

Moon and sun compasses in sandhoppers rely on two separate chronometric mechanisms

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The relationship between the chronometric system of compensation for the apparent movement of the sun and that for the moon has been the subject of several, never proven, hypotheses. Our studies on sandhoppers have demonstrated that the chronometric mechanism of the moon compass is separate from that of the sun compass. They show (i) that a period of seven days in constant darkness has no influence on the capacity for orientation, either solar or lunar, and indicates the presence of one or more continuously operating timing mechanisms; (ii) that two different shifts in the light–dark phase have no effect on the chronometric mechanism of lunar orientation, but they do affect that of solar orientation; and (iii) that exposure to an artificial moon delayed by seven days with respect to the natural cycle causes the expected change in the mean direction of individuals tested under the natural moon, but not of those tested under the sun.

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

1. INTRODUCTION

Recently, the presence of a moon compass in sandhoppers was unequivocally confirmed (Ugolini *et al.* 1999). In addition, that study revealed both the innate component of the mechanism and its independence of non-chronometric orientating factors. However, since its discovery in talitrids (Papi & Pardi 1953), lunar orientation has been the subject of heated debate (Enright 1961, 1972; see also Wallraff (1981) for a review). This is mainly due to the fact that the chronometric part of the lunar compass must deal with significant variations of the moon during the lunar month (e.g. the moon is not always present; it never rises at the same time). Despite numerous studies carried out in the past, the nature of this mechanism and its relations with the chronometric component of the sun compass have remained the subject of various controversial hypotheses (see Wallraff 1981), which to our knowledge have never been submitted to experimentation.

Therefore we have attempted to assess the relationship between the mechanism of compensation for lunar azimuthal variation and the mechanism regulating solar orientation.

2. MATERIALS AND METHODS

We used adult individuals of *Talitrus saltator* collected at Albegna (southern Tuscany, Italy, landward direction = 88°) during the new moon. The tests were carried out near Florence in the Spring–Summer of 1997 and 1998 during the next full moon after capture. In the laboratory, the animals were kept

under artificial light in containers with wet sand and food. The duration and phase of the light–dark (L:D) cycle corresponded to the natural photoperiod. The temperature was subject to natural variations.

The methods and experimental apparatus are identical to those described recently in detail elsewhere (Ugolini *et al.* 1999). In summary, the sandhoppers were released into a transparent Plexiglas bowl (15 cm in diameter) 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 cylindrical screen 1–3 cm higher than the water level, which blocked vision of the surrounding landscape (Pardi & Papi 1953; Ugolini & Macchi 1988).

Since it has been demonstrated that sandhoppers' orientation is not influenced by group releases (Scapini *et al.* 1981), groups of about ten individuals were released into the seawater inside the bowl. Release of the sandhoppers in water overcomes the problem of the animals' motivation to orientate themselves, which is often difficult to achieve in nocturnal experiments on a dry surface. Indeed, it provoked within a few seconds an orientation in the landward direction of their home beach. A single direction for each individual was determined *ca.* 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 *ca.* 2 m from the bowl. To cancel the horizontal component of the natural magnetic field (Ugolini & Pardi 1992), we equipped the device with a pair of Helmholtz coils (64 cm in diameter, 35 cm apart). All the releases were performed under a full moon (moon's phase from 85% to 100%).

The following experiments were carried out.

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(a) Releases of sandhoppers kept in constant darkness

Sandhoppers were collected under the new moon and kept for 7 d in constant darkness at a temperature of $25 \pm 2^\circ\text{C}$. The aim was to test the effect of a prolonged period of captivity in constant conditions (presumably, therefore, with the endogenous clock free-running) on the chronometric compensation for azimuthal variations of the moon and sun.

(b) Clock-shifting experiments

(i) Adults were collected under the new moon and subjected for 6–9 d to an L:D cycle of artificial illumination anticipated with respect to the natural one so as to shift the expected direction (i.e. the one expected for use of the sun compass) by 90° counterclockwise (CCW) (new expected direction, 358°).

(ii) Adults were collected under the new moon and subjected for 14 d to a 20 L:4 D cycle or a 4 L:20 D cycle in which the artificial noon and midnight corresponded with the natural ones. As recently demonstrated (Ugolini & Frittelli 1998), a shortening of the light period corresponds to an acceleration of the chronometric mechanism of compensation for the sun.

During experiments (i) and (ii), the individuals were kept in thermostatic cells at constant temperature ($25 \pm 2^\circ\text{C}$).

(iii) Adults were collected under the full moon and kept for 28 d under a false moon, phase-shifted with respect to the natural cycle. During this period, sandhoppers were subjected to an artificial L:D cycle corresponding in phase and duration to the natural one. A dim artificial light ($0.1 \mu\text{W cm}^{-2}$ at the plane of the rearing container, source diameter 4 mm), placed 40 cm from the individuals, simulated the moon with a rise–set period delayed 7 d with respect to the natural one (new expected direction, 355°). Controls were subjected to identical conditions with a false moon in phase with the natural one. The sandhoppers were kept in the usual rearing room, subject to natural temperature fluctuations. Some releases were also carried out under the sun.

Analysis of the data was carried out using methods of circular statistics (Batschelet 1981; Zar 1984). For each distribution, we calculated the length of the mean resultant vector and the mean angle. In the case of bimodal distributions, the method of doubling of the angles was applied, the limits of this procedure being taken into account. The V -test or the Rao test were applied to assess whether the distribution differed from uniformity ($p < 0.05$ at least).

3. RESULTS**(a) Releases of sandhoppers kept in constant darkness**

Sandhoppers kept for seven days in constant darkness (figure 1*b,d*) did not show any relevant difference in orientation with respect to controls kept on a normal L:D cycle, neither in the tests under the sun (figure 1*a,b*) nor in those under the moon (figure 1*c,d*) (Watson U^2 two-sample test, not significant in any case). However, in the experimentals there was a slight tendency to a greater dispersion.

(b) Clock-shifting experiments

The individuals with the clock shifted by 90° CCW (figure 2*a*) and with a 4 L:20 D cycle (figure 2*c*) exhibited a good clustering around their new expected directions when tested under the sun. When tested under the moon,

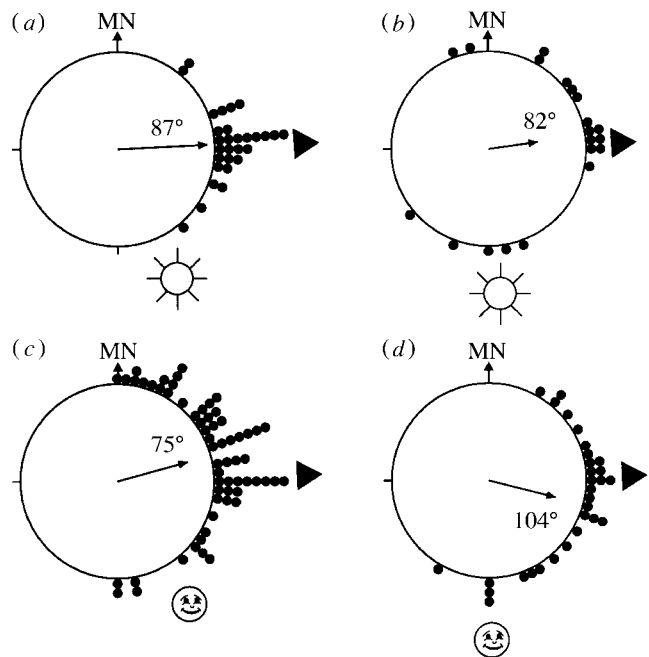


Figure 1. Adult sandhoppers tested under the sun (*a,b*) and under the moon (*c,d*); (*a*) and (*c*) show controls kept with an artificial L:D cycle corresponding to the natural one; (*b*) and (*d*) show experimentals kept in constant darkness for seven days. MN, magnetic north. The arrow inside each distribution represents the mean vector (length varies between 0 and 1 = radius of the circle); dots, sandhopper directions (each dot represents one individual); black triangle, expected landward direction. The symbols of the sun–moon azimuths. (*a*) $n = 29$, $u = 7.172$, $p < 0.0001$; (*b*) $n = 20$, $u = 3.257$, $p < 0.001$; (*c*) $n = 61$, $u = 8.207$, $p = 0.0001$; (*d*) $n = 30$, $u = 5.393$, $p < 0.0001$.

both the 90° CCW individuals (figure 2*b*) and the 20 L:4 D individuals (figure 2*d*) were well concentrated around the expected direction of their home beach.

The same is true for the individuals subjected to the shifting of the chronometric mechanism for the moon compass and then tested under the sun (figure 3*b*). They were concentrated in the landward direction of their home beach independently of the phase shifting, and their orientation was not statistically different from that of the controls ($U_{43,44}^2$, n.s.) (figure 3*a*).

It should be noted, however, that the experimentals tested under the moon (figure 3*d*) were well clustered around their new (93° apart) expected directions. The bimodality of the distribution is probably due to a photo-positive component. The controls tested under the moon (figure 3*c*) were clustered around the landward direction of their home beach.

4. DISCUSSION

That the lunar orientation of sandhoppers neither depends on an inhomogeneity of the experimental apparatus nor on non-chronometric orientating factors, such as the Earth's magnetic field, has been demonstrated elsewhere (Ugolini *et al.* 1999). Furthermore, young, inexperienced laboratory-born sandhoppers also possess the moon compass (Ugolini *et al.* 1999). In addition, it has been confirmed that adult sandhoppers are well able to use the

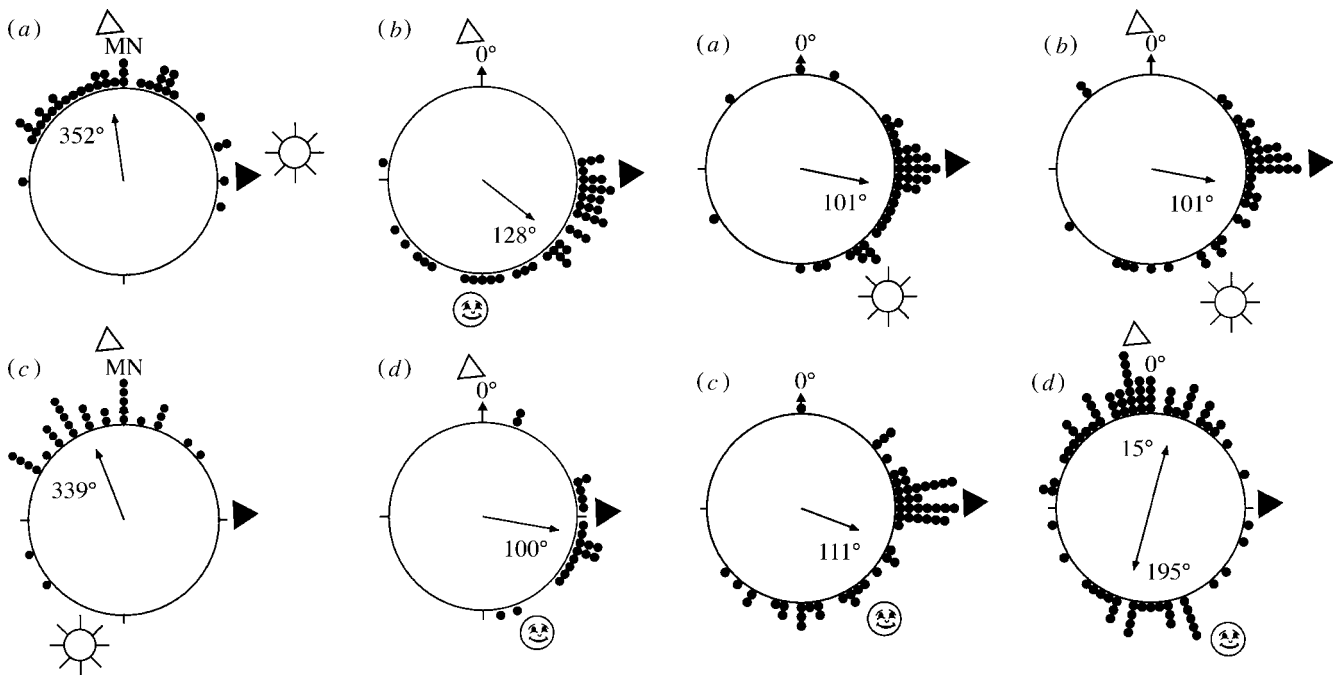


Figure 2. Clock-shifting of the sun compass. (a,b) 90° CCW tested under the sun and the moon, respectively; (c) 4L:20D tested under the sun; (d) 20L:4D, tested under the moon. The symbols of the sun and moon in (c) and (d) indicate the azimuth at the time of the test. Tests (b) and (d) were performed without the natural magnetic field (north = 0°). Black triangle, landward direction; white triangle, new expected direction. For further details, see figure 1. (a) $n = 37$, $u = 6.27$, $p < 0.0001$ (white triangle); (b) $n = 44$, $u = 5.05$, $p < 0.0001$ (black triangle); (c) $n = 31$, $u = 2.112$, $p < 0.0001$ (white triangle); (d) $n = 21$, $u = 5.235$, $p < 0.0001$ (black triangle).

moon as a chronometric orientating factor even after a period (seven days in our experiments) in the dark (figure 1) (see Enright 1972; Papi & Pardi 1959). Therefore, the hypothesis of Papi & Pardi (1959, 1963) of a continuously operating endogenous lunar periodicity can be considered confirmed.

The test performed with sandhoppers subjected to a clock-shift of the sun compass (figure 2) shows that the clock-shifting has no effect on the capacity to compensate for the azimuthal variations of the moon. Moreover, if the chronometric mechanism were the same for the sun and the moon, the sandhoppers with the 20L:4D cycle tested under the moon would have exhibited a more rapid compensation, i.e. by 30°h^{-1} rather than the 15°h^{-1} or so required by the astronomical conditions, as recently demonstrated for the solar orientation mechanism (Ugolini & Frittelli 1998) and confirmed by tests of 4L:20D individuals under the sun. Finally, the tests performed with sandhoppers subjected for 28 days to a false moon, rising and setting like the real one but delayed by seven days, show that this treatment affects only the lunar timing mechanism; the solar orientation remains correct.

Therefore, our results refute both the hourglass hypothesis (Hoffmann 1965) and the only-one-timing-mechanism hypothesis (Enright 1961). Although the ability of only one clock to drive different works has recently been ascertained (see Sassone-Corsi 1996), our

Figure 3. Clock-shifting of the moon compass. (a,b) Controls and experimentals tested under the sun; (c,d) controls and experimentals tested under the moon. The symbols of the sun-moon correspond to the mean solar-lunar azimuths. U , Rao test value. For further details, see figures 1 and 2. Black triangles: (a) $n = 43$, $u = 6.741$, $p < 0.0001$; (b) $n = 44$, $u = 6.297$, $p = 0.0001$; (c) $n = 55$, $u = 6.447$, $p < 0.0001$; white triangle: (d) $n = 80$, $U = 203$, $p < 0.001$.

clock-shifting experiments strongly support the suggestion (Papi & Pardi 1959; Papi 1960) that the chronometric mechanism of the moon compass is separate from that of the sun compass. Thus the presence of a phase-frequency transforming-coupler mechanism (e.g. see Palmer 1974) is improbable.

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