopositive behaviour, quite common in young and adults in moon or sun compass experiments, and/or to the difficulty in leaving the orientation cue behind.

When the moon was reflected with a mirror from a different azimuth (figure 2*b*), the directions assumed by the sandhoppers agreed well with the expected direction based on the reflected moon: the mean direction (175°; angular deviation, 36°) was deviated by 122° in the expected sense with respect to that of individuals tested under the natural moon (figure 2*a*; mean angle, 53°; angular deviation, 50°). The difference between the theoretically expected deflection and the deflection exhibited by the sandhoppers was only 13°.

Therefore, our results fully confirm that the direction-finding ability based on the moon is independent of the presence of the natural magnetic field and lunar azimuth variation (see also Ugolini *et al.* 1999*a*,*b*). They also clearly show that, in *T. saltator*, the capacity to use the moon as a chronometrically compensated compass cue for sea–land axis orientation is innate. It should be emphasized that *T. saltator* is currently, to our knowledge, the only animal species in which an innate setting of the moon compass has been demonstrated.

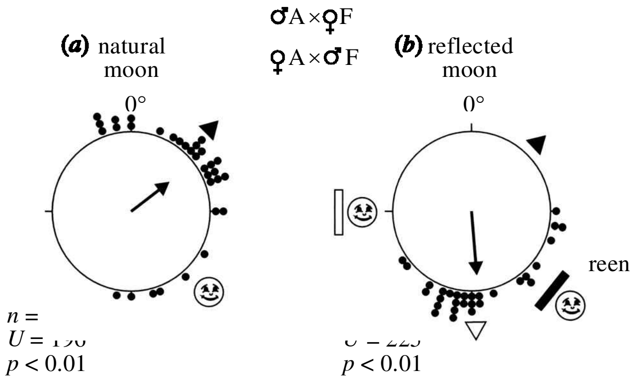

Figure 2. Lunar orientation in inexpert young sandhoppers. Mirror experiment with young from interpopulation crosses. The test was done under (*a*) the natural moon or (*b*) under a reflected moon: the lunar azimuth was deviated by 135° with a mirror, the true moon being covered with a screen. White triangle, new expected direction based on the reflected moon. See figure 1 for further details.

Indeed, *T. saltator* possesses two innate compass systems for astronomical orientation (the moon and the sun compass) and it has recently been demonstrated that their chronometric components are separate (Ugolini *et al.* 1999*b*). Because the innate ecologically efficient direction indicated by the two compasses is the same, differences between the two orienting systems are due to the chronometric mechanisms: one for compensation for the apparent motion of the sun (Papi & Pardi 1953; Pardi & Papi 1953), the other for lunar compensation (Papi & Pardi 1953; Papi 1960). This finding is also supported by the circalunidian hypothesis (Palmer 1974) which is based on the existence of a circalunidian clock working independently of the circadian clock (Palmer 1995, 2000), the latter strictly related to sun compensation (Papi 1955; Ugolini & Frittelli 1998). Regarding the zeitgeber of the lunar clock in young sandhoppers, we can at the moment only hypothesize that vision of the full moon could be an instant zeitgeber and/or that the light : dark rhythm entrains both chronometric mechanisms which will work independently in the adult.

Finally, we do not wish to discuss in detail the hypotheses about the genetic determination of the theoretical direction the young from crosses should assume. However, because the mean direction obtained with young from the interpopulation crosses tested under the moon is intermediate between the parents' expected directions and this result corresponds to that obtained in similar experiments done under the sun (Pardi & Scapini 1983), it can be hypothesized that there is oligogenic transmission based on two genes with two alleles each or one gene with four alleles, as proposed by Pardi (Pardi & Scapini 1983; see also Scapini *et al.* (1988) for a review).

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