Insect orientation to polarized moonlight

An African dung beetle uses the moonlit sky to make a swift exit after finding food.

Moonlight, like sunlight[1], scatters when it strikes tiny particles in the atmosphere, giving rise to celestial polarization patterns[2]. Here we show that an African dung beetle, *Scarabaeus zambesianus,* uses the polarization of a moonlit sky to orientate itself so that it can move along a straight line. Many creatures use the Sun's light-polarization pattern to orientate themselves[3,4], but *S. zambesianus* is the first animal known to use the million-times dimmer polarization of moonlight for this purpose.

S. zambesianus starts to forage on the wing for fresh dung at around sunset. Once a source has been located, the beetle quickly forms a ball of dung with its front legs and head (Fig. 1a), and rolls it away in a straight line — the most efficient path for escaping aggressive competition for food in the dung pile. To orientate itself to follow a straight line at dusk, *S. zambesianus* relies on the polarization pattern formed around the setting Sun to maintain its departure bearing[5].

But after astronomical twilight, when the Sun is more than 18° below the horizon, this cue is no longer available. To find out whether the beetles are able to use the polarization of moonlight instead, we monitored their movements under the night-time sky. We found that on moonlit nights, the beetles rolled radially in straight lines away from the dung (Fig. 1b), but that on nights without a moon, they no longer followed a straight-line path (Fig. 1c).

To test whether the beetles' orientation depends on the polarization of the moonlit sky, rather than on the moon itself, we hid the moon from view by shading an arena that contained the beetle and its ball from the rising moon, and placed a polarizing filter over a ball-rolling beetle. The filter had its electric vector (*e*-vector) transmission axis orientated perpendicularly to the dominant orientation of the *e*-vector present in the zenith and along the lunar vertical, so the moon's polarized-light pattern appeared to turn through 90° as the beetles continued to roll beneath the filter. In response to this light rotation, the beetles turned close to the expected 90°, either left or right (Fig. 1d, e). The symmetrical pattern of polarized light from the sky does not allow the beetles to discriminate between left and right. As a control, the experiment was repeated with the filter's *e*-vector orientation parallel to that of the sky, and this resulted in the beetle maintaining its rolling direction under the filter (Fig. 1e). We conclude that it was not the fivefold drop in light intensity experienced by beetles covered by a filter that caused them to change direction.

Our results indicate that these dung beetles orientate by using the polarization pattern of moonlight. Receptors for polarized-light analysis have recently been found in the dorsal-most part of the eye of *S. zambesianus*[5]. By using the polarization of moonlight for orientation, *S. zambesianus* is able to extend its foraging time. Although, to our knowledge, this is the first description of an animal using the moon's polarization pattern as a nocturnal compass, this ability may turn out to be widespread in the animal kingdom.

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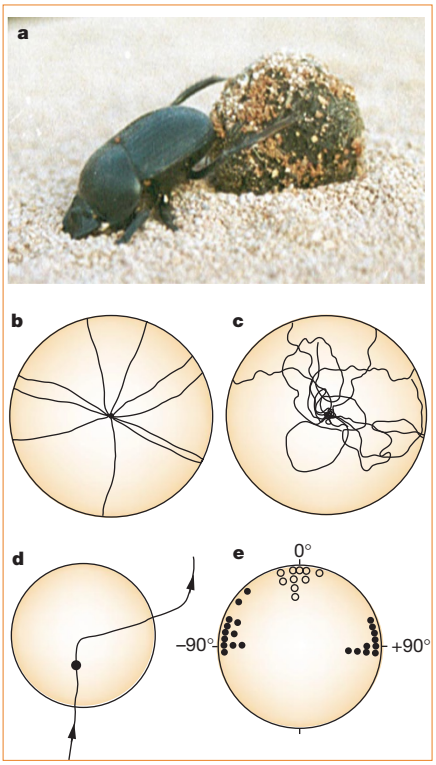

Figure 1 Polarization of moonlight and orientation in African dung beetles. **a,** The beetle rolling a ball of dung. **b, c,** Paths taken by beetles ($n = 10$) moving dung balls outwards from the centre of an arena (diameter, 3 m). On a moonlit night (**b**), beetles orientate along straight paths; on moonless nights (**c**), their direction is random. Maximum light intensities were 3.53×10^{-2} cd m^{-2} and 2.80×10^{-4} cd m^{-2} in **b** and **c**, respectively. **d,** Change in direction (turn to the right by +70°) taken by a beetle when a perpendicularly polarizing filter (Polaroid HN22; circle represents the extent of the 42-cm-diameter filter) is placed over the beetle at the point indicated by the dot; the beetle resumes its direction of travel on exposure to the open sky. **e,** Average angles of turn made by 22 beetles when covered by the same filter as in **d** (filled circles; binned in 5° intervals): left turns, $n = 12$, $-77.0° \pm 14.7°$ (mean ± s.d.); right turns, $n = 10$, $+87.9° \pm 9.3°$. Under a parallel-polarizing filter ($n = 10$), beetles deviated by an average absolute angle of $6.7° \pm 5.5°$ (open circles; binned in 5° intervals) from the path they were following before filter placement.

COMMUNICATIONS ARISING

Photosynthesis

A new function for an old cytochrome?

In many cyanobacteria and algae, cytochrome c_6 transports electrons between the cytochrome *bf* complex and photosystem I, replacing plastocyanin when copper is deficient. Higher plants, however, were thought to lack cytochrome c_6 (refs 1,2) until the existence of a modified form in several species was inferred from genomic evidence[3]. By measuring oxygen evolution with inside-out thylakoids, Gupta *et al.* inferred that heterologously expressed *Arabidopsis* cytochrome c_6 can replace plastocyanin from *Synechocystis* or *Arabidopsis* in reconstitution experiments *in vitro*[4]. From structural and kinetic evidence, however, we find that *Arabidopsis* cytochrome c_6 cannot carry out the same function as *Arabidopsis* plastocyanin or as cytochrome c_6 from the alga *Monoraphidium braunii*. This suggests that cytochrome c_6 in higher plants may have lost its original primitive function in photosynthesis.

The ultraviolet/visible-light absorption spectrum of reduced *Arabidopsis* cytochrome c_6 expressed in *Escherichia coli* is similar to that of *Monoraphidium* cytochrome c_6, except that the absorption bands of the plant cytochrome are shifted towards the red (α-peaks are at 554.5 and 552.5 nm for plant and algal cytochromes, respectively; results not shown).

As expected, the midpoint redox potential of *Arabidopsis* plastocyanin (365 mV) is

similar to that of *Monoraphidium* cytochrome c_6 (358 mV), but surprisingly, that of *Arabidopsis* cytochrome c_6 (140 mV) is lower. *Arabidopsis* cytochrome c_6 would therefore be thermodynamically unsuitable for oxidizing cytochrome *f* (redox potential, 320 mV; ref. 5), but could, in principle, donate electrons to photosystem I. However, kinetic analysis of laser-flash-induced reduction of *Arabidopsis* photosystem I reveals that *Arabidopsis* cytochrome c_6 is 100 times less effective than plastocyanin as an electron donor (Fig. 1a), whereas algal cytochrome c_6 and *Arabidopsis* plastocyanin react at similar rates. The traces for *Arabidopsis* plastocyanin and *Monoraphidium* cytochrome c_6 are both biphasic, as is typical in eukaryotic systems[6].

Figure 1b shows that the observed rate constant (k_{obs}) for the overall reaction with *Arabidopsis* cytochrome c_6 does not significantly increase even at high concentrations of electron-donor protein, whereas k_{obs} increases to a plateau for the slow phase with *Arabidopsis* plastocyanin and *Monoraphidium* cytochrome c_6.

The dependence on ionic strength of k_{obs} clarifies the lack of reactivity of *Arabidopsis* cytochrome c_6 towards photosystem I (Fig. 1c). If this were due to charge repulsion between the donor and acceptor sites on the two proteins, k_{obs} should increase with ionic strength as a result of charge screening. This was not the case. By contrast, however, *Arabidopsis* plastocyanin and *Monoraphidium* cytochrome c_6 produce profiles characteristic of other eukaryotes[6] (Fig. 1c).

In eukaryotic systems, electrostatic interactions between plastocyanin or cytochrome c_6 and photosystem I are dominated by positive charges on the acceptor and negative charges on the donor adjacent to the electron-transfer sites[2,7]. *Arabidopsis* plastocyanin and *Monoraphidium* cytochrome c_6 both have the negative surface electrostatic potential that typifies eukaryotic proteins, whereas *Arabidopsis* cytochrome c_6 has a positive area close to the solvent-exposed haem (Fig. 2). *Arabidopsis* cytochrome c_6 should therefore be unsuitable as an electron donor to photosystem I, as our results show (Fig. 1).

By contrast, Gupta *et al.*[4] reported that *Arabidopsis* cytochrome c_6 was as effective as plastocyanin or cytochrome c_6 from *Synechocystis* in stimulating electron transfer. However, the *Synechocystis* proteins are poor electron donors to higher-plant photosystem I (ref. 6). Although we cannot exclude the possibility that the *Arabidopsis* protein differs from that expressed in *E. coli* (or *Synechocystis*[4]), we know of no modification that could alter the surface electrostatic properties sufficiently to allow reaction with photosystem I. We conclude that *Arabidopsis* cytochrome c_6 is not an effective donor to its own photosystem I. The true function of this molecule may be related to the loop extension of 12 residues (shown in yellow in Fig. 2) that is a unique feature of higher-plant cytochrome c_6 (ref. 3).

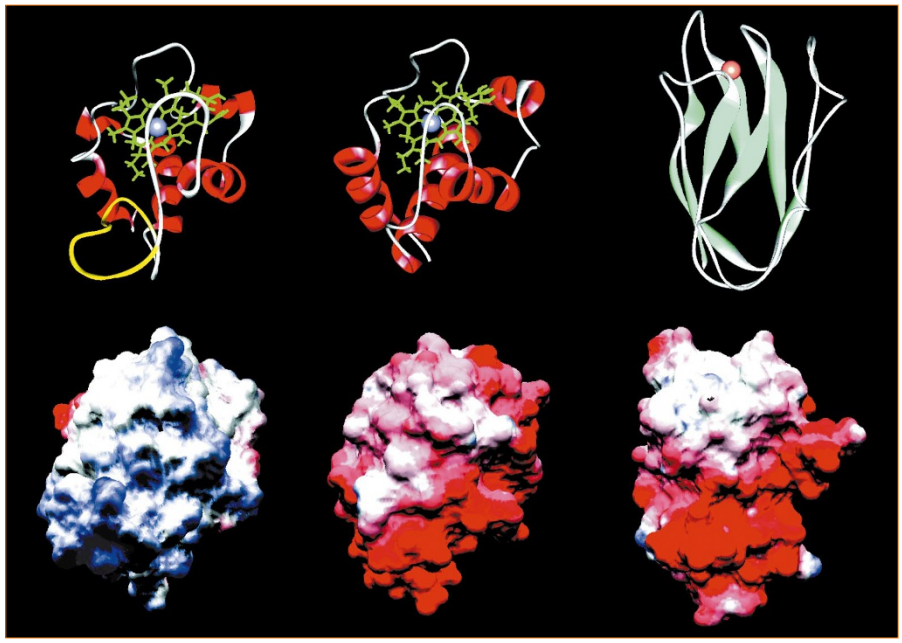

Figure 2 Structural models (top) and surface electrostatic-potential distribution (bottom) of, from left to right, *Arabidopsis* cytochrome c_6, *Monoraphidium* cytochrome c_6 and *Arabidopsis* plastocyanin. The molecules are similarly orientated: *Monoraphidium* cytochrome c_6 and *Arabidopsis* plastocyanin are placed with their respective negatively charged areas towards the front; *Arabidopsis* cytochrome c_6 is placed with its haem group in the same orientation as that of *Monoraphidium* cytochrome c_6. Haem groups are shown in green; the extra loop of *Arabidopsis* cytochrome c_6 is shown in yellow; negatively and positively charged regions are shown in red and blue, respectively. Surface electrostatic-potential distributions were calculated at an ionic strength of 40 mM and pH 7.0.

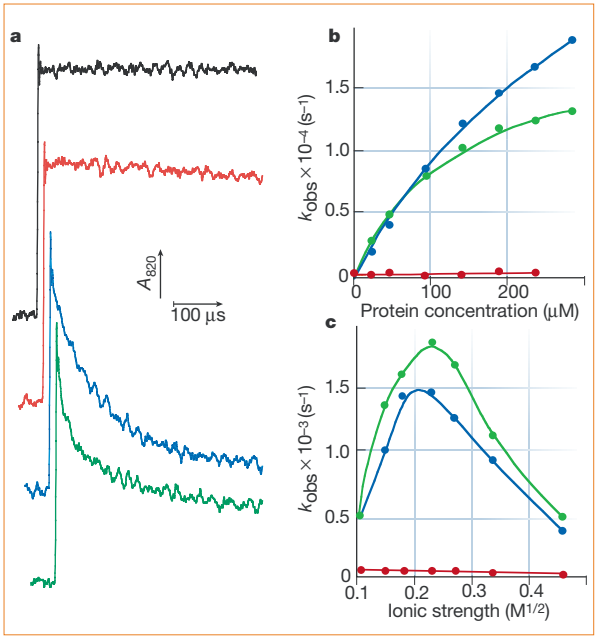

Figure 1 Reduction of *Arabidopsis* photosystem I by cytochrome c_6 of *Arabidopsis* (red) or *Monoraphidium* (blue) and by plastocyanin of *Arabidopsis* (green). **a,** Laser-flash-induced kinetic traces measured at 820 nm with 150 µM protein as electron donor, and a control without added protein (black). **b,** Dependence of the observed rate constant (k_{obs}) on concentration of electron-donor protein. **c,** Effect of ionic strength on k_{obs} with 30 µM electron-donor protein; ionic strength was adjusted with concentrated NaCl solution. Experiments were carried out at 25 °C and pH 7.5. Plant recombinant haem protein was prepared using a sequence encoding mature *Arabidopsis* cytochrome c_6 fused to that of the signal peptide from *Anabaena* cytochrome c_6, and the synthetic gene was cloned and expressed in *Escherichia coli*; *Monoraphidium* cytochrome c_6 was isolated from cell cultures. Further details are available from the authors.

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