Magnetic orientation in the mealworm beetle *Tenebrio* and the effect of light

Martin Vácha* and Helena Soukopová

Comparative Physiology Department, Faculty of Science, Masaryk University, Kotlářská 2, 611 37, Brno, Czech Republic

*Author for correspondence (e-mail: vacha@sci.muni.cz)

Summary

There is evidence for both light-dependent and light-independent mechanisms of magnetoreception of terrestrial animals. One example of a light-independent mechanism frequently cited is the magnetic compass of the mealworm beetle (*Tenebrio molitor*). We found that magnetoreception of the mealworm beetle *per se* is a replicable phenomenon but that, in contrast to earlier findings, *Tenebrio* only exhibited consistent magnetic compass orientation when light was present. The problem of whether the loss of orientation is due to a light-dependent magnetoreception mechanism or is instead an effect of motivation change is discussed.

Key words: magnetoreception, light dependency, magnetic compass, mealworm beetle, *Tenebrio*.

Introduction

Sensitivity to the earth's magnetic field has been documented in several groups of insects (Wiltschko and Wiltschko, 1995). Insects have also been used in attempts to characterize the mechanism(s) of magnetoreception in terrestrial organisms (Arendse, 1978; Phillips and Sayeed, 1993; Kirschvink et al., 2001). In only a few cases (most extensively the honeybee *Apis mellifera*), however, has the evidence for magnetic sensitivity proved to be replicable in different laboratories and using different approaches (Frier et al., 1996; Kirschvink and Kirschvink, 1991; Kirschvink et al., 1997).

There are several models of the mechanism of magnetoreception in terrestrial organisms. One mechanism involves biologically synthesized magnetite coupled to a transduction mechanism such as a stretch receptor or sensory hair (Kirschvink et al., 2001; Fleissner et al., 2003). A second type of mechanism involves a specialized photoreceptor in which the magnetic field's effect on a light-induced radical pair reaction alters the sensitivity to light (Ritz et al., 2000). Edmonds (1996) has proposed a model that has elements of both the magnetite-based and photoreceptor-based mechanisms. Edmonds' model involves elongate magnetite particles suspended in carotenoid-containing oil droplets that occur in the outer segments of vertebrate photoreceptors. Rotation of the magnetite particles as they are aligned by an external magnetic field causes changes in the transmission properties of the oil droplets and, thus, the intensity of light reaching the photopigment molecules. In both of the photoreceptor-based mechanisms (Edmonds, 1996; Ritz et al., 2000), magnetic field sensitivity is predicted to depend on light, while other mechanisms involving magnetite are predicted to be independent of light.

One of the frequently cited examples of a magnetic response that is independent of light is the magnetic compass orientation of the mealworm beetle *Tenebrio molitor* (Arendse and Vrins, 1975; Arendse, 1978). The assay developed by Arendse and Vrins takes advantage of the response of the beetles to a directional light source. Beetles exhibit either photopositive or photonegative responses depending on relative humidity (RH), orienting towards the light at very high or very low humidity and away from the light at intermediate humidity (Perttunen and Lahermaa, 1963). We will discuss the possible ecological significance of this phenomenon later.

Arendse and Vrins (1975) showed that beetles that had been exposed to a directional light source learn the direction towards or away from the light source relative to the magnetic field bearing. Arendse (1978) obtained evidence that mealworm beetles are able to use the magnetic field to orient in the 'trained' (i.e. photopositive or photonegative) magnetic direction in either an isotropic light field or in total darkness.

Since the authors interpreted their results as evidence of a light-independent magnetic compass orientation (Arendse, 1978) and the experiment is widely cited as an example of light-independent magnetic compass orientation (see review by Wiltschko and Wiltschko, 1995), we set out to replicate this work. Our goals were to determine whether use of the magnetic field for compass orientation by mealworm beetles was replicable and, if so, whether this response was dependent on the presence of light. Some experimental conditions were not described in the original works and thus we could not replicate

them. Besides, we used some more bias-resistant approaches (discussed below). Therefore, our work is not a strict replication. Its goal was to verify a concrete experimental assay, which, if confirmed, could serve for subsequent research on magnetosensory mechanisms.

Materials and methods
Experimental animals

The experiments were carried out with adult mealworm beetles (*Tenebrio molitor* L.; Coleoptera, Tenebrionidae). The beetles were reared in covered plastic boxes containing a medium that consisted of 4725 ml crushed wheat, 200 ml dried milk and 25 ml inactivated dried yeast. The boxes containing the beetles were kept in permanent darkness at a temperature of 28°C. Humidity in the rearing boxes was not controlled. Individuals intended for experiments were selected randomly from fully pigmented beetles (>2 days old).

Training

The beetles were trained in a 'cross trainer' (Fig. 1A), which consisted of a 60 W frosted light bulb at the center of four cardboard corridors (11 cm×11 cm×44 cm), with inner sides painted dull black, separated by 90°. Light from the bulb was diffused by a paper diffuser that covered the inner end of each corridor. The beetles were placed in a jar (diameter 10 cm, height 9 cm) with an airtight seal at the end of each corridor furthest from the light bulb. Each jar also contained a small dish with chemicals that maintained a constant relative humidity (see below). Beetles were held in these jars for 24 h prior to testing. While in these jars, they could perceive light coming from one of the four directions with respect to the magnetic field. Finally, the jars were temporarily (0.5–6 h)

placed into a small, light-tight, black box while tests were carried out.

Testing

Beetles were tested in a circular arena (diameter 56 cm, height 42 cm) with opaque white walls, a translucent white Plexiglas lid and a glass floor (Fig. 1B). A sheet of filter paper was laid on top of the glass surface and was replaced after each beetle was tested to eliminate possible effects of odor trails. The arena was placed inside a four-element cube surface coil (Merrit et al., 1983), which made it possible to change the intensity and direction of the local magnetic field (Fig. 2).

Tests were carried out between 08.00 h and 16.00 h under infrared light (LED array with peak output at 880 nm) or under isotropic white light produced by a 60 W frosted light bulb positioned 50 cm above the lid of the arena. A small Petri dish (diameter 4.5 cm), which could be raised up from the centre of the white lid by a string, was used as a release device. A beetle was placed into the release device under red light and left for two minutes under the dish to calm down. The dish was then drawn up and the beetle allowed to move freely in the arena.

A beetle's movements were monitored by means of its silhouette, viewed by a video camera located beneath the arena (Fig. 1B) and processed by path analysis software (EthoVision; Noldus, Wageningen, The Netherlands). Each beetle was tested only once and its sex was determined at the end of the test. As in Arendse's original experiment (Arendse, 1978), the humidity during testing was not controlled and in our experiments varied between 17% and 63%. The temperature varied between 20°C and 31°C.

Tests with untrained beetles – random check control

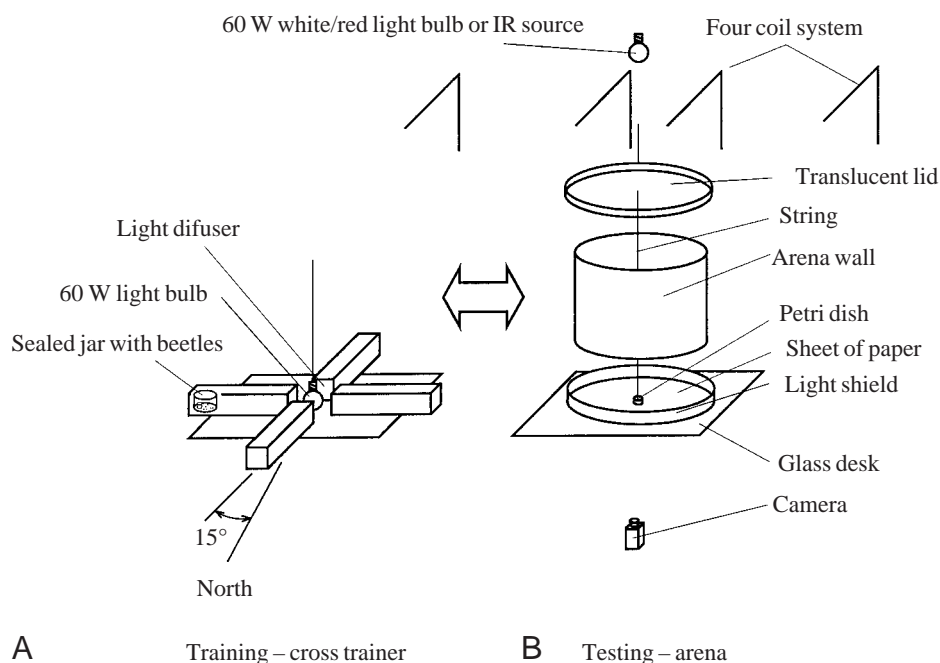We initially investigated whether beetles exhibit a

Fig. 1. Design of (A) training and (B) testing set-up. (A) For training, beetles were kept in constant humidity for 24 h, being exposed to a directional light emanating from one of four geographic directions. The light in training came from a frosted light bulb and passed through a diffuser of white paper. (B) For testing, beetles were placed in the centre of the arena under a plastic Petri dish. They were released by raising the Petri dish after a delay of 2 min, and their movement was observed by a camera located underneath the arena. Both training and testing took place in the same location in the laboratory.
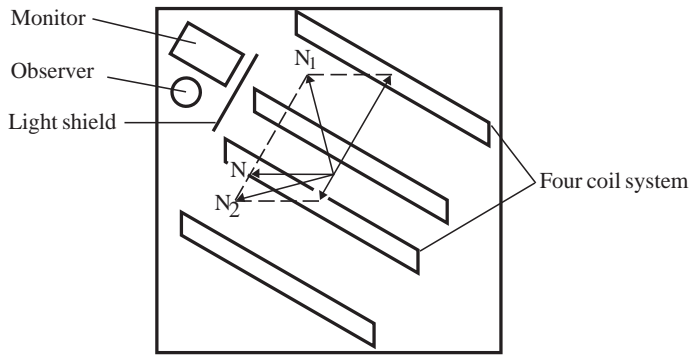
Fig. 2. Top view of a coil system and magnetic vectors. Both training and testing took place in the centre of a four-coil system that produced magnetic fields that differed in alignment by 90°. The two fields had a horizontal intensity of 20 μT and an inclination of 65°. The black light shield was placed between the observer and the test arena. N_1 and N_2 are the directions of the experimental fields; N is the direction of the local natural field.

spontaneous directional preference relative to the magnetic field. During these control experiments, beetles were put in a sealed jar, keeping a constant humidity inside. Then the jar was put into an opaque black box for 24 h. The only difference compared with the trained condition was the lack of light. Subsequent testing procedures were the same as for trained animals. In addition to testing for a spontaneous magnetic preference (e.g. similar to the spontaneous preference for the cardinal compass directions exhibited by honeybees; Altmann, 1981; Martin and Lindauer, 1977), random check control experiments were carried out to determine if there was any non-magnetic bias in the testing apparatus.

Humidity during training

We used four distinct humidities in the training jars exposed to the directional light source: two from the middle of the humidity scale – 50±4% and 75±3.5% – and two at the extremes – <5% and >95%. The constant humidities were maintained (Dambach and Goehlen, 1999) by a small dish containing dried silica-gel (<5% RH), salt solutions (50% and 75% RH) and water (>95% RH). The humidity in the training jars was checked periodically with a hygrometer probe (D 3121; Commeter, Roznov, CZ).

Photic conditions

The intensity of light was measured by an energy sensor (SKE 510; Skye Instruments, Llandrindod Wells, UK). Beetles in the cross trainer were exposed to a light intensity of 0.35 ± 0.005 W m^{-2}. During the light experiments, the light intensity in the testing arena was 0.19 ± 0.005 W m^{-2} at the center and 0.17 ± 0.005 W m^{-2} along the wall.

Magnetic conditions

Artificial fields were produced by a horizontal four-element coil surface coil (2 m×2 m×2 m; Merritt et al., 1983), and the spatial variation in the region of the arena was <2%. Magnetic

flux density was measured by a three-axis magnetometer probe (HMR 2300; Geomag software; Honeywell US and Geomag Software; Edis, Kosice, SK). The horizontal component could be rotated by 90° anticlockwise. The size and position of the coils in the laboratory did not allow the rotation of the field by a larger angle (Fig. 2). The field in which the beetles were held prior to testing, as well as both alignments of the field used in testing, had a horizontal component of 20 μT, a vertical component of 44 μT and an inclination of 65°. The 20 μT value of the horizontal component was selected on the basis of our previous experience (M. Vácha and H. Soukopová, unpublished) and outdoor measurements. The local geomagnetic field had a horizontal component of 17 μT, and its daily variation (caused mainly by the trolley bus traffic) did not exceed 0.4 μT.

Statistical methods

The directional bearings of beetles exposed to the four directional light gradients were combined by rotating the distributions so that the trained directions coincided. The pooled distributions of bearings were analysed using standard circular statistics (Batschelet, 1981). Circular statistical parameters describing obtained data were counted: sample size (60 per sex), expected direction of the sample, average direction (mean bearing) of the sample, significance level of the Rayleigh test and 95% confidence interval for mean axis. A χ^2 test was used for verifying differences between males and females. If no significant difference between sexes was obtained (all experiments except 50% RH), both samples were pooled together. Watson's U^2 test was used to compare distributions between dark and light experiments.

Results

5% RH

After being held under 5% RH, untrained beetles failed to exhibit a directional preference in any of the random check control conditions under either white light or dark conditions (Fig. 3A–C), indicating that there was no source of non-magnetic directional bias in the testing arena and also that the beetles did not exhibit a spontaneous directional preference relative to the magnetic field.

After training to a directional light source, beetles also failed to exhibit a consistent direction of orientation when tested in the dark (Fig. 3D). Under white light, however, they exhibited orientation that coincided with the direction towards the light in training (Fig. 3E). When the magnetic field was rotated 90° anticlockwise, beetles exhibited a corresponding shift in the direction of orientation (Fig. 3F). A U^2 test showed a significant difference between all trained groups ($P<0.005$).

All subsequent tests were carried out with the rotated field only, i.e. beetles were trained in one field alignment and tested in the other field, which was rotated 90° anticlockwise.

75% RH

Untrained beetles failed to exhibit a consistent direction of orientation under either light or dark conditions (Fig. 4A,B).
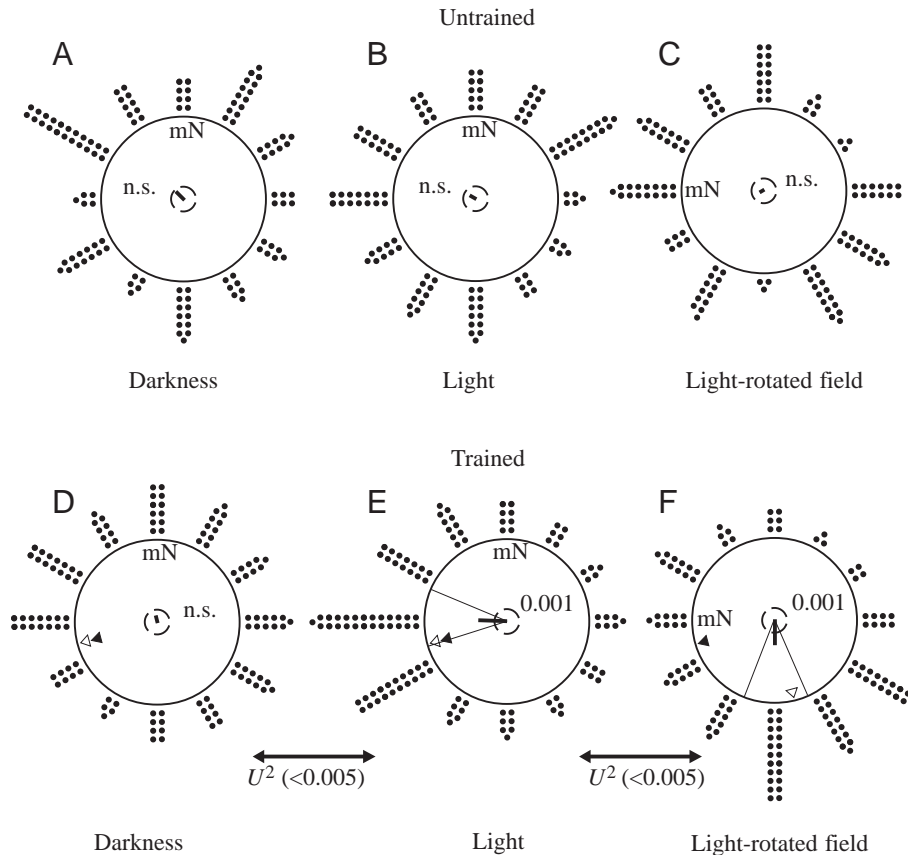
Fig. 3. 5% relative humidity (RH) – random check controls (A–C) and tests (D–F). All bearings of untrained beetles are randomly distributed, both in the magnetic field used in training (A,B) and in the magnetic field rotated 90° anticlockwise (C) (*P*>0.05, Rayleigh test; n.s. = not significant). Each dot represents one beetle (*N*=120). mN = magnetic north during testing. The line starting at the centre of each circle is the mean vector bearing; the radius of the circle corresponds to a mean vector length (*r*)=1.0; the inner broken circle gives the 5% significance level. During tests, unimodal orientation towards the trained magnetic position of light was expected. Beetles did not exhibit a consistent direction of orientation relative to the magnetic field in darkness (D). By contrast, in the light, beetles oriented in the trained magnetic direction (E). In addition, when the magnetic field was rotated 90° anticlockwise, the orientation in the light was shifted accordingly (F). All distributions after training differ from each other significantly (double-headed arrow indicates Watson's U^2 test). Filled triangles, trained topographic direction; open triangles, trained magnetic direction. Lines on either side of the mean vector indicate the 95% confidence interval for the mean vector bearing. The Rayleigh test probability level is indicated in each circle.

Animals trained to a directional light source also failed to exhibit a consistent direction of orientation relative to the magnetic field when tested in the dark (Fig. 4C). Under white light, they exhibited consistent orientation relative to the magnetic field, which was rotated 90° anticlockwise of its alignment in training (Fig. 4D). A U^2 test confirms the difference between dark and light testing conditions. The beetles' orientation coincided with the direction away from the directional light source in training.

95% RH

Untrained controls were again disoriented (Fig. 5A,B). As in the other humidity conditions, beetles trained to a directional light source were disoriented in the dark (Fig. 5C) but exhibited significant orientation relative to the rotated magnetic field under white light, with the field rotated 90° anticlockwise of its alignment in training (Fig. 5D). Distributions in darkness and light are again significantly different. The beetles' orientation coincided with the magnetic direction towards the light in training.

50% RH

Untrained controls were disoriented (Fig. 6A,B). In this humidity only, the distribution of bearings obtained from trained males and females in the dark differed significantly (χ^2 test, *P*=0.01) and samples could not been pooled. After training

to a directional light source, females tested in the dark failed to exhibit a consistent direction of orientation relative to the magnetic field (Fig. 6C). However, males tested in the dark exhibited significant bimodal orientation along a magnetic axis that was rotated by approximately 45° from the trained direction (Fig. 6E), with the field rotated 90° anticlockwise of its alignment in training. When tested in the light, females exhibited a significant direction of orientation relative to the magnetic field corresponding with the direction away from the light in training (Fig. 6D), whereas males exhibited, as expected, bimodal orientation relative to the magnetic field along the trained axis (Fig. 6F). U^2 tests showed significant differences between dark and light tests.

Discussion

The findings reported here indicate that *Tenebrio* trained in a directional light field were able to learn a direction of orientation relative to the magnetic field. In tests carried out under white light, the trained magnetic response was evident in both females and males and after training in all four humidity conditions. Moreover, the directions of orientation along the trained magnetic axis were consistent with earlier work (Arendse and Vrins, 1975), i.e. unimodal orientation towards the light under 5% RH (Fig. 3E,F) and 95% RH (Fig. 5D), unimodal orientation away from the light under 75%
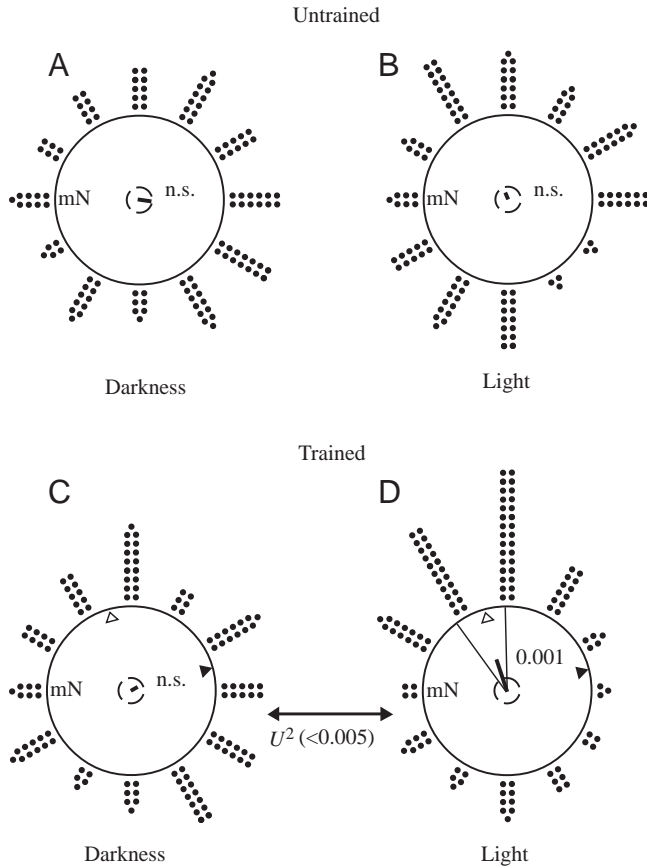
Fig. 4. 75% relative humidity (RH) – random check controls (A,B) and tests (C,D). Unimodal orientation away from the trained magnetic position of light was expected. As in the earlier experiment, controls failed to exhibit a consistent direction of orientation relative to the magnetic field in the dark (A) or in the light (B). Again, beetles trained to the directional light source and tested in the 90° rotated field were not oriented in the dark (C) but oriented in the expected magnetic direction in the light (D).
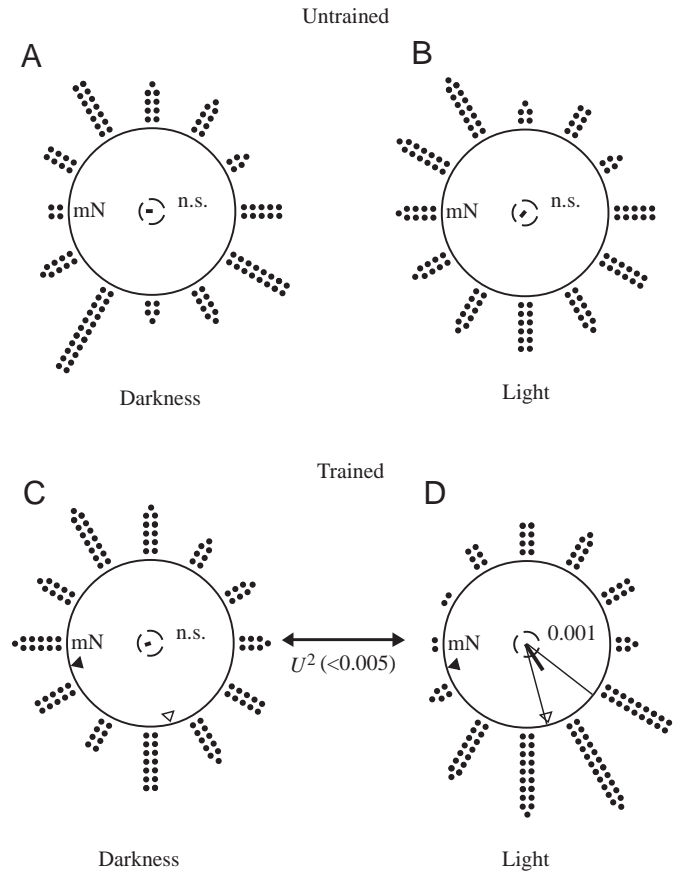

Fig. 5. 95% relative humidity (RH) – random check controls (A,B) and tests (C,D). Unimodal orientation towards the trained magnetic position of light was expected. As before, random check controls are randomly distributed (A,B). Beetles did not prefer any direction in darkness (C) but oriented in the expected magnetic direction in the light (D).

RH (Fig. 4D) and unimodal (females; Fig. 6D) or bimodal (males; Fig. 6F) orientation under 50% RH. These findings indicate that exposure to the directional light source for only ~24 h was sufficient for the beetles to learn a direction of orientation relative to the magnetic field.

In contrast with Arendse's findings (Arendse, 1978), however, beetles tested in the dark (Figs 3D, 4C, 5C, 6C) did not orient with respect to the trained magnetic axis. In only one case (Fig. 6E) was the distribution of magnetic bearings non-randomly distributed (i.e. the bimodal distribution of magnetic bearings exhibited by males trained under 50% RH), but the 95% confidence interval for the mean axis of orientation did not include the trained magnetic axis. Given that a non-random distribution was observed in only one out of the five distributions and, unlike all seven distributions obtained from beetles tested in the light, did not coincide with the trained axis, the most likely explanation is that this result occurred by chance alone.

As for the importance of light for magnetotactic behaviour of *Tenebrio*, there is considerable contrast between our results

and those of Arendse. However, the question is whether such discrepancy is due to differences in experimental protocols and whether our experiment can serve as a verification or whether it is practically a new experimental set-up. Here, we discuss what differences it concerns.

Differences between experimental protocols

Our magnetic field had the same vertical component as in the original experiments – 44 μT. However, while the horizontal component of Arendse's field was 18 μT, ours was 20 μT. We chose this stronger field to attain identical parameters to those of the natural outdoor geomagnetic field in our locality. It does not seem likely that such a small difference could elicit qualitative change in the impact of light.

Concerning light parameters, the exact values were missing from the original work and thus precise comparison was not possible. However, our results differ from those of Arendse in dark tests not in light ones.

It is difficult to consider Arendse's tests as a whole because, having no IR camera, he had to use different techniques for
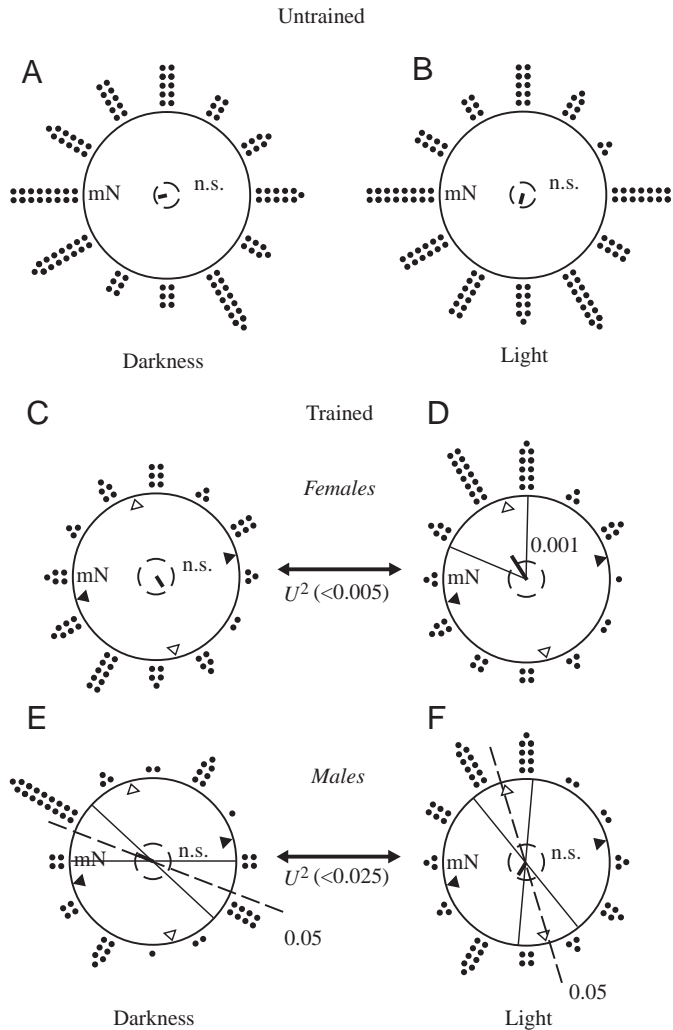
Untrained

Fig. 6. 50% relative humidity (RH) – random check controls (A,B) and tests (C–F). Bimodal orientation was expected. Untrained beetles' bearings are randomly distributed (A,B). In darkness (C), females showed no orientation but (E) males were bimodally distributed ($P<0.05$, Rayleigh test on doubled angles) and differed significantly from females (sexes could not be pooled together). However, the 95% confidence interval for the males' mean axis of orientation did not include the trained magnetic axis. In light (D), females oriented unimodally away from the magnetic bearing of light and (F) males oriented bimodally in the expected direction – confidence interval for the mean axis of orientation included the trained magnetic axis. $N=60$.

light and dark tests. None of his beetle tracing methods in dark eliminates biasing olfactory or other impacts. He used a platform covered with starch first. The trail left by the beetle in the starch was copied onto graph paper. The other design was that the platform was "completely surrounded by 12 plastic cups so that, when a beetle arrived at the edge of the platform, it would fall into one of the cups." It is likely that some beetles did not fall into the cups immediately as they hit them. Besides, there is no note about wiping or exchanging surfaces to prevent pheromone traces.

Our experiments avoided non-magnetic bias at four levels: (1) random check experiments ruled out spontaneous preference of any direction by untrained animals; (2) pooling data from four training corridors and recalculating them respectively to magnetic north made sure that possible non-magnetic directional cues from the environment were neutralized; (3) animals were tested in a magnetic field that was rotated with respect to the magnetic field used for training; our final preferred bearings corresponded with that rotated field and no other cues possibly learned during the training showed an impact; (4) comparison of results between tests with unrotated and rotated magnetic north (Fig. 3E,F) gave unequivocal evidence about the effect of nothing but the magnetic field itself.

Arendse's original work on *Tenebrio* magnetic orientation in darkness (Arendse, 1978) did not fulfil (or the author did not note it) a single case of the bias-preventing conditions mentioned above. The most prominent weakness seems to be that Arendse (1978) did not shift the alignment of the magnetic field in the dark tests. Consequently, if the beetles in Arendse's study had access to a source of non-magnetic directional biasing information (e.g. sounds or vibrations that were present in both training and testing), they may have used this, rather than the magnetic field, to orient in the trained direction in the dark.

Nevertheless, our results are perfectly in line with Arendse's in-light work. The beetles' attitude to the light, and consequently the polarity of their magnetic orientation, changed according to air humidity in the same way as in Arendse's original experiments. This correspondence confirms both his and our results. However, we state that Arendse's work did not show clear, strong and well-documented evidence of magnetic orientation in darkness and that the independence of *Tenebrio* magnetoreception on light is still a problematic hypothesis.

Nevertheless, we cannot refute that the *Tenebrio* magnetoreception mechanism is distinct from light detection. The lack of magnetoreception behaviour in darkness may result from changed motivation when beetles perceive a magnetic field but do not use its information.

Motivation and ecological significance of Tenebrio behaviour

In terms of the change in *Tenebrio* motivation, the switching from light-avoiding behaviour when RH is normal to light-seeking behaviour when RH is extreme is very interesting (Perttunen and Lahermaa, 1963). Unfortunately, the benefit of such behaviour is not quite clear. Darkness may generally work as a refuge and shield from danger. Sometimes, when humid conditions are extreme and danger of desiccation or mould infection is high, light may show the way to a better place. Beetles can remember the magnetic bearing of light whatever affinity they have to it – whether positive or negative – according to the air humidity. The relationship between humidity and magnetoreception seems to be indirect only, by means of phototactic behaviour.

In our tests in light, we found the same motivation switches as Arendse did. However, we speculate that the substantial discrepancies between our and Arendse's dark tests is not due

to different motivation by the beetles but because of some biasing problems in Arendse's work. In any case, behavioural proof of whether the lack of magnetoreception behaviour in dark is due to the loss of motivation or the mechanism itself being knocked out is not easy to obtain. More behavioural data or quite different experimental approaches are needed to solve this problem.

Possible implications for the mechanism of magnetoreception in Tenebrio

Studies of the magnetic compass orientation of migratory birds (Wiltschko and Wiltschko, 1995) showed that the slope or inclination of the magnetic field is used to distinguish between the two ends of the magnetic axis and that the birds were disoriented in a horizontal magnetic field, indicating that they were sensitive to the axis, but not polarity, of the magnetic field (the so-called inclination magnetic compass). By contrast, Arendse (1978) found that mealworm beetles were able to orient unimodally in a 'horizontal' magnetic field (inclination=0°; see below), suggesting that they were able to detect the horizontal polarity of the magnetic field. Sensitivity to polarity is not consistent with a photoreceptor-based mechanism involving either magnetite (Edmonds, 1996) or a light-induced radical-pair reaction (Ritz et al., 2000). There are a number of reports that polar compass behaviour is usually light independent in different animals (salmon – Quin et al., 1981; mole rat – Marhold et al., 1997; lobster – Lohmann et al., 1995). Consequently, Arendse's findings (Arendse, 1978) point to a light-independent mechanism, possibly involving magnetite or a similar permanently magnetic material. This type of mechanism is also consistent with Arendse's finding that mealworm beetles are able to orient with respect to the magnetic field in total darkness (Arendse, 1978).

In contrast to Arendse (1978), mealworm beetles in our experiments exhibited a consistent direction or axis of orientation relative to the magnetic field in the light (Figs 3E,F, 4D, 5D, 6D,F) but not in the dark (Figs 3D, 4C, 5C, 6C,E), which is consistent with a light-dependent magnetic compass mechanism.

One potential difficulty with the conclusion that the mealworms have a light-dependent magnetic compass is that, to date, light dependence has only been found in organisms that have inclination (rather than polar) magnetic compasses (e.g. salamander – Phillips (1986); bird – Wiltschko and Wiltschko, 1972). It will be of considerable importance, therefore, to determine whether the evidence for a polarity magnetic compass in *Tenebrio* (Arendse 1978) is replicable. There is one potential problem with the earlier experiments. The 'horizontal' field may have had a slope of as much as 2° (Arendse and Vrins, 1975). If so, this amount of inclination may have been enough for the beetles to use an inclination compass. In future experiments, it will be important to reverse, rather than attempt to eliminate, magnetic inclination, since reversal provides an unambiguous method to distinguish between inclination and polarity compasses (Wiltschko and Wiltschko, 1972; Phillips, 1986).

Conclusions

Findings from the present experiments confirm that *Tenebrio* has a well-developed magnetic compass and is able to learn a direction of orientation relative to the magnetic field in as little as 24 h. These results confirm previous experiments done on *Tenebrio* under light (Arendse and Vrins, 1975). In contrast to the earlier work by Arendse (1978), however, beetles failed to exhibit a consistent response to the magnetic field in the dark. Our findings, therefore, are more consistent with *Tenebrio* exhibiting a light-dependent, rather than a light-independent, magnetic compass behaviour. Nevertheless, whether the failure to orient magnetically in darkness is due to a light-dependent magnetoreception mechanism or to a non-specific effect of motivation cannot yet be determined. Additional research will be needed to solve this problem.

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