The sun compass of the sandhopper *Talitrus saltator*: the speed of the chronometric mechanism depends on the hours of light

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Summary

Experiments on solar orientation were conducted with adult amphipods (*Talitrus saltator*) subjected to a reduction and/or phase shift of the hours of light (L) or dark (D) with respect to the natural photoperiod: 15 h:9 h L:D (controls), 15 h:9 h inverted (i.e. phase-shifted by 12 h and tested with the sun during the subjective night), 4 h:20 h, 20 h:4 h inverted. The sandhoppers were released in a confined environment, and individual orientation angles were recorded. The results confirm the continuous operation, through the entire 24-h period, of a chronometric mechanism of compensation for apparent solar motion. They show excellent agreement with a recently proposed model of compensation for the sun at constant (not differential) speed and they demonstrate a dependence of the speed of the chronometric mechanism on the L:D ratio in the 24-h period.

Key words: orientation, sun compass, chronometric mechanism, sandhopper, *Talitrus saltator*.

Introduction

The ability of *Talitrus saltator* to use chronometrically compensated astronomic references has been recognised for some time (Pardi and Papi, 1952). The sun and moon are the main compass orientation references, and the use of these sources of information allows the sandhoppers to return to their preferred zone (the band of wet sand) along the shortest path, i.e. the *y*-axis (sea–land) of the beach (Papi and Pardi, 1953; Pardi and Papi, 1953). This decreases the effect of numerous biological and physical stress factors characteristic of the coastal ecotone (a boundary line between two ecosystems; see Ugolini, 1996).

The sun compass mechanism in sandhoppers has been the subject of thorough investigations (Papi and Pardi, 1953; Pardi and Papi, 1953; Papi, 1955; Pardi and Grassi, 1955). These revealed the mechanism's chronometric basis, which results in compensation for the azimuthal variation of the sun. Experiments utilising photoperiod phase-shifting (Pardi and Grassi, 1955; Marchionni, 1958) and the 'longitudinal jump' (i.e. Italian sandhoppers tested in Argentina; Papi, 1955) showed that the ability to compensate for apparent solar motion is not due to local orientation factors. On the basis of experimental observations made on freshly collected individuals, Pardi and Papi (1953) hypothesized that this mechanism compensates precisely for the azimuthal variation of the sun (= differential compensation). However, it should be remembered that the azimuthal speed of the sun varies during the day and the year depending on its height above the horizon. These variations could affect the accuracy of sandhopper orientation (for example, see Ugolini, 2001).

It has been hypothesised (Ugolini and Frittelli, 1998) that compensation for apparent solar motion does not vary during the day but is based on the mean speed of the sun (determined on the basis of the sun's daily azimuthal variation and the number of hours of light).

We have therefore carried out experiments to test the two hypotheses of sun compensation in *T. saltator*. In particular, we tested whether the light:dark (L:D) ratio affects the speed of compensation of the sun compass chronometric mechanism.

Materials and methods

This study used adult *Talitrus saltator* (Montagu) collected 10–15 days prior to experimental manipulation at a locality in southern Tuscany near the mouth of the Albegna River. The direction of the *y*-axis (sea–land) of the beach is 268° (sea) – 88° (land). The individuals were transported to the laboratory and maintained in plastic containers with wet sand and food until the tests. Groups of animals were subjected to different artificial photoperiods, for 7–10 days as follows. (i) A photoperiod corresponding to the natural photoperiod in duration and phase (15 h:9 h L:D). (ii) A photoperiod corresponding to the natural photoperiod in duration, but phase-shifted by a number of hours sufficient to cause a day–night inversion. Thus, these individuals were tested under the sun during their subjective night (see Pardi and Grassi,

1955). (iii) A photoperiod with only 4 h of light and 20 h of dark (4 h:20 h L:D), maintaining the subjective noon coincident with local noon. Unfortunately, in this case, it was impossible to carry out experiments in the afternoon. (iv) A photoperiod with 4 h of dark and 20 h of light (20 h:4 h L:D inverted). These individuals were tested under the natural sun during the 4 h of their subjective night (subjective midnight corresponds to local noon).

The experiments were conducted from August to October in 1998 and 1999 in Florence in conditions of natural sun and sky. The tests were performed every 15–30 min throughout the animals' subjective day or night.

The sandhoppers were released into an apparatus described previously (Ugolini and Macchi, 1988; Ugolini, 2001) composed of a transparent Plexiglas bowl (diameter 18 cm) set on a transparent Plexiglas plate placed horizontally on a tripod. A cylindrical white Plexiglas screen (3 cm high) around the bowl prevented the sandhoppers from viewing the surrounding landscape but allowed them to see the sun and sky. Groups of approximately five individuals were released into the bowl containing approximately 1 cm of seawater. Each individual was tested only once, and a single direction per individual was recorded 2 min after release by means of a video camera under the bowl.

Statistical analyses of the circular distributions deriving from each release were performed using the procedure reported by Batschelet (1981). For each distribution, we calculated the mean resultant vector. Rao's test was applied to assess whether the distribution differed from uniformity ($P \leq 0.05$). The bimodality of each distribution was assessed by the possible increase in length of the mean vector using the method of doubling angles (Batschelet, 1981). In cases of bimodality, only the landward resultant was considered. Uniform distributions were excluded from further analysis. We chose the two-dimensional Cartesian axes form rather than the circular form to represent the results because we believed it would describe the results more effectively.

To test the time course of variation in compensation for apparent solar motion, we used least-squares polynomial regression, testing the successive powers of the independent variable (time) as separate predictor variables. The fit of functions to the data was quantified both by adjusted r^2 (i.e. the adjusted coefficient of determination, the percentage of the total variability explained by the particular function taking account of the fact that the parameters are estimated from the data) and by testing the highest term in the polynomial for significance by Student's t-test. From the different polynomials tested for the same values, the one with maximum r^2 and the lowest t probability was chosen.

To compare the fitting of the selected curves in Fig. 2C, we chose the following method. For each curve, we calculated the sum-of-squared differences between the mean angle and the corresponding value on the curve; we then divided this sum by the degrees of freedom to obtain a variance value quantifying the variability about the regression. To compare these variabilities with the variability about the polynomial regression, we calculated the variance ratio: the variance about the regression for a single curve divided by the variance about the regression for the polynomial regression; the result was compared with the F table for N–1 and N–3 degrees of freedom. The F-probability gives a measure of similarity between curves, the highest probability indicating the greatest similarity (see Armitage et al., 2002).

Tests of the slopes and intercepts and of whether the regression lines were parallel were carried out with the usual Student's t-test methods.

Theoretical models of the sandhoppers' sun compass chronometric mechanism

The theoretical variation in the angle of orientation that individuals should assume with respect to the sun to maintain a constant direction was calculated according to the following criteria. For individuals tested when their subjective day corresponds to the natural day, we considered the model proposed by Ugolini and Frittelli (1998), i.e. that the mechanism of compensation for the movement of the sun has a constant speed during the period of light (or dark) and that its speed is regulated by the duration of the photoperiod of the previous day (or a few days) and the azimuthal variation of the sun in that period of the year. Obviously, a constant speed during the same time period will cause theoretically predictable orientation 'errors' by the animals, as a result of the discrepancy between the speed of the internal chronometric mechanism and the azimuthal speed of the sun (which is not constant during the day or year). This can be represented by the following expression, given that the mean direction of orientation of the sandhoppers and the solar azimuth at the time of the release are parameters that derive from the experiment itself:

$$Ey_L(t) = Kt + AZs(t), \qquad (1)$$

in which $Ey_L(t)$ is the expected y-axis landward direction, expressed in degrees from north, that the sandhoppers must assume after t min from sunrise, $AZs(t)$ is the sun's azimuth at the time of the release. The angular speed of correction K is:

$$K = (Azs_S - Azs_R)/ML, \qquad (2)$$

where AZs_S and AZs_R are the azimuth of the sun at sunset and sunrise, respectively, and ML indicates the minutes of light from sunrise to sunset. Since the sun's azimuthal speed in the period of the releases is not constant during the day, Ey_L will assume a curvilinear form (not a horizontal line) because of the discrepancy between the speed of the compensation mechanism and the azimuthal speed of the sun.

To simplify interpretation of the experiments in which the tests occurred during the sandhoppers' subjective night (inverted photoperiod), it should be remembered that there are two models of compensation for apparent solar motion at night: (i) the 'Apis mellifera model' (Lindauer, 1954, 1957): at night, the sun passes from west through north to its position in the east in the morning (Fig. 1A); and (ii) the 'Talitrus model' proposed by Pardi (1954) and not since tested in amphipods,

although confirmed in other riparian or littoral arthropods (Birukow, 1957; Ercolini and Scapini, 1976; Pardi, 1958): compensation for the sun in the nocturnal period of solar orientation occurs as if the sun, once it has set in the west, retraces the path covered during the day, i.e. passing from west to south to east at sunrise (Fig. 1B). It should be emphasised that, during the subjective night, the sandhoppers were tested under the natural sun (which appears to move from east to west). Therefore, the expected direction of orientation will necessarily be different from that of the home beach (Fig. 1C).

Results

The results of tests with individuals subjected to a photoperiod corresponding to the natural photoperiod during the day (Fig. 2A) were analysed using the polynomial regression method described above (Table 1). Introduction of the cubic term into the regression equation produces a marked increase in adjusted r^2 and a significant decrease in the residual sum of squares, marked by the highly significant t value for this coefficient. This process, with the introduction of subsequent terms, could be continued, but because of our sample size (N=16), it is doubtful whether any useful purpose would be achieved. The effect of the fifth-degree term is only marginally significant, and there are five terms in introducing the polynomial regression and only 16 values. The final model chosen was the third-degree polynomial (Table 1; Fig. 2A).

Tests with individuals kept under an artificial L:D cycle corresponding to the natural photoperiod, during the subjective night

(Fig. 2B), gave the best fit to the regression line (adjusted r^2=0.335). The slope, which is positive and statistically significant (P=0.029), is significantly different from that of the
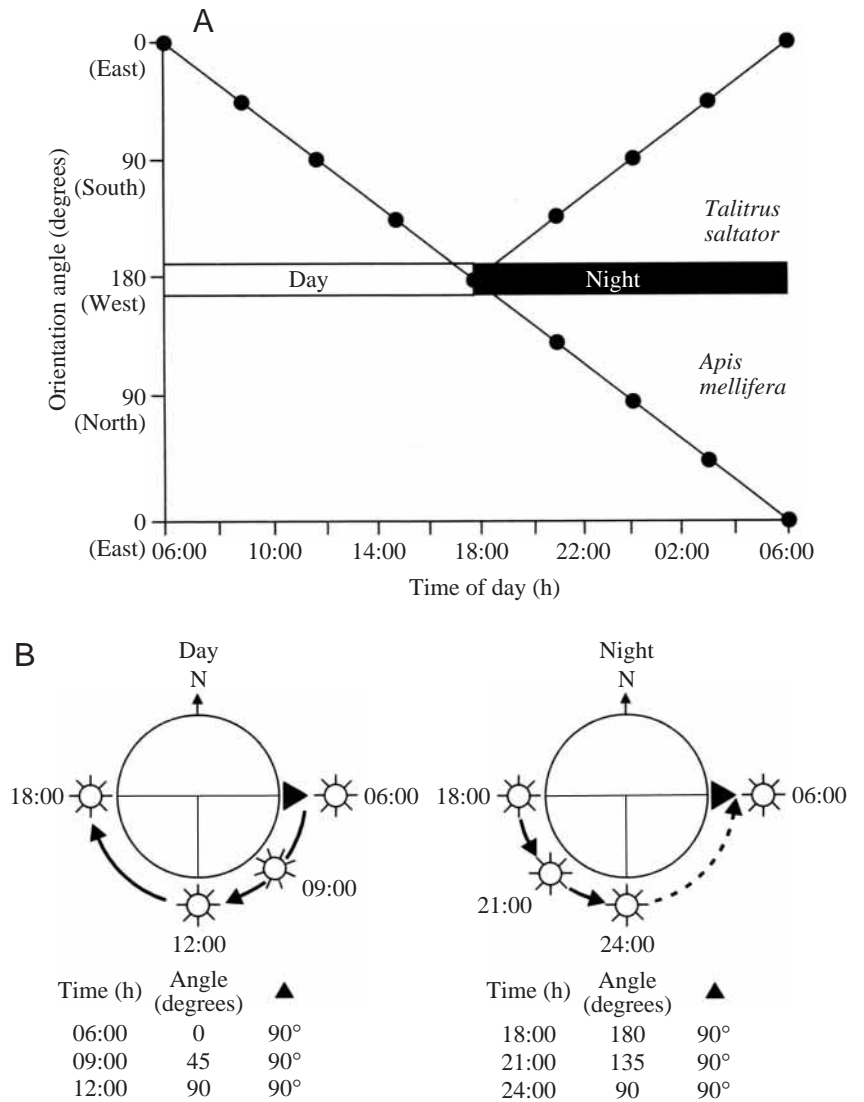

Fig. 1. Schematic representation of the angular variation in orientation angle for solar orientation in *Talitrus saltator* and *Apis mellifera* (A) (modified from Pardi and Ercolini, 1986). The theoretical direction of orientation is eastward. The orientation angle of *T. saltator* returns to 0° from 180°, passing through south at night. The same data are represented in the two distributions below (B), in which the path of the sun during the day (solid line) and at night (dashed line) is represented. Black triangle, expected direction of orientation. The theoretical angles between the sun and the expected direction of orientation are also reported for some hours of the day. (C) However, since the sandhoppers were released during the real day the sun's path is from east to west. Therefore, the expected direction of orientation varies with the time of release.
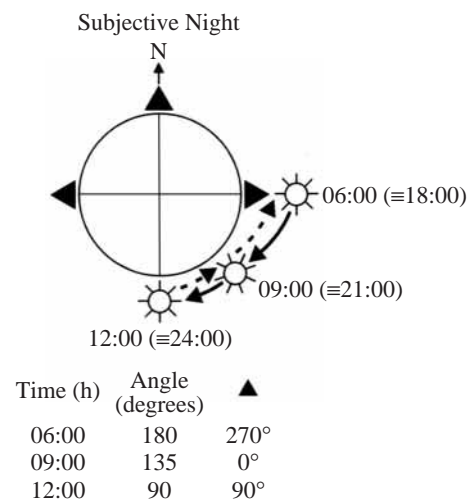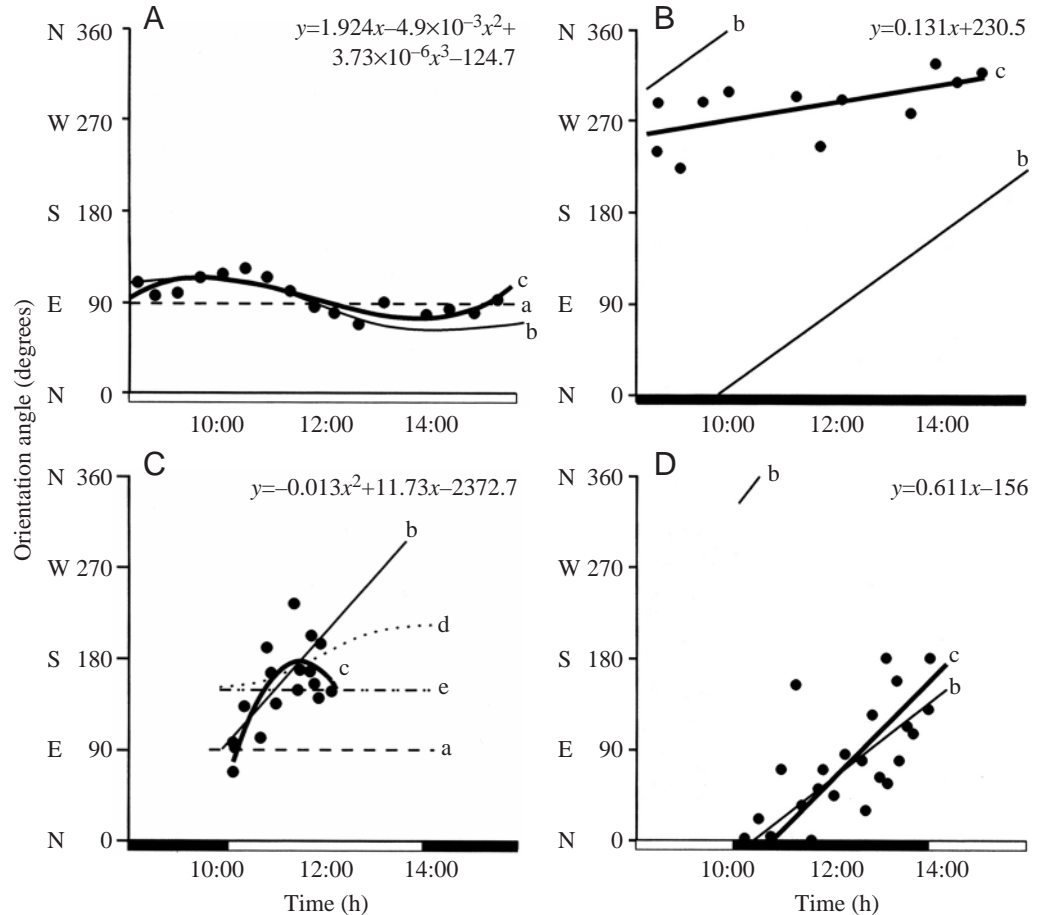
Fig. 2. (A) Orientation angles of sandhoppers subjected to an artificial L:D cycle (15h:9h) corresponding to the natural cycle in phase and duration. (B) Orientation angles of individuals subjected to an artificial L:D cycle (15h:9h) inverted with respect to the natural cycle: the sandhoppers were released during their subjective night. (C) Orientation angles of sandhoppers subjected to an artificial 4h:20h L:D cycle; and (D) to an artificial 20h:4h inverted L:D cycle. The diagrams represent the relationship between the mean angle resulting from each release (filled circles) and time of day. White and black bars represent the natural or subjective hours of light and dark, respectively. a, expected direction towards land (i.e. y_L) for differential compensation; b, expected y_L according to the model of compensation proposed in this study; c, interpolation of the data. In C, curve d represents the expected direction according to the proposed model in the case of clock-shifting affecting subjective noon (clockwise shifting of 6h and 17min). Line e indicates the same thing in the case of differential compensation. In B and D, the line for y_L based on differential compensation is not represented since we do not know the curve of the sun at night. The equations of the regressions are also given.

Table 1. *Determination of the degree of polynomial regression of Fig. 2A*

Degree	r^2 adjusted	Residual sum of squares	d.f.	F	$P(F)$	t for the highest term	$P(t)$
1	0.394	2240	14	10.75	0.0055	–3.28	0.005
2	0.349	2232	13	5.03	0.0240	0.20	0.843
3	0.634	1159	12	9.67	0.0016	3.34	0.006
4	0.602	1155	11	6.68	0.0056	–0.19	0.853
5	0.708	770	10	8.28	0.0025	–2.23	0.050

Total sum of squares 3959, d.f. 15.

The table includes the following: the degree of polynomial regression (Degree); the adjusted coefficient of determination (r^2); the sum of squares about the regression (Residual sum of squares) and degrees of freedom (d.f.); the variance ratio (F) and the probability value, $P(F)$, testing the significance of the decrease in residual mean square; Student's t-test (t for the highest term) and its $P(t)$ value for the introduction of the last term in the regression equation.

expected direction ($P<0.0001$). In fact, there is a tendency for the animals to assume angles of orientation that are constantly less than the expected ones.

Fig. 2C,D illustrates the results of experiments with sandhoppers subjected to 4h:20h L:D and 20h:4h L:D inverted (i.e. tested under the natural sun during the 4h of subjective night).

To determine the degree of the polynomial model for the regression of Fig. 2C, we used the method described above. The second-degree polynomial gave the best fit: adjusted r^2=0.548; residual sum of squares=11940 (d.f.=14); F=10.7; $P(F)$=0.0015; t for the highest term=–2.61; $P(t)$=0.020. Comparisons of the fitting of the curves to the second-degree polynomial are reported in Table 2. The highest F-probability

Table 2. *Comparison of the fitting of the curves of Fig. 2C to the second-degree polynomial regression*

Curves	Variance	d.f.	F	$P(F)$
c	853	14	–	–
a	5637	16	6.61	0.00048
b	1408	16	1.65	0.176
d	1789	16	2.10	0.085
e	1891	16	2.22	0.070

The Table includes the following: identifiers of the curves in Fig. 2C (a–e); mean squares about the regression (Variance) and degrees of freedom (d.f.); the variance ratio (F) and the probability value, $P(F)$, for comparison of variability about each curve and polynomial regression.

indicates the highest similarity between the model and the second-degree polynomial. The final model chosen (curve c) was the second-degree polynomial (Fig. 2C).

For sandhoppers subjected to 20h:4h L:D inverted (Fig. 2D), the fit to the regression line is given by r^2 adjusted=0.597; the slope, which is positive and significant ($P<0.0005$), is not significantly different from that of the expected direction ($P=0.109$).

The slopes of the regression lines in Fig. 2B,D are significantly different ($t=4.18$; d.f.=28; $P=0.0003$).

Discussion

For nocturnal solar orientation (Fig. 2B,D), the data do not allow us to deduce the form of the curve of the sandhoppers' angular variation. However, for diurnal orientation (Fig. 2A), it appears to be a non-linear function (as it would be if it agreed with the model of differential compensation). Our results support those obtained in previous experiments carried out at a different time of year (June; Ugolini and Frittelli, 1998). Therefore, even though we cannot exclude other sources of error in orientation for sandhoppers, such as (modest) changes in the ephemerids between the date of capture and the date of testing, our model of sun compensation represents a valid alternative to that of differential compensation, at least in sandhoppers.

It is well documented that sunrise is an important *Zeitgeber* for sandhoppers (Williams, 1980). Therefore, it should be emphasised that in our experiments the imposed time of sunrise does not cause a deviation in the mean directions of orientation corresponding to the theoretically predicted deviation in the case of a clock-shifting of 6h and 17min with respect to the natural sunrise, which affects the subjective noon; in this case, the mean directions represented in Fig. 2C should correspond to lines d or e but not to line b.

Therefore, the relationship between the number of hours of light and the number of hours of dark influences the speed of the chronometric mechanism of compensation for apparent solar motion. However, this implies that the sandhoppers use information about the total azimuthal variation in the sun in that particular period of the year but not about the daily variation in the sun's azimuthal speed. In other words, the speed of the solar compensation mechanism is independent of the height of the sun above the horizon. Although experiments on this topic were not conducted in the present study, this hypothesis is supported by the results of previous experiments in which the solar azimuth was deflected with a mirror: the height of the reflected sun had no influence on the sandhoppers' choice of direction (see Pardi, 1957; Pardi and Ercolini, 1986).

We do not wish to enter the debate about the existence of an ephemerid's function in crustaceans, as demonstrated for insects and birds (see Wehner and Lanfranconi, 1981; Neuss and Wallraff, 1988; Schmidt-Koenig et al., 1991; Wehner and Müller, 1993; Dyer and Dickinson, 1994; Towne and Kirchner, 1998; Wiltschko et al., 2000). However, we would like to emphasize that sandhoppers are neither 'homers' nor 'central place foragers'; instead, they use a unidirectional, non-vectorial orientation in their zonal recovery (i.e. to return as quickly as possible to the belt of damp sand near the sea). Therefore, it would not be surprising if they used a chronometric system for sun compensation that differed somewhat from (i.e. was simpler than) that used by other animals with different spatio-temporal problems to solve.

Moreover, the present study shows that a single chronometric mechanism provides for compensation for apparent solar motion both during the day and at night. Concerning nocturnal compensation for the movement of the sun, our results do not fully confirm the '*Talitrus* model' proposed by Pardi (1954); a larger number of releases is necessary to clarify the matter of sun compensation at night. However, for the purposes of the present research, it is sufficient to note the difference between Fig. 2B and Fig. 2D: the slope of the regression line in Fig. 2D is significantly different from that in Fig. 2B, in agreement with the expected effect of a reduction in the number of hours of dark.

References

Armitage, P., Berry, G. and Matthews J. N. S. (2002). *Statistical Methods in Medical Research*. London: Blackwell.

Batschelet, E. (1981). *Circular Statistics in Biology*. London: Academic Press.

Birukow, G. (1957). Lichtkompaßorientierung beim Wasserläufer *Velia currens* F. (Heteroptera) am Tage und zur Nachtzeit. I. Herbst-und Winterversuche. *Z. Tierpsychol.* **13**, 463-484.

Dyer, F. C. and Dickinson, J. A. (1994). Development of sun compensation by honeybees: How partially experienced bees estimate the sun's course. *Proc. Natl. Acad. Sci. USA* **91**, 4471-4474.

Ercolini, A. and Scapini, F. (1976). Fototassia negativa e orientamento astronomico solare in due specie di stafilinidi ripari (*Paederus rubrothoracicus* Goeze e *Stenus bipunctatus* Erichson). *Redia* **59**, 135-153.

Lindauer, M. (1954). Dahertänze im Bienenstock und ihre Beziehung zur Sonnenbahn. *Naturwissenschaften* **41**, 506-507.

Lindauer, M. (1957). Sonnenorientierung der Bienen unter der Äquatorsonne und zur Nachtzeit. *Naturwissenschaften* **44**, 1-6.

Marchionni, V. (1958). Modificazione sperimentale della direzione innata di fuga in *Talorchestia deshayesei* Aud. (Crustacea, Amphipoda). *Boll. Ist. Mus. Zool. Univ. Torino* **6**, 29-39.

Neuss, M. and Wallraff, H. G. (1988). Orientation of displaced homing pigeons with shifted circadian clocks: prediction versus observation. *Naturwissenschaften* **75**, 363-365.

Papi, F. (1955). Experiments on the sense of time in *Talitrus saltator* (Montagu) (Crustacea, Amphipoda). *Experientia* **11**, 201.

Papi, F. and Pardi, L. (1953). Ricerche sull'orientamento di *Talitrus saltator* (Montagu) (Crustacea, Amphipoda). II. Sui fattori che regolano la variazione dell'angolo di orientamento nel corso del giorno. L'orientamento di notte. L'orientamento diurno di altre popolazioni. *Z. Vergl. Physiol.* **35**, 490-518.

Pardi, L. (1954). Esperienze sull'orientamento di *Talitrus saltator* (Montagu) (Crustacea, Amphipoda): l'orientamento al sole degli individui a ritmo nictiemerale invertito, durante la 'loro notte'. *Boll. Ist. Mus. Zool. Univ. Torino* **4**, 127-134.

Pardi, L. (1957). L'orientamento astronomico degli animali: risultati e problemi attuali. *Boll. Zool.* **24**, 473-523.

Pardi, L. (1958). Esperienze sull'orientamento solare di *Phaleria provincialis* Fauv. (Coleoptera): il comportamento a luce artificiale durante l'intero ciclo di 24 ore. *Atti Accad. Sci. Torino* **92**, 1-8.

Pardi, L. and Ercolini, A. (1986). Zonal recovery mechanism in talitrid crustaceans. *Boll. Zool.* **53**, 139-160.

Pardi, L. and Grassi, M. (1955). Experimental modifications of direction-finding in *Talitrus saltator* (Montagu) and *Talorchestia deshayesei* (Aud.) (Crustacea, Amphipoda). *Experientia* **11**, 202-210.

Pardi, L. and Papi, F. (1952). Die Sonne als Kompass bei *Talitrus saltator* (Montagu). *Naturwissenschaften* **39**, 262-263.

Pardi, L. and Papi, F. (1953). Ricerche sull'orientamento di *Talitrus saltator* (Montagu) (Crustacea, Amphipoda). I. L'orientamento durante il giorno in una popolazione del litorale tirrenico. *Z. Vergl. Physiol.* **35**, 459-489.

Schmidt-Koenig, K., Ranvaud, R., Ganzhorn, J. U. and Gasparotto, O. C. (1991). Retardation of homing pigeons' ephemerides? *Naturwissenschaften* **78**, 330-333.

Towne, W. F. and Kirchner, W. H. (1998). Honey bees fail to update their solar ephemerids after a displacement. *Naturwissenschaften* **85**, 459-463.

Ugolini, A. (1996). Jumping and sun compass in sandhoppers: an antipredator interpretation. *Ethol. Ecol. Evol.* **8**, 97-106.

Ugolini, A. (2001). Relationship between compass systems of orientation in equatorial sandhoppers. *Anim. Behav.* **62**, 193-199.

Ugolini, A. and Frittelli, F. (1998). Photoperiod length and the chronometric mechanism of the sun compass in Mediterranean sandhoppers. *J. Mar. Biol. Ass. UK* **78**, 1155-1165.

Ugolini A. and Macchi, T. (1988). Learned component in the solar orientation of *Talitrus saltator* Montagu (Amphipoda – Talitridae*). J. Exp. Mar. Biol. Ecol.* **121**, 79-87.

Wehner, R. and Lanfranconi, B. (1981). What do the ants know about the rotation of the sky? *Nature* **293**, 731-733.

Wehner, R. and Müller, M. (1993). How do ants acquire their celestial ephemerids function? *Naturwissenschaften* **80**, 331-333.

Williams, J. A. (1980). The light-response rhythm and seasonal entrainment of the endogenous circadian locomotor rhythm of *Talitrus saltator* (Crustacea, Amphipoda). *J. Mar. Biol. Ass. UK* **60**, 773-785.

Wiltschko, R., Walker, M. and Wiltschko, W. (2000). Sun-compass orientation in homing pigeons: compensation for different rates of change in azimuth? *J. Exp. Biol.* **203**, 889-894.