Polarised Moonlight Guides Nocturnal Bull Ants Home

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Abstract

For the first time in any animal, we show that nocturnal bull ants use the exceedingly dim polarisation pattern produced by the moon for overnight navigation. The sun or moon can provide directional information via their position; however, they can often be obstructed by clouds, canopy or the horizon. Despite being hidden, these bodies can still provide compass information through the polarised light pattern they produce/reflect. Sunlight produces polarised light patterns across the overhead sky as it enters the atmosphere, and solar polarised light is a well-known compass cue for navigating animals. Moonlight produces an analogous pattern, albeit a million times dimmer than sunlight. Here we show the first evidence that polarised moonlight forms part of the celestial compass of navigating nocturnal ants. Nocturnal bull ants leave their nest at twilight and rely heavily on the overhead solar polarisation pattern to navigate. Yet many foragers return home overnight when the sun cannot guide them. We demonstrate that these bull ants use polarised moonlight to navigate home during the night, by rotating the ambient polarisation pattern above homing ants, who alter their headings in response. Furthermore, these ants can detect this cue throughout the lunar month, even under crescent moons, when polarised light levels are at their lowest. Finally, we show long-term incorporation of this moonlight pattern into the ants' path integration system throughout the night for homing, as polarised sunlight is incorporated throughout the day.

Keywords: ant navigation, lunar cues, route maintenance, cue detection, polarised light, vector

Introduction

Many navigating animals attend to the position of the sun or moon to guide their movement (Jander 1957; Koltz and Reid 1993; Perez et al. 1997; Dacke et al. 2014; Warrant and Dacke 2016; Freas and Cheng 2022). Yet these celestial bodies are not always directly visible, often obscured by clouds, the canopy or after passing below the horizon, resulting in gaps for navigation relying solely upon direct visual detection. Animals hence rely on the pattern of polarised skylight which is accessible even when the celestial bodies are occluded to some extent (Horváth et al., 2014). Polarised light comprises light waves which occur along a single plane and are produced as a by-product of light passing through the upper atmosphere (Horváth & Varjú 2004; Horváth et al., 2014). E-vectors are arranged in concentric circles around the sun/moon with the maximum degree of polarisation located 90° from the source. Hence when the sun/moon is near the horizon, the pattern of polarised skylight is particularly simple with uniform direction of polarisation approximately parallel to the north-south axes (Dacke et al., 1999, 2003; Reid et al. 2011; Zeil et al., 2014). The pattern's stability makes the sky's polarisation a useful directional cue for orientation (Wehner and Müller 2006; Reid et al. 2011; Lebhardt and Ronacher 2013; Warrant and Dacke 2016; Freas et al. 2017a, 2019; Freas and Spetch 2023), which insects detect through specialised photoreceptors located in the dorsal rim area of their eyes (Labhart and Meyer 1999; Homberg and Paech 2002; el Jundi et al. 2015). Like solar polarisation, though a million times weaker, the moon reflects sunlight, producing a polarised moonlight pattern emanating from the moon's position in the night sky (Gál et al. 2001). Given that the moon creates a much dimmer version of the polarisation pattern formed around the sun, only night-navigating insects with eyes highly specialised for low light detection may be able to rely on this pattern to orient and navigate to goals.

Currently, only nocturnal dung beetles (*Scarabaeus satyrus* and *S. zambesianus*) are known to attend to moonlight polarisation patterns during their movement (Dacke et al. 2003, 2004, 2011; Foster et al. 2019). Yet interestingly, these beetles do not use moonlight to navigate, instead relying on this pattern to keep moving straight in order to roll their dung balls expeditiously away from a central dung pile. As such, this cue has only been documented for heading maintenance over short periods. While it has been theorised that this ability to detect the much dimmer polarisation pattern produced by the moon may be present across nocturnal insects more broadly, including nocturnal bees and crickets (Herzmann and Labhart 1989; Greiner et al. 2007; Rost and Honegger 1987), there is currently no behavioural evidence for its use in goal-directed navigation.

The large-eyed *Myrmecia* ants have several species that restrict majority of their navigation to evening twilight (outbound) and morning twilight (inbound) respectively (Narendra et al., 2017). We know that two well-studied nocturnal ant species, *Myrmecia pyriformis* and *Myrmecia midas*, use the overhead solar polarised light pattern, which is still visible during the twilight period to derive compass information (Reid et al. 2011; Freas et al. 2017ab; Freas and Cheng 2018). Because the information required for visual navigation degrades beyond twilight, it has been suggested that animals tend to be less active at night.

However, a small proportion of *M. pyriformis* foragers leave the nest (10.7% of daily foraging force) or return home during night (13.3% of daily foraging force) (Reid et al., 2013). In *M. midas,* this nocturnal activity is even more pronounced with a majority of the foraging force returning during night (62.8% of daily foraging force) along with a minority of foragers leaving the nest during night (26.2% of daily foraging force) (Freas et al. 2017a).

Nocturnal bull ants navigate using a combination of learned visual cues and homing vectors obtained by integrating pedometric and celestial compass information. This 'true nocturnal navigation' is likely aided by the increase in the light intensity provided by the moon's presence. In *M. pyriformis*, on 'full-moon' nights there was a significantly greater proportion of foragers leaving the nest at night compared to a 'new-moon' night (Reid et al., 2013). This additional light at night may enhance terrestrial visual features foragers have learned and therefore assist in visual guidance. In addition, the moon and the lunar polarisation pattern also likely provides compass information, allowing foragers acquire or follow a homing vector. Several arthropods, including ants and bees directly track the moon's position to obtain compass information (Jander 1957; Koltz and Reid 1993; Dacke et al. 2004; Ugolini et al. 2003). But given the moon may be occluded by clouds or overhanging canopy we aim to identify here whether the lunar polarised skylight can also be used by ants for homing.

In the current study, we provide the first evidence of polarised moonlight detection and guidance in ants for goal-directed navigation. We studied foraging ants' ability to orient during lunar twilight, by placing and rotating a linear polarising filter over them as they returned to the nest (Figure 1A). We also explored whether these navigators weigh their attendance to polarised moonlight across the lunar calendar, since during quarter-moon and crescent-moon nights, smaller portions of the moon's surface reflect sunlight (as well as moonless nights when no ambient e-vector is present). Finally, we characterised changes in weighting that these ant navigators give polarised moonlight, as a function of the moon's overnight consistency in the sky (waxing vs. waning) and the length of their accumulated path integrator, which should increase the weight given to celestial compass portion of the path integrator when in conflict with terrestrial visual cues (Burkhalter 1972; Narendra 2007; Wystrach et al. 2015; Freas and Cheng 2019).

Methods

Study site

Experiments were conducted from April through October 2023 on two *Myrmecia midas* nests on the Macquarie University Wallumattagal campus in Sydney, Australia (33°46 11S, 151°06 40E). *M. midas* nests are typically located within stands of *Eucalyptus* trees with the nest entrance located near (<30cm) a tree. *M. midas* is nocturnal, with foraging onset occurring ~20min after sunset when foragers leave the nest to travel to and up one of several surrounding foraging trees overnight (Freas et al. 2018). Inbound navigation is more variable with foragers returning to the nest entrance overnight and into morning twilight (Freas et al. 2017a). For this study, we chose two nests under open canopies to ensure

foragers had unobstructed visual access to the overhead sky (Figure 1B). The understory of these areas is naturally barren of vegetation, and we cleared the foraging column of debris to aid visual tracking.

Apparatus: Polarisation filter

For each condition, we altered the ambient pattern of polarised moonlight by rotating a linear polarisation filter (30cm diameter; same apparatus used in Freas et al. 2019) above each ant along their inbound journey. This rotation alters the dominant ambient e-vector direction of the sky above the navigator. This filter was held by a circular 1cm thick ring and lifted 10cm off the ground by four equally spaced thin metal legs (Figure 1). All testing was conducted overnight before morning solar twilight, during the lunar twilight for each tested lunar phase. For each night we obtained the moon's position as it reached the horizon based on the Astronomical Almanac (http://asa.usno.navy.mil) and set the ambient lunar e-vector perpendicular from the moon's position at moonset. We relied on a digital compass mobile application (Apple™) confirmed by an analogue compass to locate the ambient e-vector and rotated the linear polariser by ±45° from this direction for each ant.

Across all conditions, we recorded four positions: the ant's release point, position when the filter was placed overhead, the filter exit point, and reorientation after ~1m, taking care to slowly follow the ant and mark their positions so as to not disturb their travel. These positions determine each forager's initial orientation, exit orientation and reorientation directions (Figure 1). After testing, each forager was marked with acrylic paint (Tamiya™) to prevent retesting. Testing was conducted at distinct lunar phases which predicably occur throughout the lunar month cycle (29.5 days).

Full moon

For full moon testing we chose nights during which the waxing moon's lunar phase was near full but with clear separation between solar and lunar twilights. Testing on true full moon nights is problematic as solar and lunar twilights fully overlap and the solar polarised light pattern would overpower the lunar counterpart. Testing during the nights preceding the full moon (waxing phase) ensured the moon's presence in the night sky overnight and testing occurred on nights in which the lunar twilight (1am – 4am) was clearly separated from the start of morning solar twilight (5:22am) with illumination above 80% of the lunar surface.

Outbound *M. midas* foragers from two nests were followed as they left the nest during evening twilight and collected as they climbed onto their foraging tree, (Nest 1: 6.0m; Nest 2: 3.1m from the nest entrance). Each forager was provided a small amount of honey and held within a clear plastic phial on the ground 5m from the foraging tree with an unobstructed view of the sky (Figure 1B). Foragers were held in these phials overnight until the moon's position was within ±10º of the horizon (large stands of trees and buildings near the western horizon occluded the moon's position during all testing; Figure 1B).

Waxing lunar phase

We observed clear shifts in forager headings at both nests under *Full Moon* conditions, yet nights with over 80% lunar illumination only account for nine nights per lunar cycle. To assess if polarised moonlight can be used throughout the lunar month, we tested foragers in three further conditions representing distinct lunar phases: a *Waxing Quarter Moon, a Waxing Crescent Moon,* and a *No Moon* control. For the *Quarter Moon* and *Crescent Moon* conditions, we tested ants identically to full moon conditions; for overnight testing, however, the moon has a different temporal period when it is positioned near the horizon (12am and 9pm, respectively).

The procedure was slightly modified for *No Moon* testing as we did not test these foragers on the new moon night (the new moon is only present during the day). We hypothesised that foragers with no available ambient polarisation and suddenly presented with an e-vector pattern might fall back on a memory of the morning solar e-vector as many foragers return during morning twilight and this direction remains consistent across nights. In order to test on a night when there were distinct directional differences between the lunar and morning solar e-vectors (Lunar e-vector: 329º; Morning solar e-vector: 7º; Evening Solar e-vector: 353º), we chose to test on a quarter moon night when the moon was well below the horizon. Testing commenced at 9:00pm, when the moon was 30º below the horizon and we rotated the filter around to the future lunar e-vector direction (moonrise at 12:39am). If foragers were relying on a solar e-vector memory, we expected to see unequal shifts between ±45° (smaller shifts in the +45º condition and larger shifts in the −45º condition).

Waning lunar phases

While there is a consistent presence of polarised light during the waxing phase, during waning lunar phases there is a gap which may impede this pattern's use as a compass cue. Testing during the waxing lunar phase, as the moon's illuminated surface increases, corresponds with periods in which the moon rises prior to sunset and sets overnight. This creates a consistent presence of polarised light (solar then lunar) that foragers could use to continuously update their celestial compass and path integrator. In contrast, the waning lunar phase corresponds with the moon rising overnight, leading to a gap in this cue as solar twilight ends. We hypothesised that the absence of the moon's presence as a cue overnight might decrease its weighting or degrade its integration into the celestial compass. We added two conditions to test this hypothesis: a *Waning Full Moon* and *Waning Quarter Moon* conditions. Foragers were tested identical to previous conditions; only, they were tested during moonrise (10pm and 1am) overnight rather than moonset.

Vector testing

After noticing differences in heading shift magnitude between nests which correlated with PI vector lengths, we tested the hypothesis that, similar to solar polarisation (Freas et al. 2017b), the navigator's accumulated vector length impacts orientation to rotated lunar polarised light. While foragers at Nest 1 (6.0m vector) reoriented almost fully, those at Nest

2 (3.1m vector) reoriented only halfway on near-full-moon nights. To test this hypothesis, we collected foragers of Nest 1 on near (waxing) full moon nights and tested them at a site close (2m) to their nest tree with either a 2.0m remaining vector, or with a larger (~6m) full corridor remaining vector (*Halfway Collect & Release* or *Halfway Release* conditions). In the *Halfway Collect & Release* condition, outbound foragers were collected at the halfway point, held overnight and released at this site to be tested (±45° rotations) at ~2m from the nest. In the *Halfway Release* condition, we followed outbound foragers to their foraging tree, collected and held them until lunar twilight (2-4am) identically to previous full moon conditions. These foragers were released along the route at the halfway point (3.0m) from the nest/foraging tree and we tested each forager with their vector state near full but their true location close (~2m) to the nest tree. In both conditions, we again recorded the initial orientation, filter exit orientation and post-filter reorientation of each forager.

Statistical analysis

Data were analysed with circular statistics with the statistics package Oriana Version 4 (Kovach Computing Services). Each ant had a slightly different inbound heading due to their stereotypical route along the foraging corridor and we corrected this variance by designating the initial headings (pre-filter) as 0° for calculating shift magnitudes under the filter. To assess shift magnitude between −45° and +45° foragers within conditions, we calculated the mirror of shift in each −45° condition, allowing shift magnitude comparisons within each condition. As −45° and +45° shifts were not significantly different in any condition (Watson–Williams F-tests), they were combined for between condition comparisons. Within-individual comparisons (Initial Orientation vs. Filter Exit and Filter Exit vs. Reorientation) were analysed using Moore's Paired Tests. Across-condition shift magnitudes were analysed using Watson–Williams F-tests. In the lunar phase comparisons where full, quarter and crescent shift magnitudes, as well as Waning and Waxing phases were compared, Holm-Bonferroni corrections were applied to the *p*-value to account for multiple comparisons.

Results

Full moon testing

Under a (waxing) *Full Moon* with lunar illumination above 80%, when the linear polariser was rotated clockwise (+45°), exit orientations were shifted to the right of initial headings (mean ± s.e.m. Nest 1: 38.4±4.8°; Nest 2: 23.4±4.2°; Figure 2AB), and these changes were significant (Moore's Paired Test, Nest 1: R=1.639, p<0.001; Nest 2: R=1.592, p<0.001). Forager headings also changed predictably when the filter was rotated counter-clockwise (–45°) with exit orientations to the left of initial headings (Nest 1: –41.1±5.5°; Nest 2: –27.1±7.4°; Figure 2AB), and these changes were significant (Moore's Paired Test, Nest 1: R=1.794, p<0.001; Nest 2: R=1.310, p<0.01). After exiting the +45° or –45º rotated filter, foragers reoriented significantly to the left (Moore's Paired Test, Nest 1: R=1.598, p < 0.001; Nest 2: R=1.383, p<0.005) or right respectively (Moore's Paired Test, Nest 1: R=1.604,

p<0.001; Nest 2: R=1.328, p<0.005). Shift magnitudes did not differ between +45° and –45° conditions (Watson–Williams F-test, Nest 1: $F_{(1,23)}$=0.155, p=0.697; Nest 2: $F_{(1,20)}$=0.234, p=0.634). Shift magnitudes were significantly larger at Nest 1 compared to Nest 2 (Watson–Williams F-test, $F_{(1,45)}$=8.672, p=0.005), exhibiting shifts near the full e-vector change (39.7±3.2°) while foragers at Nest 2 only exhibited shift magnitudes at about half the 45º rotation (25.2±3.7°).

Waxing lunar phases

Under a *Waxing Quarter Moon* (lunar illumination ~50%), when the e-vector was rotated clockwise (+45°), exit orientations were again significantly shifted (Moore's Paired Test, R=1.787, p<0.001) to the right of their initial heading (mean ± s.e.m.; 37.6±4.1°; Figure 3A). Forager headings were similarly significantly altered (Moore's Paired Test, R=1.734, p<0.001) when the overhead e-vector was rotated counter-clockwise (–45°) with exit orientations to the left of initial headings (mean ± s.e.m. –38.5±6.4°; Figure 3A). After exiting the filter in both conditions, foragers reoriented significantly back to the ambient lunar e-vector either to the left (+45°: Moore's Paired Test, Nest 1: R=1.616, p<0.001) or right (–45°: Moore's Paired Test, Nest 1: R=1.664, p<0.001) of their filter exit heading direction.

Foragers continued to show evidence of attending to the lunar polarisation pattern even under a *Waxing Crescent Moon* (lunar illumination ~20%). Here, when the ambient e-vector was rotated clockwise (+45°), exit orientations were again significantly shifted (Moore's Paired Test, R=1.175, p<0.025) to the right of their initial heading (mean ± s.e.m.; 29.3±9.9°; Figure 3B). Forager headings were similarly significantly altered (Moore's Paired Test, R=1.39, p<0.005) when the overhead e-vector was rotated –45º counter-clockwise, with exit orientations to the left of initial headings (mean ± s.e.m.; –27.6±4.7°; Figure 3B). After exiting the filter in both conditions, foragers reoriented significantly back to the ambient lunar e-vector either to the left (+45°: Moore's Paired Test, Nest 1: R=1.324, p<0.005) or right (–45°: Moore's Paired Test, Nest 1: R=1.223, p<0.025) of their filter exit heading direction. Shift magnitudes were not significantly different between ±45° conditions (Watson–Williams F-test, $F_{(1,20)}$=0.03, p=0.863).

When no ambient lunar e-vector was present (*No Moon*) and the polariser was rotated either clockwise (+45°) or counter-clockwise (–45°), foragers did not significantly alter their paths under the filter (+45°: Moore's Paired Test, R=0.226, p>0.50; mean ± s.e.m.: –5.4±7.4°; –45°: Moore's Paired Test, R = 0.650, p > 0.10; mean ± s.e.m.: 0.7±7.6°; Figure 3C). Foragers also did not significantly reorient after exiting the filter (+45°: Moore's Paired Test, R=0.294, p>0.50; mean ± s.e.m.: –7.1±5.6°; –45°: Moore's Paired Test, R=0.611, p>0.10; mean ± s.e.m.: 1.8±5.1°). Shift magnitudes were not significantly different between ±45° conditions (Watson–Williams F-test, $F_{(1,24)}$=0.016, p=0.899).

When comparing shift magnitudes across lunar phases, *Full Moon f*oragers were not significantly different from either the *Waxing Quarter Moon* or *Waxing Crescent Moon* (Watson–Williams F-test, p>0.05; Figure 5).

Waning lunar phases

Under a *Waning Full Moon*, when the ambient e-vector was rotated clockwise (+45°), exit orientations were shifted significantly to the right of initial headings (mean ± s.e.m. = 16.5±5.3°; Moore's Paired Test, R=1.468, p<0.005; Figure 4A). Headings also shifted significantly to the left of initial headings (mean ± s.e.m.: –15.4±5.1°; Moore's Paired Test, R=1.513, p<0.001; Figure 4A) when the overhead e-vector was rotated –45º counter-clockwise. After exiting the filter in both conditions, foragers reoriented significantly back to the ambient lunar e-vector either to the left (+45°: Moore's Paired Test, Nest 1: R=1.548, p<0.001) or right (–45°: Moore's Paired Test, Nest 1: R=1.247, p<0.025) of their filter exit heading direction. Shift magnitudes were not significantly different between the ±45° conditions (Watson–Williams F-test, $F_{(1,30)}$=0.022, p=0.884).

Under a *Waning Quarter Moon* (lunar illumination ~50%), when the ambient e-vector was rotated clockwise (+45°), exit orientations were shifted to the right of initial headings (mean ± s.e.m. = 20.5±8.4°; Figure 4B), and these changes were significant (Moore's Paired Test, R=1.33, p<0.005). Headings also changed significantly (Moore's Paired Test, R=1.31, p<0.01) when the overhead e-vector was rotated counter-clockwise (–45°) with exit orientations to the left of initial headings (mean ± s.e.m.: –22.5±11.7°; Figure 4B). After exiting the filter in both conditions, foragers reoriented significantly back to the ambient lunar e-vector either to the left (+45°: Moore's Paired Test, Nest 1: R=1.504, p<0.001) or right (–45°: Moore's Paired Test, Nest 1: R=1.246, p<0.025) of their filter exit heading direction. Shift magnitudes were not significantly different between the ±45° conditions (Watson–Williams F-test, $F_{(1,18)}$=0.154, p=0.700).

When comparing shift magnitudes between Waxing and Waning phases, shift magnitude was significantly higher in both *Waxing Full Moon* and *Waxing Quarter Moon* foragers when compared to their *Waning* counterparts (39.8º vs. 16.0º and 38.1º vs. 22.7º respectively; Full Moon: Watson–Williams F-test, $F_{(1,55)}$=21.62, p<0.001; Quarter Moon: Watson–Williams F-test, $F_{(1,44)}$=5.889, p=0.038; Figure 5).

Vector testing

Both *Halfway Release* (~6m vector) and *Halfway Collection & Release* (~2m vector) foragers exhibited significant shifts (*Halfway Release*: Moore's Paired Test, R=1.728, p<0.001; *Halfway Collection & Release:* Moore's Paired Test, R=1.380, p<0.005) to the right of initial headings (mean ± s.e.m.: 41.9±4.9° and 6.8±4.3°; Figure 6AB) when the ambient e-vector was rotated clockwise (+45°). Forager headings also significantly shifted (*Halfway Release*: Moore's Paired Test, R=1.664, p<0.001; *Halfway Collection & Release:* Moore's Paired Test, R=1.07, p<0.05) when the overhead e-vector was rotated counter-clockwise (–45°) with exit orientations to the left of initial headings (mean ± s.e.m.: –46.4±6.3° and 12.7±8.5°; Figure 6AB). After exiting the filter in both conditions, foragers reoriented significantly back to the ambient lunar e-vector either to the left (+45º: *Halfway Release*: Moore's Paired Test, R=1.692, p<0.001; *Halfway Collection & Release:* Moore's Paired Test, R=1.600, p<0.001) or right (–45°: *Halfway Release*: Moore's Paired Test, R=1.604, p<0.001; *Halfway Collection &*

Release: Moore's Paired Test, R=1.274, p<0.01) of their exit heading direction. Shift magnitude was significantly higher in *Halfway Release* foragers compared to *Halfway Collection & Release* foragers tested at the same location 2m from the nest entrance (44.1º and 14.8º respectively; Watson–Williams F-test, $F_{(1,40)}$ = 29.105, p < 0.001; Figure 6AB).

Discussion

These results constitute the first instance of polarised moonlight use for homing and only the second reported instance of its use for orientation in any animal (Figure 7). *Myrmecia midas* foragers predictably altered their heading directions in response to experimental rotations in the ambient overhead lunar polarisation pattern. This ability to detect and attend to polarised moonlight persisted throughout the lunar cycle, with foragers attending to the pattern even under a crescent moon with ~20% lunar illumination. This indicates that polarised moonlight is detectable across the lunar month, making it a stable cue these ants can use when moving or updating their path integrator overnight.

While they can utilise the lunar polarisation pattern through the lunar cycle, foragers exhibited reduced heading shifts during the waning lunar phases, during which the moon's absence for a portion of the night leads to a cue gap overnight. These reductions in observed shifts are likely due to either decreased weighting or a degradation in the celestial compass due to periods when no celestial cues are available. The shift magnitude differences between conditions also point to moonlight polarisation being continuously tracked throughout the overnight period, in line with this celestial cue being integrated into the navigator's path integrator. Further evidence of this cue's integration into the path integrator is illustrated in our *Halfway* testing conditions. Here, foragers with longer home vectors under a full moon responded almost fully to e-vector changes regardless of their distance to the nest, while short vector distances led to less than half the e-vector shift, corresponding with decreases in vector cue strength at small distances (Wystrach et al. 2015). These changes align exactly with how *M. midas*, uses polarised sunlight as part of its vector-based homing during evening and morning twilight (Freas et al. 2017b; Figure 7), suggesting that polarised moonlight is detected and integrated into its path integrator along the same visual pathways as sunlight.

Moonlight vs. sunlight

The nocturnal bull ants *Myrmecia pyriformis* and *Myrmecia midas* are known to use the solar polarised light pattern during the twilight periods (Reid et al. 2011; Freas et al. 2017ab), yet both species are active after twilight, when solar polarisation cues are absent (Reid et al. 2011, 2013; Freas et al. 2017ab). The observed true nocturnal navigation in these animals could be driven by the moon's presence with *M. pyriformis*, showing more foraging activity on full moon nights (Reid et al., 2013). *M. midas* exhibits a high level of overnight activity, with almost half of foragers returning before the morning twilight. *M midas* also navigates through heavily canopied forest habitat where the moon may be occluded but its

polarisation pattern across the sky remains unobstructed. Thus, *M. midas* makes for an interesting species to characterise lunar and solar polarised light detection.

While we cannot compare solar and moonlight polarisation navigation in outbound ants (outbound foraging is highly correlated with evening twilight when the solar light would overpower any moonlight polarisation pattern), striking similarities occur when comparing solar and moonlight polarisation navigation in ants homing to the nest (Figure 7). In the solar polarisation study (Freas et al. 2017b), inbound foragers tested during morning twilight at 4–6 m from their nest altered their paths under the filter for almost the full 45º solar e-vector manipulation (–45° rotation: –41.16°/ +45° rotation: 34.13°; Figure 7) but only compensated for around half of the rotation when tested 1–2 m from the nest (–45° rotation: –24.86°/ +45° rotation: 19.73º). We see the same pattern with polarised moonlight with foragers exhibiting near full shifts 5m from Nest 1 (–45° rotation: –41.4°/ +45° rotation: 38.4°) and half shifts 2m from Nest 2 (–45° rotation: –27.1°/ +45° rotation: 23.4°; Figure 7).

We also observed consistent slight under-estimation in the shifts even when foragers had a long vector. Observations of ants after the filter was placed overhead suggest that heading updates are not immediate, occurring only after the ant travels along its original heading a few centimetres (~5cm). This means that even if the ant fully shifts its heading, the delay will cause our measurements at filter exit to slightly underestimate each individual's position since we measure the angle from where the lunar cue changes (as filter was placed overhead) and not the position at which the ant altered its heading.

Moonlight and the path integrator

We also see the same full heading changes to both solar- and moonlight-polarisation-pattern rotations when foragers were released halfway to the nest and tested 2m with a larger vector (Solar: –45° rotation: –35.77°/ +45° rotation: 39.42°; Moonlight: –45° rotation: –46.4°/ +45° rotation: 41.9; Figure 7). In the *Halfway collect & Release* condition, this shift significantly decreased at the same testing location, indicating that the vector length and not the testing site dictated this adjustment in cue weighting. These findings suggest that under the filter, foragers use any available celestial and terrestrial cues that are still visible, yet the weighting of the polarisation pattern appears to change in accordance with the vector state (Burkhalter 1972; Narendra 2007) and not its test locaiton close to the nest tree, which could be a potentially highly salient landmark. This leads to several interesting implications. First, these ants weight the polarisation cue more highly and perform larger heading shifts when their current path integrator distance is longer. These increased shift magnitudes align with the hypothesis that longer accumulated vectors, ants increase the weighting given this cue (Burkhalter 1972; Narendra 2007; Wystrach et al. 2015; Freas et al. 2017b). Secondly, these ants are using polarised moonlight precisely the same way they use solar polarisation, meaning that just as polarised moonlight is likely integrated into the forager's path integrator throughout the night. Moonlight polarised light is likely processed through the same visual pathways as polarised sunlight, meaning that these ants can use the same underlying neural architecture for polarised solar light and polarised moonlight cues. The

only limiting factor would be the navigator's detection threshold (at least 20% lunar luminance), which can be accomplished through the specialised eye morphology of these ants (Zeil et al. 2014).

Polarised moonlight and lunar phase

Foragers showed clear evidence of detecting and employing polarised moonlight when homing to the nest across the lunar cycle, even on waxing crescent moon nights. This aligns with polarised moonlight's use in dung beetles, with individuals able to maintain their straight-line paths under quarter and crescent moon e-vectors (Dacke et al. 2004). Furthermore, the lack of a shift-magnitude reduction between full and crescent nights suggests no reduction in detection. While we could have continued to test with smaller portions of the moon surface illuminated, a reduction in shift magnitude could result from either physiological limits in detection or 'decisional' processes in how much weight to accord the cue. Behavioural responses and physiological limits cannot be untangled behaviourally, and detection thresholds would require intracellular recordings under dim polarised light. Finally, the lack of shifts with *No Moon* foragers indicates that these navigators do not fall back on memories of the evening or morning solar e-vector when presented one overnight.

One unexpected finding was the reduction in shift magnitude under waning moons relative to waxing moons. When we first tested foragers under *Waning Full Moons* and Waning *Quarter Moons* they showed clear evidence of attending to the polarisation cue, but shift magnitudes were significantly smaller compared to the *Waxing Moon* conditions. This reduction in shift magnitude suggests that polarised moonlight was being detected but it was weighted weakly, perhaps due to the celestial compass incorporation into the path integrator being interrupted. This observed reduction in shift magnitude likely results from the period overnight in which the waning moon was absent from the night sky, meaning foragers could not attend to a consistent polarisation pattern throughout the night. The moonlight pattern only becomes visible in the sky once the moon reaches –18º below the horizon; thus, waning-moon nights present periods when there are neither solar nor lunar cues available to maintain the compass, potentially degrading the compass portion of the vector estimate. This suggests that when the moon is waxing and present throughout the overnight period (at least until moonset), *M. midas* foragers are continuously tracking it and integrating this compass cue into their path integrator. When there are periods overnight when the moon and its polarisation pattern are absent, it is either weighted weakly or it becomes misaligned with the true homing direction since the celestial compass incorporation into the path integrator has been interrupted with overnight periods with no detectable celestial cues.

Conclusions

In the first evidence of polarised moonlight being used to navigate to a goal location, inbound *M. midas* foragers detect and respond predictably to rotations of the moonlight e-

vector orientation under a filter and reorient back to the ambient e-vector after filter exit. This ability occurs across lunar phase, suggesting that polarised moonlight is a detectable cue throughout the lunar month. Heading changes due to polarised moonlight align with responses to polarised sunlight as part of the path integrator during solar twilight. This indicates that polarised moonlight is likely detected and integrated into the ant's path integrator for inbound homing along the same visual pathways as polarised sunlight. Reductions in heading shifts due to differences in PI vector lengths, and periods without access to polarised light patterns suggest that these animals can weight the information provided by celestial polarised light. In so doing these foragers can cater their navigational decisions proportionate to closely match the reliability of available navigational information.

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Conflicts of interest

The authors declare no conflicts of interest associated with this work.

Ethics

There are no state or federal governmental regulations guiding the research of invertebrates in Australia.

Data Availability

All data, documentation and code will be made available online at osf.io

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Figure Captions

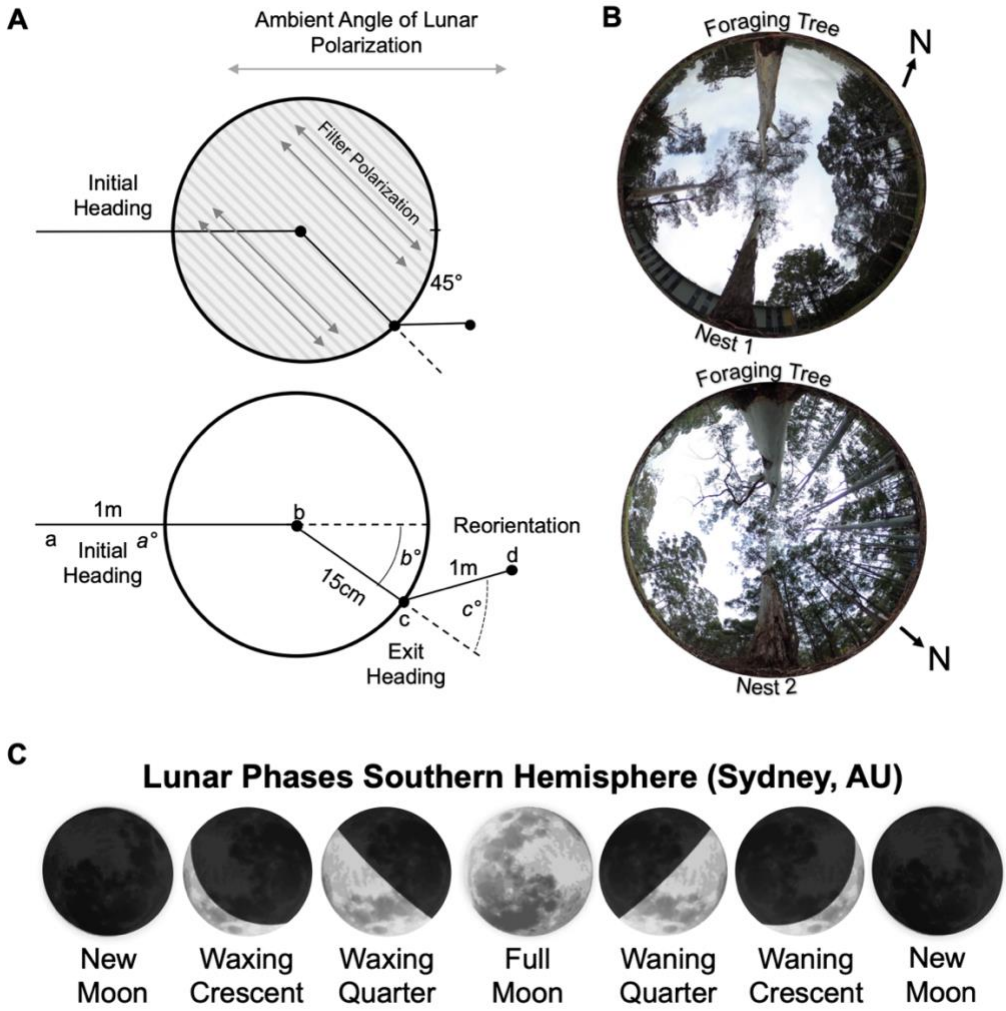


Figure 1. Diagram of the polarisation filter and changes to the ambient lunar polarisation pattern. (A) During the inbound journey, a linear polarisation filter was placed over the forager, rotating the overhead e-vector by ±45°. Panel depicts the positional measurements recorded during testing. Initial orientation routes were measured from the foraging tree release point (a) to when the polarisation filter was placed over each forager (b). Exit orientations were measured from the filter centre (b) to the forager's exit location at the filter edge (c). Route directions under the filter (b°) were calculated from the forager's initial route direction zeroed. Reorientations were measured from the forager's exit location from the polarisation filter (c) to the forager's path 1m after exit (d). Reorientation directions (c°) were calculated from the under-filter route direction zeroed. (B) Images of the sky and canopy cover at both nests. Photos were taken at the on-route midpoint between the foraging and nest trees. (C) Lunar phases denote the sunlit part of the moon's surface and where this area is increasing (Waxing) or decreasing (Waning). The lunar phase cycle repeats every 29.5 days. moon images are public domain art accessed through wiki commons (https://commons.wikimedia.org/).

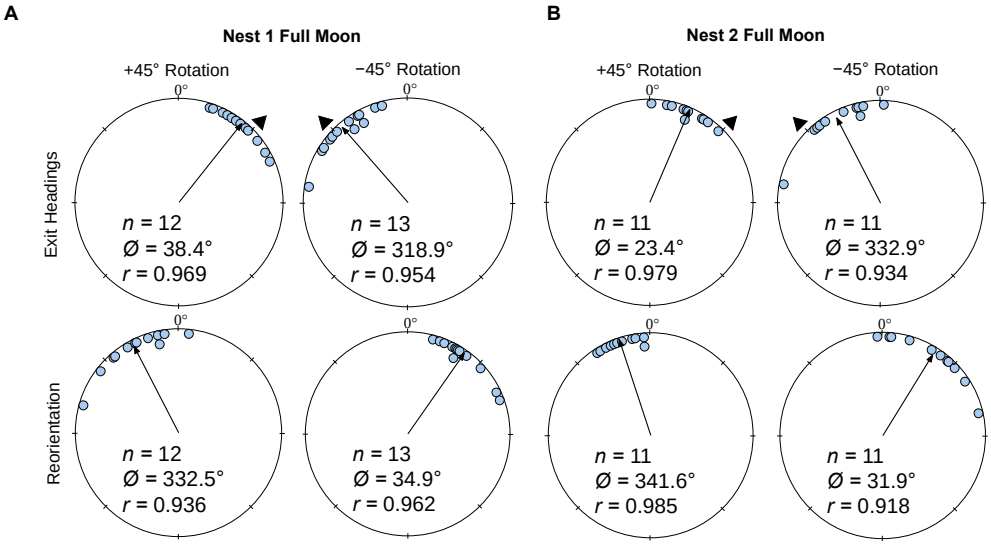


Figure 2. Circular distributions of headings during the original full moon conditions. In both conditions, testing occurs in the nights preceding the full moon (illumination > 80%) with the moon waxing. Circular plot shifts show the exit orientations of individual foragers from their initial headings while the reorientation represents the change in headings 1m after exiting the filter. Triangles denote the ±45° e-vector rotation. The arrow denotes the length and direction of the mean vector. (*A*) Nest 1 foragers, 5m from the nest (6.0m foraging route). (*B*) Nest 2 foragers, 2m from the nest (3.1m foraging route). *n*, number of individuals; *Ø*, mean vector; *r*, length of the mean vector.

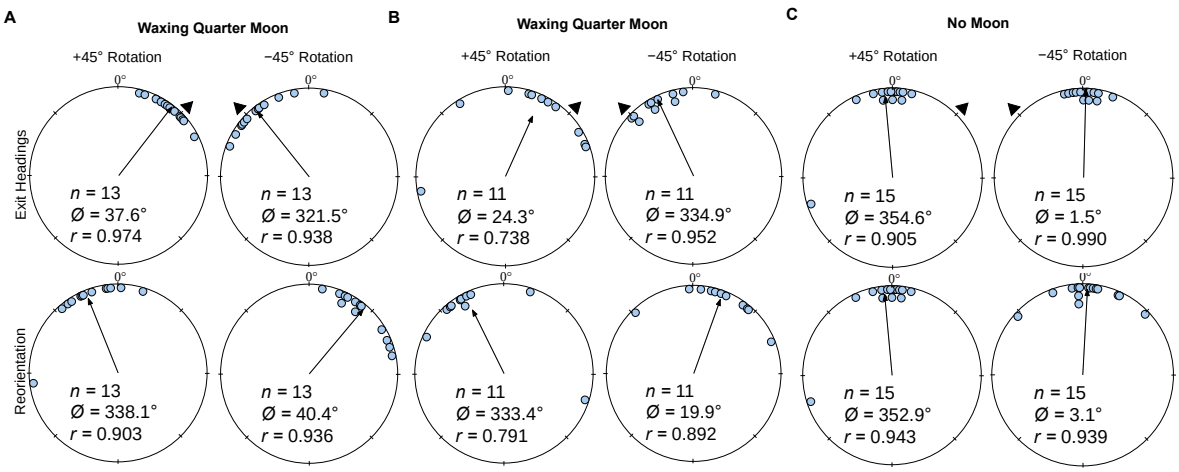


Figure 3. Circular distributions of headings during (A) Waxing *Quarter Moon,* (B*) Waxing Crescent Moon* and (C) *No Moon* conditions. Circular plot shifts show the exit orientations of individual foragers from their initial headings while the reorientation represents the change in headings 1m after exiting the filter. Triangles denote ±45° e-vector rotation. The arrow denotes the length/direction of the mean vector. *n*, number of individuals; *Ø*, mean vector; *r*, length of the mean vector.

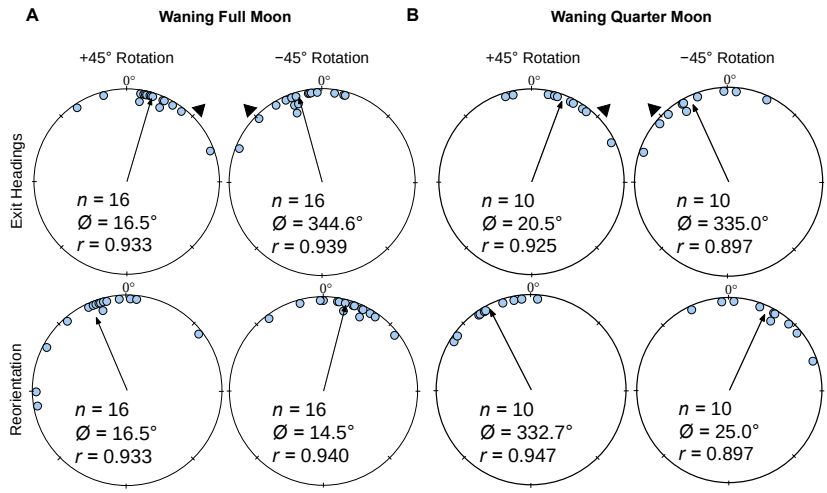


Figure 4. Circular distributions of headings during (A) Waning Full *Moon,* (B*) Waning Quarter Moon* conditions. Circular plot shifts show the exit orientations of individual foragers from their initial headings while the reorientation represents the change in headings 1m after exiting the filter. Triangles denote ±45° e-vector rotation. The arrow denotes the length/direction of the mean vector. *n*, number of individuals; *Ø*, mean vector; *r*, length of the mean vector.

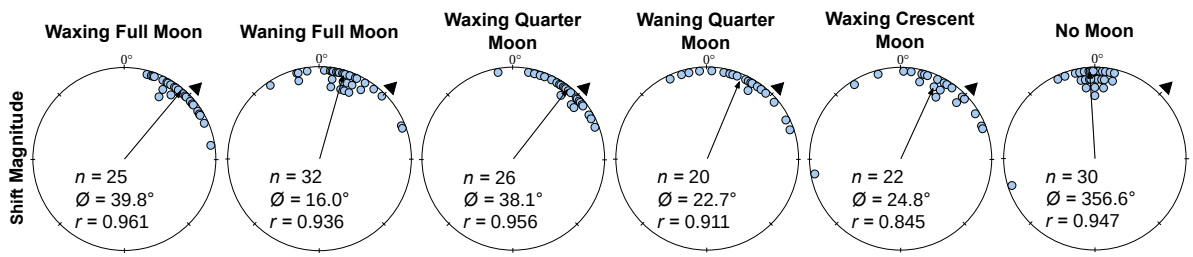


Figure 5. Shift magnitudes for lunar phase conditions at Nest 1. Each circular plot shows the ±45° combined shifts for each condition. Triangles denote +45° e-vector rotation; data from –45º were mirrored and combined). The arrow denotes the length and direction of the mean vector. *n*, number of individuals; *Ø*, mean vector; *r*, length of the mean vector.

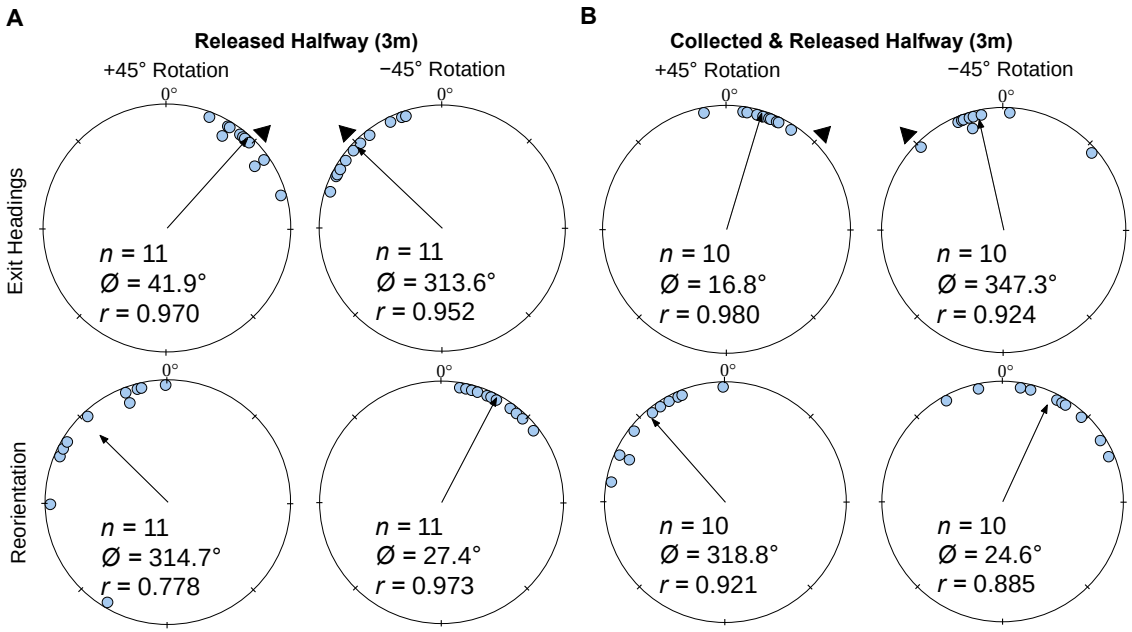


Figure 6. Circular distributions of *M. midas* headings during (A) *Released Halfway, (*B*) Collected & Released Halfway* conditions. Foragers in both conditions were tested at 2m from the nest with *Released Halfway* foragers having a long 6.0m vector and *Collected & Released Halfway* foragers having a 3.1m vector. Circular plot shifts show the exit orientations of individual foragers from their initial headings while the reorientation represents the change in headings 1m after exiting the filter. Triangles denote ±45° e-vector rotation. The arrow denotes the length and direction of the mean vector. *n*, number of individuals; *Ø*, mean vector; *r*, length of the mean vector.

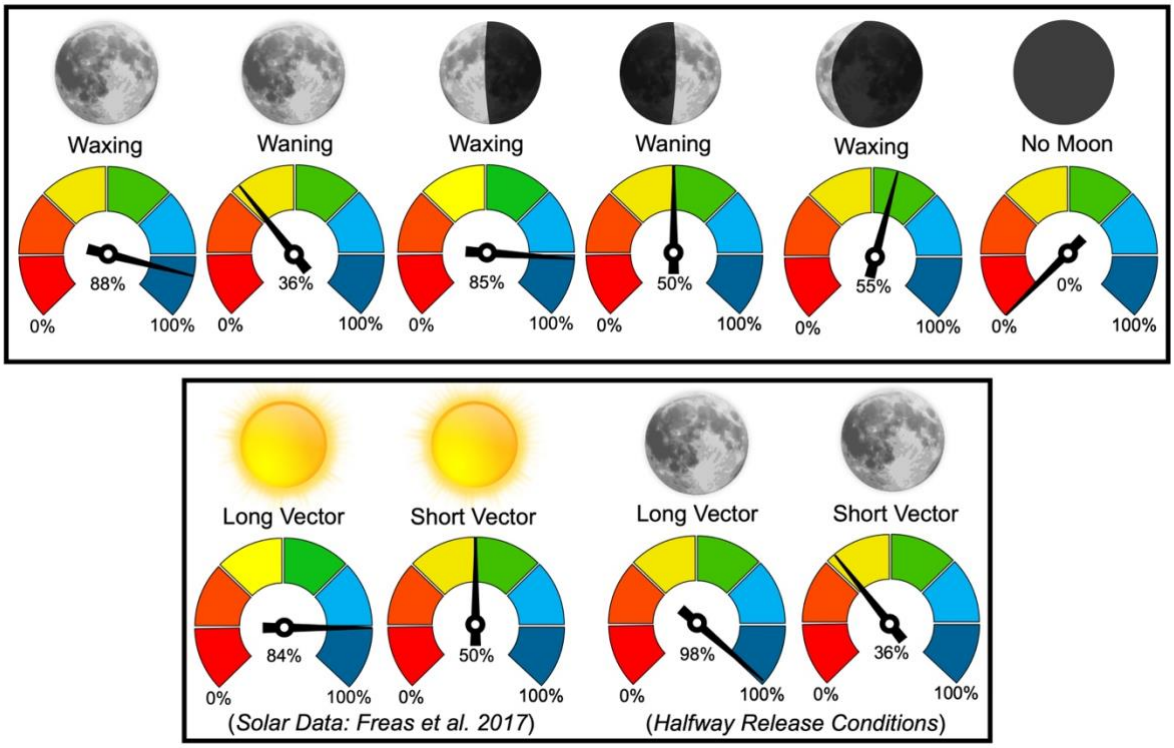
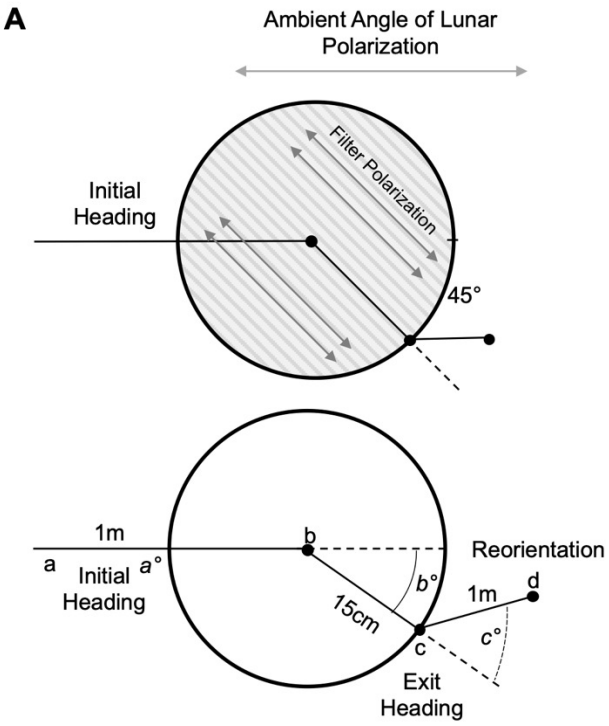
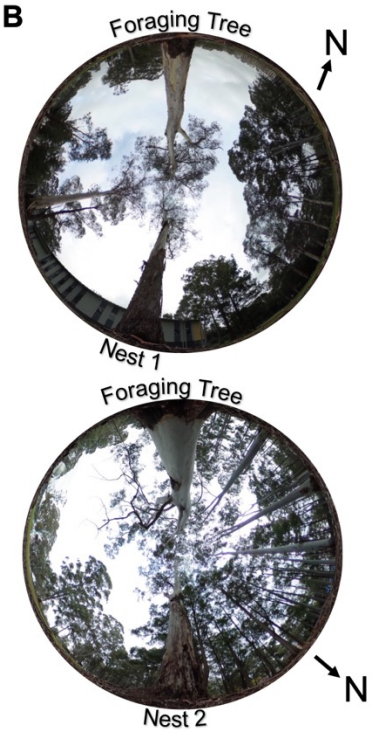
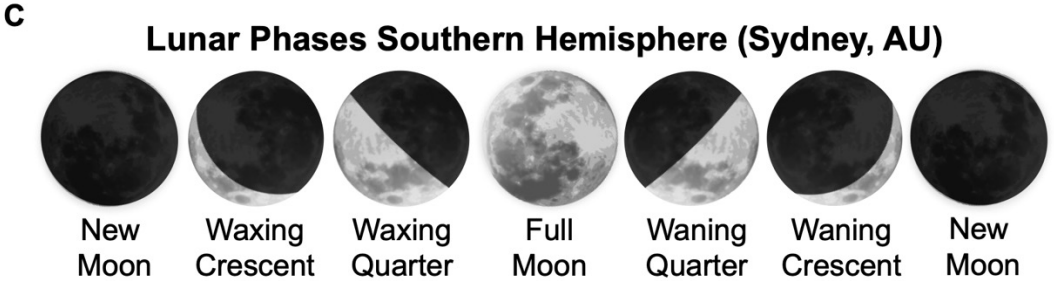
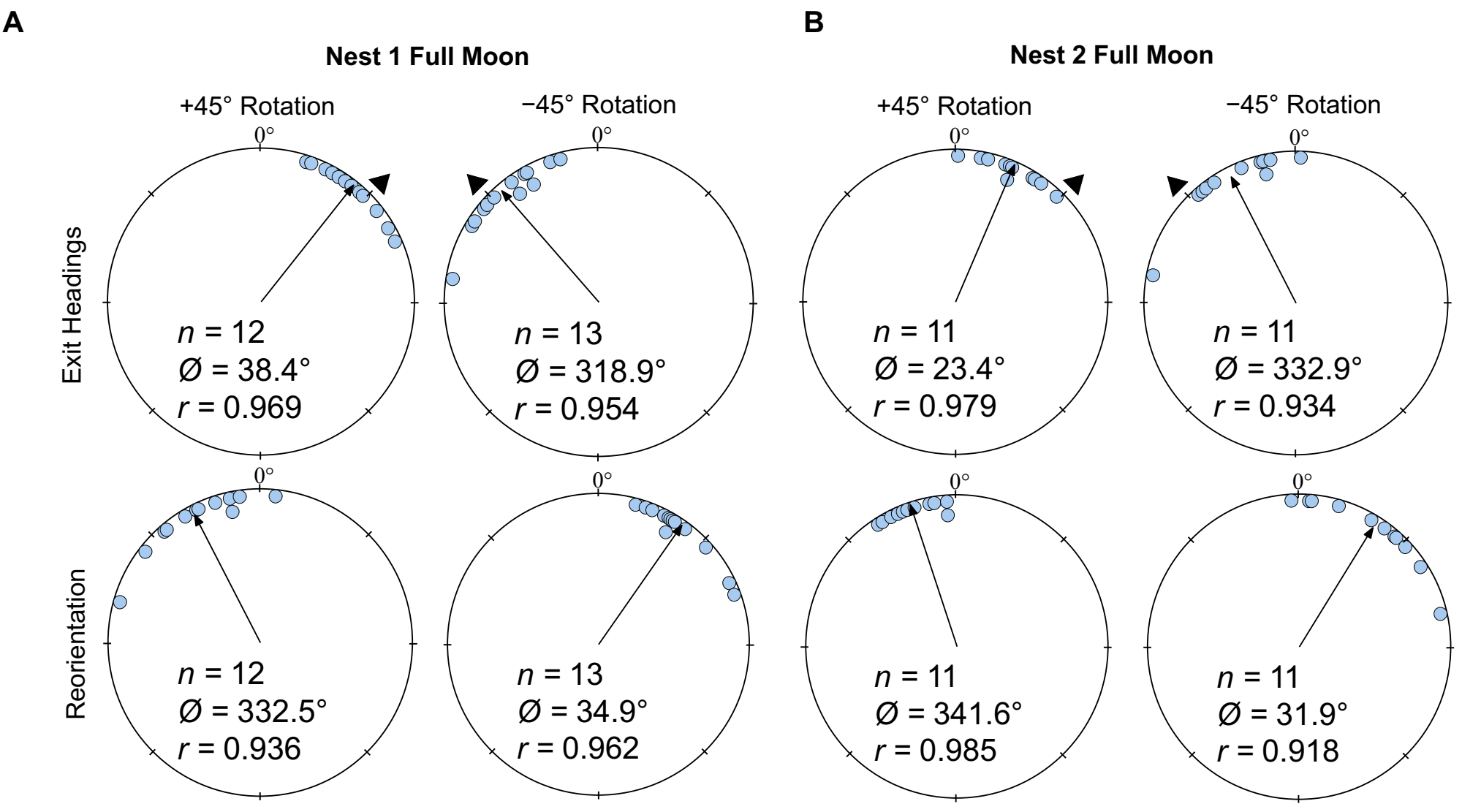


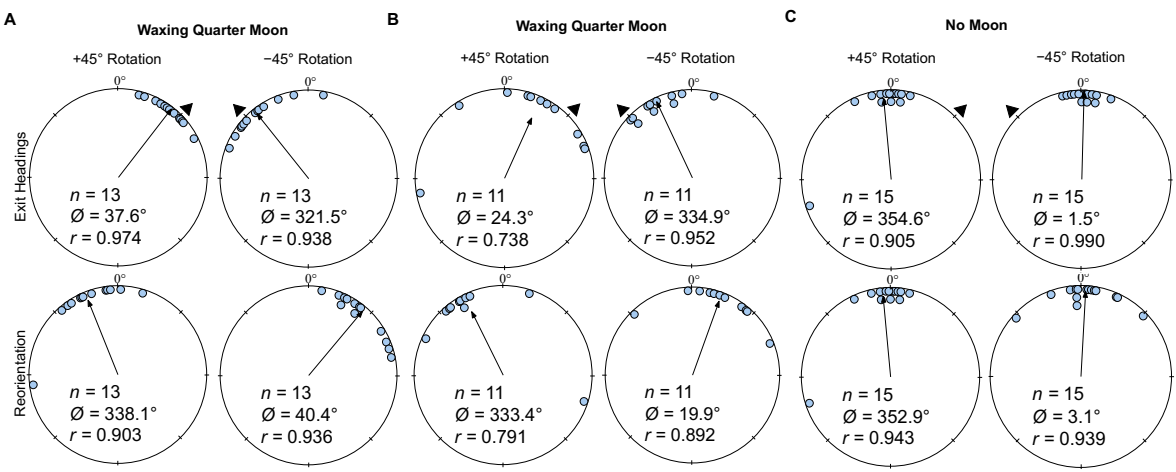
Figure 7. Mean shift magnitudes, +45º and –45º combined, reported as percentages of the 45º e-vector rotation (100% = 45º). Vector data for solar polarised light is reported from Freas et al. 2017, Royal Society Open Science. Sun and moon images are public domain art accessed through wiki commons (https://commons.wikimedia.org/).

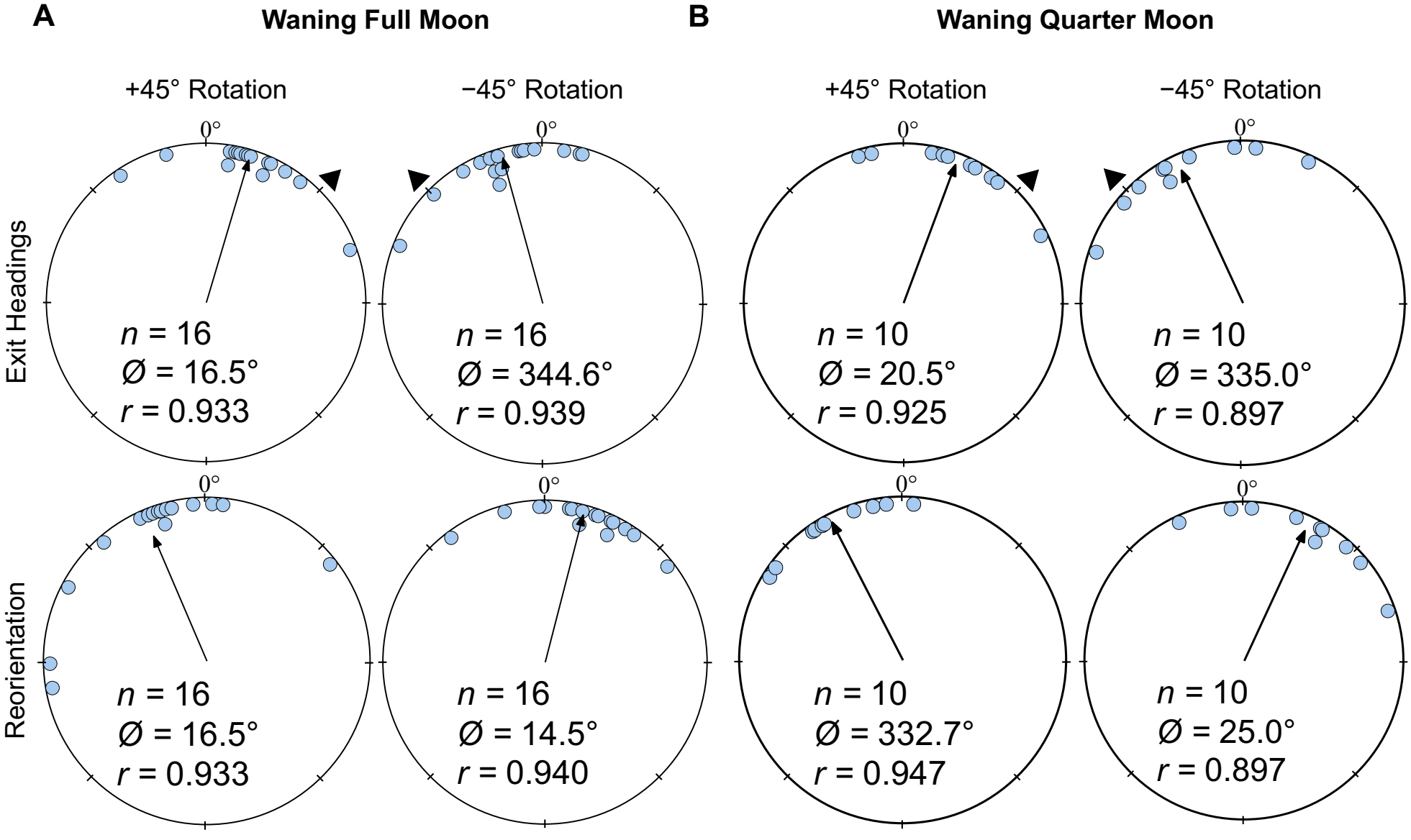

A
Ambient Angle of Lunar Polarization
Filter Polarization
Initial Heading
45°
1m
a
Initial Heading
a°
b
15cm
b°
Reorientation
1m
d
c
c°
Exit Heading

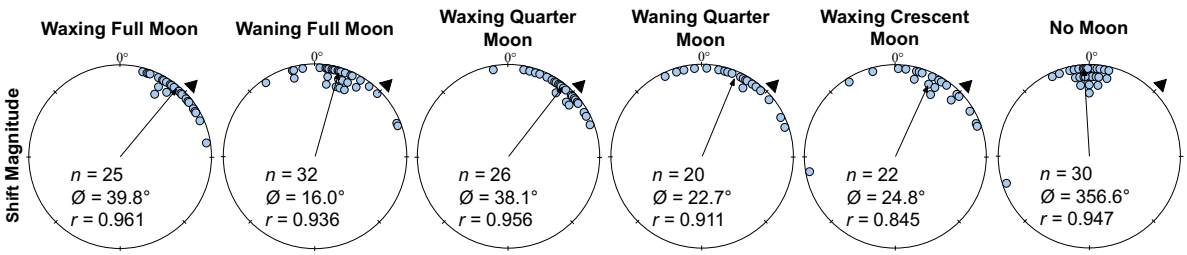

B
Foraging Tree
N
Nest 1
Foraging Tree
N
Nest 2

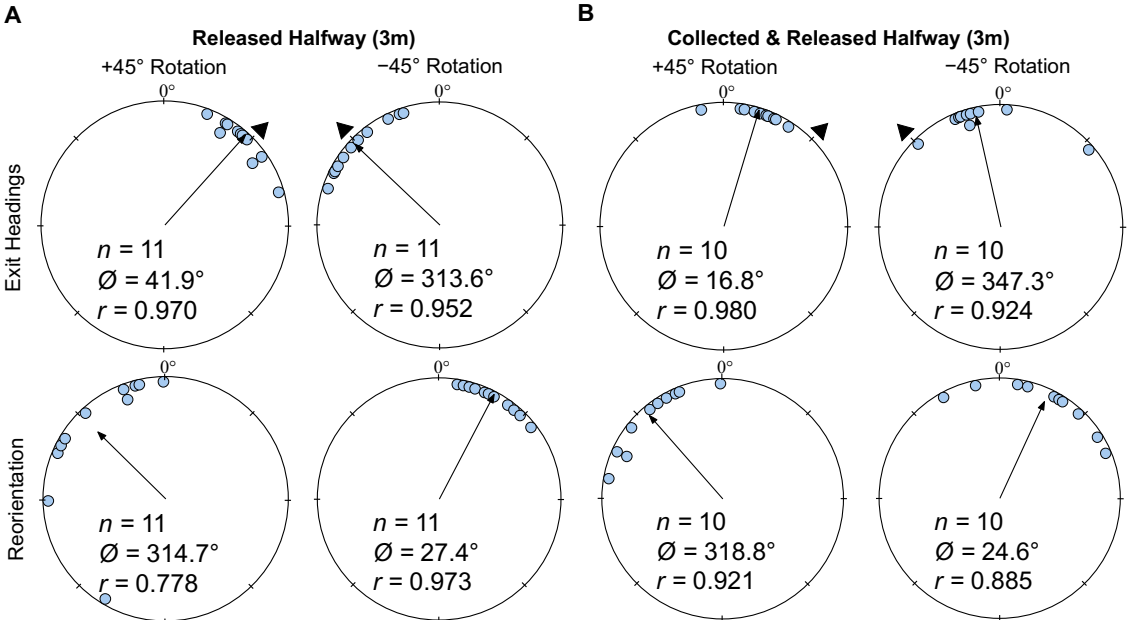

C
Lunar Phases Southern Hemisphere (Sydney, AU)
New Moon
Waxing Crescent
Waxing Quarter
Full Moon
Waning Quarter
Waning Crescent
New Moon

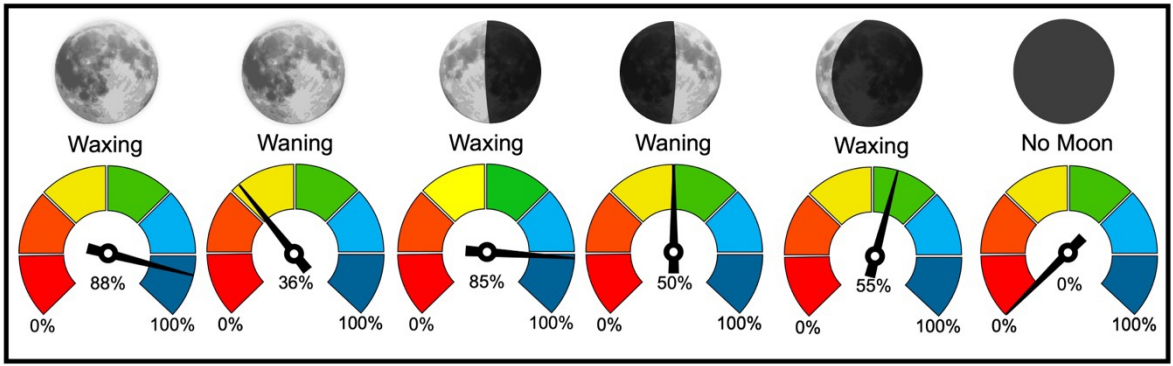

A
Nest 1 Full Moon
+45° Rotation
−45° Rotation
Exit Headings
Reorientation
0°
n = 12
Ø = 38.4°
r = 0.969
n = 13
Ø = 318.9°
r = 0.954
n = 12
Ø = 332.5°
r = 0.936
n = 13
Ø = 34.9°
r = 0.962
B
Nest 2 Full Moon
+45° Rotation
−45° Rotation
n = 11
Ø = 23.4°
r = 0.979
n = 11
Ø = 332.9°
r = 0.934
n = 11
Ø = 341.6°
r = 0.985
n = 11
Ø = 31.9°
r = 0.918

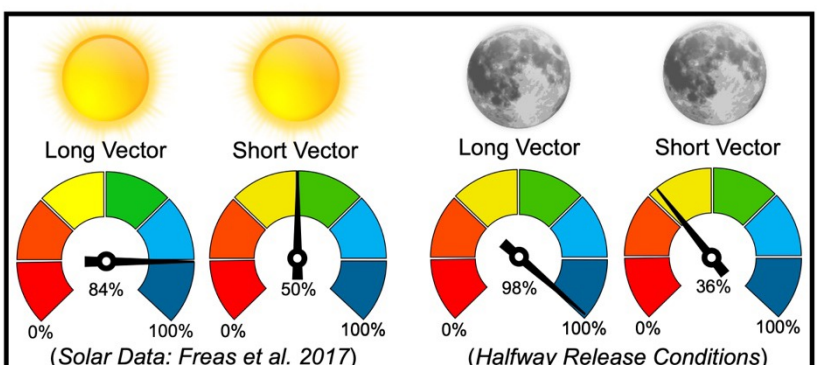

A
Waxing Quarter Moon
+45° Rotation
−45° Rotation
B
Waxing Quarter Moon
+45° Rotation
−45° Rotation
C
No Moon
+45° Rotation
−45° Rotation
Exit Headings
Reorientation
0°
n = 13
Ø = 37.6°
r = 0.974
n = 13
Ø = 321.5°
r = 0.938
n = 11
Ø = 24.3°
r = 0.738
n = 11
Ø = 334.9°
r = 0.952
n = 15
Ø = 354.6°
r = 0.905
n = 15
Ø = 1.5°
r = 0.990
n = 13
Ø = 338.1°
r = 0.903
n = 13
Ø = 40.4°
r = 0.936
n = 11
Ø = 333.4°
r = 0.791
n = 11
Ø = 19.9°
r = 0.892
n = 15
Ø = 352.9°
r = 0.943
n = 15
Ø = 3.1°
r = 0.939


A
Waning Full Moon
B
Waning Quarter Moon
+45° Rotation
−45° Rotation
+45° Rotation
−45° Rotation
Exit Headings
Reorientation
0°
n = 16
Ø = 16.5°
r = 0.933
n = 16
Ø = 344.6°
r = 0.939
n = 10
Ø = 20.5°
r = 0.925
n = 10
Ø = 335.0°
r = 0.897
n = 16
Ø = 16.5°
r = 0.933
n = 16
Ø = 14.5°
r = 0.940
n = 10
Ø = 332.7°
r = 0.947
n = 10
Ø = 25.0°
r = 0.897


Waxing Full Moon
Waning Full Moon
Waxing Quarter Moon
Waning Quarter Moon
Waxing Crescent Moon
No Moon
Shift Magnitude
0°
n = 25
Ø = 39.8°
r = 0.961
n = 32
Ø = 16.0°
r = 0.936
n = 26
Ø = 38.1°
r = 0.956
n = 20
Ø = 22.7°
r = 0.911
n = 22
Ø = 24.8°
r = 0.845
n = 30
Ø = 356.6°
r = 0.947


A
Released Halfway (3m)
+45° Rotation
−45° Rotation
B
Collected & Released Halfway (3m)
+45° Rotation
−45° Rotation
Exit Headings
Reorientation
0°
n = 11
Ø = 41.9°
r = 0.970
n = 11
Ø = 313.6°
r = 0.952
n = 10
Ø = 16.8°
r = 0.980
n = 10
Ø = 347.3°
r = 0.924
n = 11
Ø = 314.7°
r = 0.778
n = 11
Ø = 27.4°
r = 0.973
n = 10
Ø = 318.8°
r = 0.921
n = 10
Ø = 24.6°
r = 0.885


Waxing
88%
0%
100%
Waning
36%
0%
100%
Waxing
85%
0%
100%
Waning
50%
0%
100%
Waxing
55%
0%
100%
No Moon
0%
0%
100%


Long Vector
84%
0%
100%
Short Vector
50%
0%
100%
(Solar Data: Freas et al. 2017)
Long Vector
98%
0%
100%
Short Vector
36%
0%
100%
(Halfway Release Conditions)