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No evidence for behavioral responses to circularly polarized light in four scarab beetle species with circularly polarizing exocuticle

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ABSTRACT

The strongest known circular polarization of biotic origin is the left-circularly polarized (LCP) light reflected from the metallic shiny exocuticle of certain beetles of the family Scarabaeidae. This phenomenon has been discovered by Michelson in 1911. Although since 1955 it has been known that the human eye perceives a visual illusion when stimulated by circularly polarized (CP) light, it was discovered only recently that a stomatopod shrimp is able to perceive circular polarization. It is pertinent to suppose that scarab beetles reflecting LCP light in an optical environment (vegetation) being deficient in CP signals may also perceive circular polarization and use it to find each other (mate/conspecifics) as until now it has been believed. We tested this hypothesis in six choice experiments with several hundred individuals of four scarab species: *Anomala dubia*, *Anomala vitis* (Coleoptera, Scarabaeidae, Rutelinae), and *Cetonia aurata*, *Potosia cuprea* (Coleoptera, Scarabaeidae, Cetoniinae), all possessing left-circularly polarizing exocuticle. From the results of our experiments we conclude that the studied four scarab species are not attracted to CP light when feeding or looking for mate or conspecifics. We demonstrated that the light reflected by host plants of the investigated scarabs is circularly unpolarized. Our results finally solve a puzzle raised over one hundred years ago, when Michaelson discovered that scarab beetles reflect circularly polarized light.

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1. Introduction

The occurrence of circularly polarized (CP) light in nature is rare relative to the partially linearly polarized light [10]. In the biotic optical environment, the larvae of the fireflies *Photuris lucicrescens* and *Photuris versicolor* can be mentioned, the left and right lanterns of which emit weak left- and right-circularly polarized (LCP and RCP) bioluminescent light [27], and the function of which (if any) is unknown. The birefringent cuticle of certain crustaceans reflects CP light [18]. Due to a special structure of the outer cuticle (exocuticle) being optically analogous to the cholesteric liquid crystals, the metallic colored body surface of many scarab beetle species reflects LCP light ([3,15–17,19], pp. 83–85; [26], p. 189; [9,12]) (Fig. 1A–D). This selective reflection of LCP light is rare in nature, and the natural environment of these scarab beetles is also deficient in CP signals (Fig. 1E–F). [7] reported on the reflection–
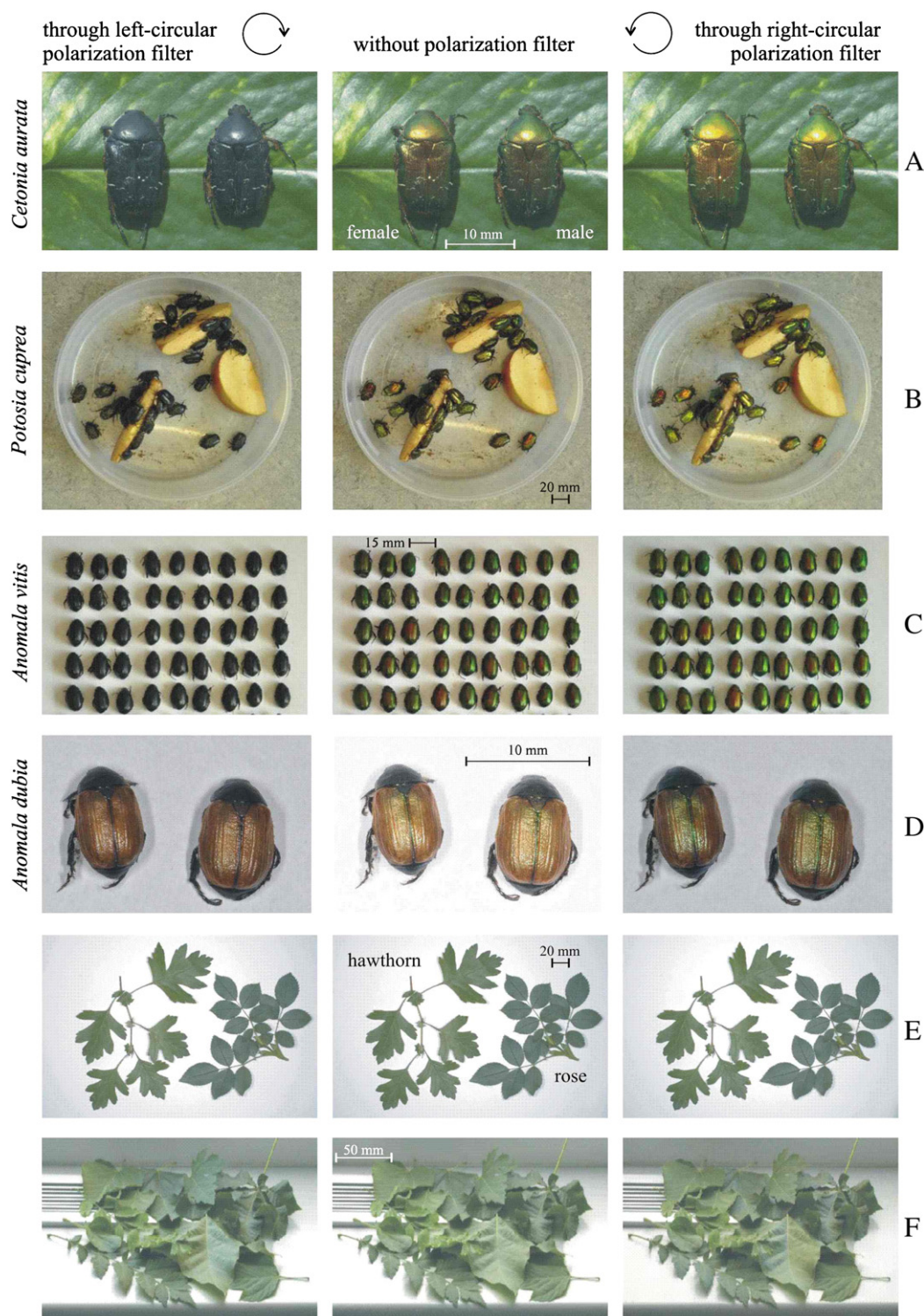
polarization properties of scarab beetles and discussed the history of this research.

Earlier, the potential biological function of CP light emitted, transmitted, or reflected by different organisms was completely enigmatic, because it was unknown whether these animals are at all able to perceive circular polarization. Although [20] observed that the human eye stimulated by CP light can perceive a visual illusion similar to the Haidinger's brushes induced by linearly polarized light [8], the discovery of an animal species being sensitive to circular polarization happened only recently: [5,14] showed that the stomatopod shrimp *Gonodactylus smithii* is able to detect CP light at the receptor level. [5] also reported that the carapace of this marine shrimp reflects CP light, furthermore they demonstrated behaviorally that these crustaceans can also be conditioned to CP stimuli when foraging.

In spite of the fact that the degree of circular polarization is weak in the underwater optical environment of *G. smithii* [5,14], they possess circular polarization sensitivity/vision. Thus, it would be pertinent to suppose that those scarab beetles (Fig. 1A–D) possessing left-circularly polarizing metallic shiny exocuticle in an optical

Fig. 1. Photographs of scarab beetles (A, C, D: dead; B: living beetles feeding on apple slices in a plastic container) and their host plants (freshly cut) taken without a polarization filter (middle column), and through an LC (left column) and an RC (right column) polarization filter. In the left and right columns the circular arrows show the handedness of CP light transmitted by the polarization filter. Note that an LC (or RC) polarization filter transmits RCP (LCP) light, while it blocks LCP (RCP) light. (A) *Cetonia aurata* (left: female, right: male) on a centipede tongavine (*Epipremnum pinnatum*) leaf. (B) *Potosia cuprea*. (C) *Anomala vitis*. (D) *Anomala dubia*. (E) Hawthorn (*Crataegus monogyna*) and wild rose (*Rosa canina*) leaves. (F) 12 different green plant leaves: black poplar (*Populus nigra*), London plane (*Platanus acerifolia*), common whitebeam (*Sorbus aria*), field maple (*Acer campestre*), small-leaved lime (*Tilia cordata*), European rowan (*Sorbus aucuparia*), wild cherry (*Prunus avium*), staghorn sumac (*Rhus typhina*), common elm (*Ulmus campestris*), pagoda tree (*Sophora japonica*), sweet chestnut (*Castanea sativa*), and European birch (*Betula pendula*). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

environment (vegetation) being poor in CP light (Fig. 1E–F) may also perceive circular polarization, and use it to find each other (mate/conspecific), which could be a relevant behavioral context of their possible circular polarization sensitivity/vision.

Recently, [2] obtained that jewel scarab beetles (*Chrysina gloriosa*) have a differential response to CP light and thus may be sensitive to circular polarization. [25] made an attempt to explain how beetles and other invertebrates could perceive circularly polarized light. If

scarabs had, indeed, circular polarization vision, the CP light reflected from their exocuticle (Fig. 1A–D) could help them to find each other in the circularly unpolarizing foliage (Fig. 1E–F) without the risk to be recognized by predators insensitive to circular polarization. In this way the camouflaging metallic green left-circularly polarizing body surface in a green foliage reflecting circular-polarization-deficient light could be perceived only by scarabs.

Cetonia and *Anomala* scarabs, for example, are common worldwide and are usually serious pests in the horti- and agriculture. Until now their sensitivity to circular polarization has not been investigated. To fill this gap, we performed six choice experiments to study whether *Anomala dubia* Scop., *Anomala vitis* F. (Coleoptera, Scarabaeidae, Rutelinae), and *Cetonia aurata* L., *Potosia cuprea* F. (Coleoptera, Scarabaeidae, Cetoniinae), all possessing left-circularly polarizing exocuticle, are attracted to LCP and RCP light stimuli in two different behavioral contexts: finding mate/conspecifics and foraging. We chose these scarab species, because their metallic green exocuticle reflects LCP light (Fig. 1A–D), and they are abundant in Hungary from May to July. To demonstrate circularly polarizing characteristics, we took photographs about the studied scarabs and their host plants without and with circular polarization filters.

The left-circularly polarizing ability of scarab cuticles has been discovered by [16]. Until now one could believe that this circular polarization could be an optical cue for scarab beetles. One hundred years after Michelson's discovery we now show that CP light reflected from four scarab species has no visual function.

2. Materials and methods

Throughout this work we used consequently the following nomenclature accepted in physics (optics): a left-circular (LC) polarization filter blocks LCP light and transmits RCP light. Similarly, a right-circular (RC) polarization filter blocks RCP light and transmits LCP light. A circular polarization filter is the complement of a circular polarizer: an LC polarizer transmits LCP light and blocks RCP light, while an RC polarizer transmits RCP light and blocks LCP light.

Experiment 1 was conducted between 8 and 10 May 2009 between 10:00 and 15:00 h (UTC + 2 h) every day in our Laboratory with 120 *C. aurata* (65 females, 55 males) collected on 7 May 2009 in the field, during preliminary experiments 1 and 2 (see Electronic Supplement). The beetles were kept together with some flowering hawthorn branches in a glass terrarium under natural illumination conditions, where some of them mated and many other individuals performed copulation attempts. Prior to the test the beetles were kept for 6 h in an empty translucent white plastic container. The test chamber was composed of a paper box ($50 \times 50 \times 30$ cm), the inner walls of which were covered by matte white paper. On one of the vertical walls of the box two test windows (15×15 cm) were set up 20 cm apart from each other (Supplementary Fig. S1). Both test windows were illuminated by homogeneous diffuse white light transmitted through a white curtain of a window (2×2 m) of the laboratory. One of the test windows was covered with an LC polarization filter (thickness = 0.8 mm, type: P-ZN/L-43186, Schneider, Bad-Kreuznach, Germany), while the other window with an RC filter (thickness = 0.8 mm, type: P-ZN/R-12628, Schneider), the transmission spectrum of which is seen in Supplementary Fig. S2. The outer surface of the polarizers was covered by a diffuser (common white paper) in order to completely depolarize the light falling onto the polarizers. The bottom of the test chamber was covered by a sheet of white paper, which was replaced by a new one after each test run, in order to eliminate the possible influence of odor marks left by the scarabs tested. On the bottom of the test chamber there was a releaser at the wall opposite to the two test windows. The releaser was composed of a non-transparent paper cup (height = 2 cm, diameter = 5 cm). At the beginning of a given choice a scarab was put from the empty container to below the releaser of the test chamber.

The beetle was left on its back side (to motivate the beetle for movement) in darkness beneath the releaser for 1 min, then the releaser was moved away. After some seconds the beetle turned from its back side to its legs, and began to crawl toward the wall with the two test windows. The experiment with a given *Cetonia* ended when the beetle reached one of the test windows covered by an LC or RC polarization filter and a diffuser. Each individual beetle was tested only once. The order of the LC and RC polarization filters in the two test windows was randomly changed between the test runs. At the end the number of beetles choosing a given test window was counted.

Experiment 2 was done with the following four scarab species: *C. aurata*, *P. cuprea*, *Anomala vitis* and *A. dubia*. The whole cuticle (both dorsal and ventral) of *Cetonia*, *Potosia* and *A. vitis* is metallic shiny green and reflects intense LCP light (Fig. 1A–C). In the case of *A. dubia* the brownish elytrae reflect weak LCP light, while other parts of the cuticle reflect intense LCP green light (Fig. 1D). On 26 April 2010 between 11:00 and 12:00 h (UTC + 2 h) 196 swarming *C. aurata* (both females and males) were captured in a field (Török-mező: 47° 88′ N, 18° 93′ E), where hawthorn (*Crataegus monogyna*) bushes bloomed. The captured beetles were kept among pieces of cardboard in translucent, white plastic containers (with numerous holes on their top for airing) under natural light and thermal conditions in a garden in Göd (Hungary), where some of them mated. Slices of fresh apple were given to them as food. Between 27 and 29 April 2010 from 10:00 to 16:00 h (UTC + 2 h) every individual beetle was tested three times in a choice-box (Supplementary Fig. S3A–F).

The choice-box was a flat cylinder (height = 10 cm, diameter = 60 cm) composed of 6 radial sectors divided by vertical walls (26×10 cm) hanging from a top disc (radius = 30 cm) with a circular hole (diameter = 5 cm) at its center. Each sector was divided by a short vertical wall (13×13 cm). Both halves of the sector were barred by a vertical wall (15×15 cm), called "window" further on. The inner surface of these windows was covered by a color picture, a circular polarizer and a 2 mm thick glass pane of the same size (7.5×13 cm). In the left and right windows of a given sector an LC polarizer (P-ZN/L-43186) and an RC polarizer (P-ZN/R-12628) were placed, respectively. All the walls of the choice-box were composed of a 5 mm thick milky white translucent plexiglass, which functioned as a depolarizer of the incident light. Many such materials could act as quarter wave plates, which when illuminated by the linearly polarized skylight during the experiment would actually produce CP light. We tested for this possibility by observing the polarization of transmitted linearly polarized light through LC and RC polarization filters, but we did not experience any trace of CP light in the choice-box. In a given choice experiment the choice-box contained the same $6 \times 2 = 12$ color pictures: in the 1st, 2nd and 3rd part of this experiment these pictures showed (1) a *C. aurata* sitting on a hawthorn (*C. monogyna*) flower (Fig. S3G), (2) hawthorn flowers and leaves (Fig. S3H), and (3) a blooming hawthorn bush (Fig. S3I). Prior to a test the beetles were kept for 6 h in an empty translucent white plastic container. During the experiment, performed open-air in the mentioned garden in Göd, a given beetle was put on the middle of a horizontal wooden board, and the insect was covered by an opaque releasing cylinder (diameter = 4.5 cm, height = 17 cm), onto which the choice-box was drawn through its central hole. The releasing cylinder was covered by the palm of hand of the investigator person for 30 s, then the cylinder was removed from the choice-box, by which the experiment began: (i) the released beetle (standing at the beginning at the center of the choice-box) could select one of the 6 sectors, toward which it began to crawl or fly, and (ii) in a given sector it could choose either the left or the right window with the same picture seen through an LC or an RC polarization filter. The behavior of the beetle in the choice-box was observed from above by the naked eye through the circular hole on the top of the box. When the beetle reached (by crawling or flying) the left/right window of the selected

sector (within 20–200 s), the experiment ended. Then the tested beetle was removed from the choice-box, the underlying wooden board of which was cleaned by a cloth impregnated with ethyl alcohol (to eliminate the possible odor cues left by the tested beetle), the circular choice-box was rotated by a random angle around its vertical symmetry axis, and the experiment was repeated by a new test beetle. This whole procedure was done three times (27, 28, 29 April 2010, 10:00–16:00 h) with the use of the three different picture types (Fig. S3G-I) in the 12 windows of the choice-box. During the experiments some of the *Cetonia* beetles escaped, thus the number of beetles decreased (N = 196, 141 and 131 for the 1st, 2nd and 3rd parts, respectively).

Between 30 June and 2 July 2010 the 1st part of this choice experiment was repeated with 100 individuals of each *P. cuprea*, *A. vitis* and *A. dubia* that were caught with traps baited by synthetic attractants (see experiment 4) and kept in the laboratory similarly as *C. aurata* described above. In captivity, several individuals of these scarab species copulated. These experiments were performed in the laboratory under bright and isotropic artificial illumination conditions (drawn-in white curtains on the windows, lamps on the ceiling switched off, choice-box placed in the center of the room) with the same pictures taken about a blooming hawthorn bush (Fig. S3I) that were used in the 12 sectors of the choice-box.

Experiment 3 was performed in the laboratory between 3 and 8 July 2010 with 100 individuals of each *C. aurata*, *P. cuprea*, *A. dubia* and *A. vitis* captured with traps baited by synthetic attractants (see experiment 4). The beetles were kept among cardboard sheets inside translucent white plastic containers under natural light conditions in the laboratory, where many of them mated. Slices of fresh apple were given to them as food. Prior to a test the beetles were kept for 6 h in an empty translucent white plastic container. Every individual was tested only once in the choice-box shown in Fig. 2.

The box had three main parts: (1) release and choice arena (length = 20 cm, width = 30 cm, height = 10 cm), where the test beetle was released from an opaque cylinder (height = 21 cm, diameter = 4 cm) after kept for 30 s in the dark. The released beetle could crawl (or fly) on a horizontal paper sheet toward the left or right window facing the arena. The paper sheet was replaced by a new one after each choice run in order to eliminate any possible scent cue left by the tested beetles. After the release cylinder was removed from the box, and the test beetle was observed from above by the naked eye through a circular hole on the top of the box. A given test ended when the beetle reached a cardboard bar (width = 14 cm, height = 1.5 cm) at the opening of the left and right stimulus compartments of the box. (2) The arena continued in the left and right stimulus boxes, which were separated from the arena by the narrow cardboard bar mentioned. The left box (length = 21 cm, width = 14.5 cm, height = 10 cm) was empty with a small removable matte white cardboard window on its rear vertical wall. On this rear window the visual stimuli were two dead beetle carcasses (one female and one male) of the same species as the test beetle. These stimulus beetles were illuminated by four light-emitting diodes (two OSSV53E1A violet-blue LEDs with an emission peak at 400 nm and emitting also some ultraviolet light, and two 530XW8C VIS LEDs emitting light in the visible part of the spectrum) placed in a small box on the ceiling. The spectrum of light emitted by both diodes can be seen in Supplementary Fig. S4. (3) The lower part of the right stimulus box (length = 10 cm, width = 14.5 cm, height = 10 cm) contained the same light source (2 violet-blue LEDs and 2 VIS LEDs) in the ceiling as the left box, and a common plane glass-silver mirror (13.5 × 14.5 cm) tilted at 45° from the horizontal. The upper part of the right stimulus box was a small tower with a small removable matte white cardboard window on its top horizontal wall. On this top window the visual stimuli were two dead beetles (one female and one male) of the same species as the test beetle. These stimulus beetles were illuminated by the four LEDs through the tilted mirror. The function of this mirror

was to convert the LCP light originating from the shiny metallic green exocuticle of the stimulus beetles to RCP light after reflection. Due to the 45° tiltness of the mirror the test beetle in the choice arena saw the two stimulus beetles in the right box as if they were on the rear vertical wall, similarly to the case of the left stimulus box. The only relevant difference between the left and right stimuli was the handedness (left-handed in the left box, and right-handed in the right box) of the CP light originating from the stimulus beetles (Supplementary Fig. S5).

The test beetles had to choose either the stimulus beetles of the same species seen directly (i.e. seen the LCP light reflected from the exocuticle), or seen through the tilted mirror (i.e. seen the RCP light converted after reflection from the mirror). The walls of the choice-box were composed of matte brownish cardboard, and the inner surfaces of which were sprayed by a matte white paint to eliminate any disturbing polarization of wall-reflected light. Since the stimulus beetles in the left and right stimulus boxes were not exactly identical (slightly differed in size, color, brightness and posture), they were replaced with each other after every 5 choices, in order to eliminate any choice bias due to these differences. Hence, during an experiment with 100 individuals of a given scarab species the left and right stimuli were changed 100/5 = 20 times.

Experiment 4 was conducted to see whether the strongly LCP light from dead adult scarabs evokes attraction from three feral scarab populations in the field. Two trapping experiments were conducted: one with *Anomala* scarabs (*A. vitis* and *A. dubia*), and the second with *C. aurata*. CSALOMON® VARb3 traps with transparent upper funnels (produced in the Plant Protection Institute of the Hungarian Academy of Sciences, Budapest, Supplementary Fig. S6A) were used, which proved to be excellent for the capture of related scarabs ([11]). Treatments in the experiment included: (A) traps with dead beetles (8 specimens of *C. aurata* or *A. vitis*, respectively, or 10 specimens of *A. dubia*) glued (by Super Bond, Henkel Ltd., Dublin, Ireland) to the inside of the transparent funnel of the trap (Fig. S6B–D). Dead beetles were washed in an excess of hexane for 5 min before gluing them to remove semiochemicals with possible behavioral activity. Dead beetles were glued roughly in a rectangle at distances of 1–2 mm from each other on the middle portion of the funnel. (B) Unbaited traps for negative control. (C) Traps baited with a synthetic attractant for positive control.

For attractant, commercially available CSALOMON® lures were used. *A. vitis/dubia* sex attractant baits were applied [active ingredient: (*E*)-2-nonen-1-ol, [21]] in the experiment with *Anomala* scarabs, where exclusively male individuals were caught due to the sex-attractant. In the *Cetonia* test the ternary floral attractant [active ingredients methyl eugenol, (*E*)-anethol, 1-phenethyl alcohol [22,24]] were used, which lures both females and males with an approximate sex ratio of 1:1. Traps were set up in a randomized complete block design. Both experiments included 3 replicate blocks. The distance between traps within a block was 10–15 m. Blocks were set 30–50 m apart. Traps were inspected twice weekly, when captured insects were identified, recorded and removed. Catches recorded at such occasions were regarded as data points in statistical analyses. The *Anomala* experiment was conducted in a sour cherry orchard at Halásztelek (Pest county, Hungary), between 21 and 28 June 2010. Traps were suspended from branches at 1.5 m height in the crown of trees. The *Cetonia* experiment was set up near the bushy edge of a mixed oak forest with mostly *Rosa canina* and *Crataegus* spp. at Julia major (Budapest, Hungary) from 10 to 25 June 2010. Traps were suspended from the vegetation at the height of 1.5 m in sunny places.

In pilot experiments, prior to experiments 5 and 6, we tested with all the later investigated scarab individuals if a positive phototaxis can be elicited from them. For this purpose we applied the choice-box used in experiment 6, but modified in such a way that one of the two windows was covered by a black cardboard sheet. Thus, the
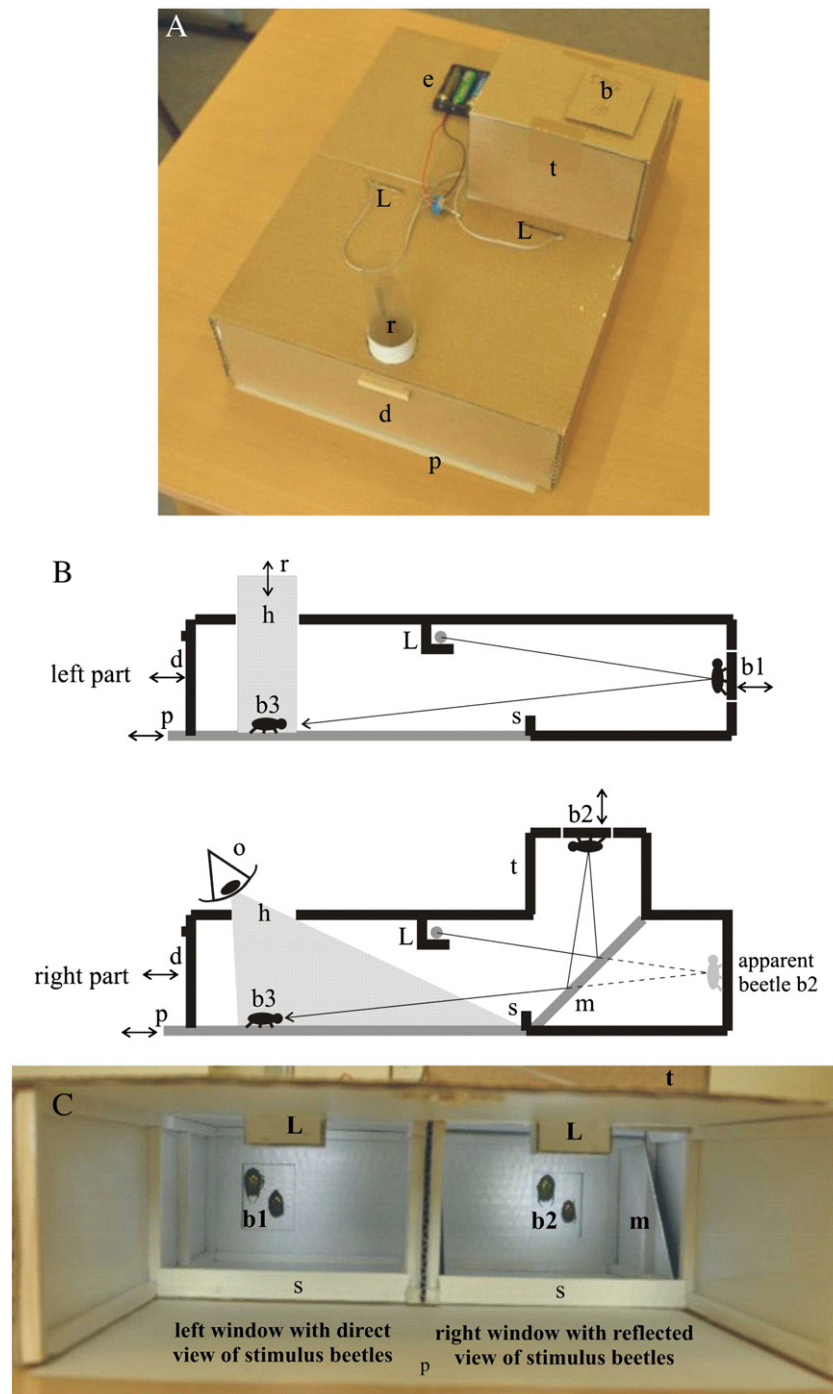
Fig. 2. Structure of the choice-box used in experiment 3. (A) Photograph of the choice-box taken from above. d: door, p: paper sheet, r: release cylinder, L: light-emitting diodes, e: electrical batteries as power supplies for the diodes, t: tower, and b: holder of dead beetles. (B) Cross-section of the left and right parts of the choice-box showing the inner structure. Double-headed arrows represent that the concerned component is removable. h: circular hole through which the test beetle can be observed; b3: test beetle; s: a bar of cardboard closing the choice arena; b1: holder of dead beetles seen directly; o: observer; m: tilted plane mirror; b2: holder of dead beetles seen through the tilted mirror. (C) The view seen by a test beetle. L: box of the light-emitting diodes; b1: left holder of dead scarab beetles seen directly, presenting LCP light stimuli originating from the beetles' exocuticle; b2: right holder of dead scarabs seen through the tilted plane mirror, presenting RCP light stimuli originating from the exocuticle of beetles being in the tower.

scarabs could choose between a dark window and a bright window, both windows transmitting unpolarized white light. During this experiment the left and right positions of the dark and bright windows were randomized. For experiments 5 and 6 we used only those scarabs (85–90% of the individuals tested in the pilot experiment) that selected the bright window, and thus evidently had a positive phototaxis. These pilot tests showed that light levels, arena size, measurement accuracy and the behavioral state of the beetles were appropriate to observe a behavioral effect if it exists.

Experiment 5 was performed in the laboratory between 15 and 30 June 2011 with 100 individuals of each *C. aurata*, *P. cuprea*, *A. vitis* and *A. dubia*. The same choice-box was used as in experiment 2 (Fig. S3A–C), but in each sector the left window transmitted LCP light while the right window transmitted totally linearly polarized (TLP) light, whose direction of polarization was 45° from the horizontal (Supplementary Fig. S7). The same picture, a blooming hawthorn bush (Fig. S3I) was presented in each window. In all six sectors this color picture was seen through a left-circular polarizer (the quarter-wavelength

retarder layer of which faced the test beetle and the linear polarizing sheet of which faced the color picture) in the left window, and through a reversed left-circular polarizer (with its quarter-wavelength retarder layer facing the color picture, while its linear polarizing sheet facing the test beetle) in the right window. Thus, the spectral (intensity and color) characteristics of both stimuli were the same, and only their polarization characteristics (LCP and TLP) were different. Other details of this experiment were the same as those of experiment 2.

Experiment 6 was conducted in the laboratory from 1 and 13 July 2011 with 100 individuals of each *C. aurata*, *P. cuprea*, *A. vitis* and *A. dubia*. We used a one-sector choice-box in which one of the windows transmitted LCP light and the other window transmitted unpolarized (UP) light (Supplementary Fig. S8). In both windows there was an LC polarizer and a white diffuser (a common paper sheet, reducing the degree of linear and circular polarization to zero, which was tested by imaging polarimetry). Seen from the box, in one of the windows the LC polarizer was inside and the diffuser was outside, while in the other window their order was reversed. Thus, the spectral characteristics of both stimuli were the same, and only their polarization characteristics (LCP and UP) were different. During this experiment the left and right positions of the LCP and UP windows were randomized. The box was composed of a light brown wooden sheet, and the surface of which was matte to reduce its reflection polarization. Other details of this experiment were the same as those of experiment 2.

Photographs through an LC and an RC polarizer were taken in the laboratory. Pictures demonstrating (i) the intense LCP light reflected from the exocuticle of *C. aurata*, *P. cuprea*, *A. vitis* and *A. dubia* scarabs (Fig. 1A–D), and (ii) the circularly unpolarized light reflected from green leaves of hawthorn and wild rose (Fig. 1E), furthermore 12 other host plants of scarabs (Fig. 1F) were captured by a digital camera (Fujifilm FinePix S2 Pro) through an LC and an RC polarizer (P-ZN/L-43186, P-ZN/R-12628). The scarabs and the leaves were illuminated by unpolarized white light transmitted through a white curtain in the window of the laboratory.

Reflection spectra of the exocuticle of scarab beetles were measured with the use of a fiber-optic UV–VIS spectrometer (Avaspec 2048/2), in a similar setup as used earlier to measure the reflectance of various butterfly wings [1,13]. Both in specular arrangement under normal incidence (Fig. 3) and using an integrating sphere (radius = 3 cm) in order to collect all the light reflected under any angle of emergence. The integrating sphere can be regarded as an equivalent to a diffuse illumination. All optical measurements were carried out with unpolarized light. An Avaspec diffuse, white standard was used as comparison sample for reflection factor measurements.

Statistical analyses were performed with Statistica 7.0 (one-way ANOVA and binomial χ^2 tests) and StatView 4.01 (non-parametric Kruskal–Wallis test).

3. Results

According to Fig. 1A, the cuticle of *Cetonia* scarabs reflects LCP light, while the underlying *Epipremnum* leaf reflects circularly unpolarized light. Fig. 1B–D shows that the exocuticle of *P. cuprea*, *A. vitis* and *A. dubia* scarabs also polarizes left-circularly the reflected light, since the whole body surface of *Cetonia*, *Potosia* and *A. vitis* is black seen through an LC polarization filter, furthermore, apart from the brownish elytra the body surface of *A. dubia* is also black through such a filter. On the other hand, the light reflected by host plants (hawthorn: *C. monogyna*, wild rose: *R. canina*, black poplar: *Populus nigra*, London plane: *Platanus acerifolia*, common whitebeam: *Sorbus aria*, field maple: *Acer campestre*, small-leaved lime: *Tilia cordata*, European rowan: *Sorbus aucuparia*, wild cherry: *Prunus avium*, staghorn sumac: *Rhus typhina*, common elm: *Ulmus campestris*, pagoda tree: *Sophora japonica*, sweet chestnut: *Castanea sativa*, European birch: *Betula pendula*) of the investigated scarabs is circularly unpolarized (Fig. 1E–F).

In experiment 1 (Fig. S1) 61 (40 crawling, 21 flying) and 59 (42 crawling, 17 flying) *C. aurata* individuals chose the LCP and RCP light stimulus, respectively. This tiny difference is statistically not significant ($\chi^2 = 0.033$, df = 1, p = 0.86). The reaction of *Cetonia* in this experiment corresponded to a simple phototactic random choice between the LCP and RCP stimuli.

In experiment 2 (Table 1) 94 *C. aurata* chose the LCP and 102 the RCP light stimulus when confronted with pictures of a *C. aurata* on a hawthorn flower (stimulus 1, Fig. S3G). For pictures of hawthorn leaves and flowers (stimulus 2, Fig. S3H), and a blooming hawthorn bush (stimulus 3, Fig. S3I) *Cetonia* chose the LCP/RCP stimulus in ratio of 67/74 and 67/64, respectively. In total, the LCP stimulus was chosen 228 times, while the RCP stimulus 240 times. All these choices were not significantly different from 50–50%. None of the six sectors was preferred by *Cetonia*. The solar half of the choice-box (when the sky was cloudless) was slightly more (189) preferred than the antisolar half (160), but this difference is not significant ($\chi^2 = 2.41$, df = 1, p = 0.12).

When reaching the LCP/RCP stimulus, *Cetonia* preferred crawling (285) against flying (183). Quite similar results were obtained for *P. cuprea*, *A. dubia* and *A. vitis* (Table 2): the choice ratio of the LCP/RCP stimulus was 50/50 (*Potosia*), 48/52 (*A. dubia*) and 51/49 (*A. vitis*), respectively. These choice ratios are not significantly different from 50/50%. Neither of the six sectors was preferred, and the test beetles crawled (*Potosia*: 72, *A. dubia*: 62, *A. vitis*: 63) more frequently than flew (*Potosia*: 28, *A. dubia*: 38, *A. vitis*: 37).

In experiment 3 (Table 3) the choice ratios of the LCP/RCP dead beetles were 48/52, 51/49, 46/54 and 50/50 for *Cetonia*, *Potosia*, *A. dubia* and *A. vitis*, respectively. Again, these rates are statistically not significantly different from 50/50%. Beetles preferred again crawling (*Cetonia*: 90, *Potosia*: 79, *A. dubia*: 72, *A. vitis*: 76) against flying (*Cetonia*: 10, *Potosia*: 21, *A. dubia*: 28, *A. vitis*: 24).

In experiment 4 traps with sex attractant caught a high number of *A. dubia* and *A. vitis*, significantly different from all other treatments (Table 4). Single *A. vitis* specimens were recorded in traps with dead *A. vitis* or *A. dubia* scarabs, whereas unbaited traps caught no beetle. Catches of *A. dubia* showed the same picture, with high number of catches in traps baited with sex attractant, and no or a single beetle in the other treatments (Table 4). The floral attractant-baited traps caught significantly more *C. aurata* than the other treatments (Table 5). In fact, no beetles were captured in traps with dead *Cetonia*
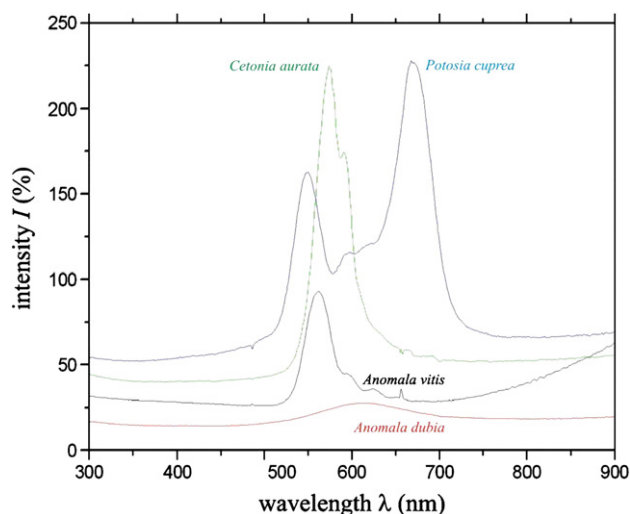

Fig. 3. Typical reflection spectra of the metallic shiny, left-circularly polarizing exocuticle of *Anomala vitis*, *A. dubia*, *Cetonia aurata* and *Potosia cuprea* scarab beetles measured at normal reflection. The value of reflected intensity *I* is measured relative to the diffuse reference reflection standard of the instrument. *I*-values can be larger than 100%, if a surface reflects a greater amount of light than the diffuse reference standard.

Table 1

Number of *Cetonia aurata* choosing either the LCP or the RCP light stimulus in the six sectors of the choice-box used in experiment 2 performed in the field with three different stimuli under sunny or totally overcast sky conditions. *Sun*: choices of beetles were on the solar half of the choice-box. *Anti-sun*: choices of beetles were on the antisolar half of the choice-box. *Overcast*: the sky was totally overcast, thus the choice-box was illuminated isotropically by cloudlight. According to the statistical analyses (Table S1), the differences are not significant. More details in Table S1.

	LCP light stimulus	RCP light stimulus
Stimulus 1: picture of *Cetonia aurata* on a hawthorn (*Crataegus monogyna*) flower (Fig. S3G)		
Sum ($N_{beetle} = 196 = 100$ female $+ 96$ male)	94 (sun = 34, anti-sun = 27, overcast = 33) (crawling = 59, flying = 35)	102 (sun = 33, anti-sun = 29, overcast = 40) (crawling = 62, flying = 40)
Stimulus 2: picture of hawthorn (*Crataegus monogyna*) leaves and flowers (Fig. S3H)		
Sum ($N_{beetle} = 141 = 73$ female $+ 68$ male)	67 (sun = 34, anti-sun = 33) (crawling = 39, flying = 28)	74 (sun = 39, anti-sun = 35) (crawling = 48, flying = 26)
Stimulus 3: picture of a blooming hawthorn (*Crataegus monogyna*) bush (Fig. S3I)		
Sum ($N_{beetle} = 131 = 69$ female $+ 62$ male)	67 (sun = 23, anti-sun = 17, overcast = 27) (crawling = 37, flying = 30)	64 (sun = 26, anti-sun = 19, overcast = 19) (crawling = 40, flying = 24)
Total	228 (sun = 91, anti-sun = 77, overcast = 60) (crawling = 135, flying = 93)	240 (sun = 98, anti-sun = 83, overcast = 59) (crawling = 150, flying = 90)

Table 2

Total numbers of *Potosia cuprea*, *Anomala dubia* and *A. vitis* choosing either the LCP or the RCP light stimulus in the six sectors of the choice-box used in experiment 2 using stimulus 3 (picture of a blooming hawthorn bush, Fig. S3I) in the 12 windows of the choice-box. According to the statistical analyses (Table S2), the differences are not significant. More details in Table S2.

Species	LCP light stimulus	RCP light stimulus
Potosia cuprea (row 2 in Fig. S1)	50	50
$N_{beetle} = 100 = 52$ male $+ 48$ female	(crawling = 35, flying = 15)	(crawling = 37, flying = 13)
Anomala vitis (row 3 in Fig. S1)	51	49
$N_{beetle} = 100 =$ male	(crawling = 32, flying = 19)	(crawling = 31, flying = 18)
Anomala dubia (row 4 in Fig. S1)	48	52
$N_{beetle} = 100$ male	(crawling = 30, flying = 18)	(crawling = 32, flying = 20)

beetles or unbaited. Catches of the closely related *P. cuprea* showed the same trend (Table 5).

In experiment 5 (Fig. S7) all four scarab species studied showed no preference between the LCP and the totally linearly polarized stimuli (Table 6). The minimal differences in their choices were not significant. The same was the situation in experiment 6 (Fig. S8), where all four species chose practically equally either the LCP or the unpolarized stimulus, and their choice differences were again not significant (Table 7). Experiments 5 and 6 support our main conclusion that circular polarization does not attract *C. aurata*, *P. cuprea*, *A. vitis* and *A. dubia* when they look for food or mate/conspecifics.

Table 3

Number of *Cetonia aurata*, *Potosia cuprea*, *Anomala vitis* and *A. dubia* choosing either the dead beetles of the same species seen directly (i.e. seen the LCP light reflected from the exocuticle) or their reflections viewed from a plane mirror (i.e. seen the RCP light reflected from a mirror) in the choice-box used in experiment 3. When testing each species, the stimuli were two pairs of dead beetles of the same species: one pair was seen directly, while in the case of the other pair their reflections from a mirror were viewed. According to the statistical analyses (Table S3), the differences are not significant.

Species ($N_{beetle} = 100$)	LCP beetle (seen directly)	RCP beetle (seen through a mirror)
Cetonia aurata (50 male + 50 female)	48 (crawling = 44, flying = 4)	52 (crawling = 46, flying = 6)
Potosia cuprea (52 male + 48 female)	51 (crawling = 45, flying = 6)	49 (crawling = 34, flying = 15)
Anomala vitis (100 male)	50 (crawling = 41, flying = 9)	50 (crawling = 35, flying = 15)
Anomala dubia (100 male)	46 (crawling = 30, flying = 16)	54 (crawling = 42, flying = 12)

According to Fig. 3, the reflection spectra (measured at normal incidence) of the left-circularly polarizing exocuticle of *A. vitis*, *A. dubia*, and *C. aurata* have a single peak in the green (*A. vitis*: 562 nm, *A. dubia*: 614 nm, *Cetonia*: 574 nm) part of the spectrum, while *P. cuprea* possesses a main peak in the red (670 nm) and a secondary peak in the green (549 nm). The only peak results in the metallic green color of *Anomala* and *Cetonia*, and the two peaks result in the brownish green color of *Potosia*.

4. Discussion

In the optical environment of the studied scarab species the strongest source of CP light is their exocuticle, since their vegetable habitat reflects generally circularly unpolarized light (Fig. 1). Consequently, circular polarization sensitivity/vision in these scarabs in principle could be exploited to look for mate/conspecifics by means of detecting the exocuticle-reflected LCP light. However, from the results of our experiments we conclude that *C. aurata*, *P. cuprea*, *A. vitis* and *A. dubia* are not attracted at all to CP light under the conditions present in our tests, which involved feeding stimuli (flowers and leaves of the host plants, or their pictures) and mate/conspecific stimuli (pictures or dead beetles of the conspecifics), in spite of the fact that their exocuticle reflects LCP light in the visible part of the spectrum (Fig. 1A–D).

The results of experiment 4 suggest that the visual stimuli, including the LCP light from dead *A. vitis*, *A. dubia*, *C. aurata* and *P. cuprea* scarabs do not evoke attraction or arrestation from conspecifics in the field, despite the fact that high catches in sex-attractant-baited traps (for *Anomala* scarabs) as well as in floral attractant-baited traps (for *Cetonia* and *Potosia*) showed that the studied species were

Table 4
Number of catches of *Anomala vitis* and *A. dubia* in traps baited with dead beetles, *A. vitis/dubia* sex attractant, or in unbaited traps in experiment 4. p-values derived from Kruskal–Wallis test.

Bait	*Anomala vitis*	*Anomala dubia*
Dead *Anomala dubia* beetles	1 (male)	0
Dead *Anomala vitis* beetles	1 (male)	0
Sex attractant	867 (male)	381 (male)
Unbaited	0	1 (male)
p-value	0.0002	0.0002

Table 5
Number of catches of *Cetonia aurata* and *Potosia cuprea* in traps baited with dead *Cetonia* beetles, *Cetonia/Potosia* floral attractant, or in unbaited traps in experiment 4. p-values derived from Kruskal–Wallis test.

Bait	*Cetonia aurata*	*Potosia cuprea*
Dead *Cetonia aurata* beetles	0	0
Floral attractant	415 (205 female + 210 male)	47 (24 female + 23 male)
Unbaited	0	0
p-value	<0.0001	<0.0001

present in relatively high population density at the experimental site. In experiment 4 the scarabs seemed to find each other by means of pheromones, rather than by visual cues, e.g. the exocuticle-reflected LCP light. Cross-attraction by sex attractant bait is well known and has been published previously for both *A. vitis* and *A. dubia* [21]. Similarly, attraction of *Cetonia* and *Potosia* (both species of similar size and shape) by the applied floral attractant, has been documented earlier [22,24].

If these scarabs can find each other by means of odors, they may not need visual cues to find mate/conspecifics. Note, however, that the use of pheromones does not necessarily influence the potential importance of visual cues, since several animal species are multimodal in their signaling [4]. In our opinion, the circular polarization of the exocuticle-reflected light may only be a byproduct of the helicoidal structure of the exocuticle of these scarabs. Instead, the major function of this helicoidal structure could be, for example, a mechanical or chemical one (in order to enhance the resistance of the exocuticle against mechanical stresses and/or acidic/alkaline damages), rather than optical (to produce LCP light for visual communication). The mechanical/chemical hypothesis should be tested in the future, while the optical hypothesis is highly improbable in light of the results presented here. Another unsolved problem is how the unique structure of scarab cuticles – resembling cholesteric liquid crystals and occurring exclusively in scarabs [23] – can be so much different from that of other beetles.

The lack of positive response (i.e. attraction to LCP stimuli) of scarabs in our experiments could also be explained by the lack of

Table 6
Number of *Cetonia aurata*, *Potosia cuprea*, *Anomala vitis* and *A. dubia* choosing either the left-circularly polarized (LCP) or the totally linearly polarized (TLP) light stimulus in the six sectors of the choice-box used in experiment 5. According to the statistical analyses (Table S4), the differences are not significant. More details in Table S4.

Species	LCP light stimulus	TLP light stimulus
Cetonia aurata (N = 100)	50 (crawling = 48, flying = 2)	50 (crawling = 47, flying = 3)
Potosia cuprea (N = 100)	52 (crawling = 51, flying = 1)	48 (crawling = 46, flying = 2)
Anomala vitis (N = 100)	52 (crawling = 52, flying = 0)	48 (crawling = 48, flying = 0)
Anomala dubia (N = 100)	45 (crawling = 45, flying = 0)	55 (crawling = 55, flying = 0)

Table 7
Number of *Cetonia aurata*, *Potosia cuprea*, *Anomala vitis* and *A. dubia* choosing either the left-circularly polarized (LCP) or the unpolarized (UP) light stimulus in the choice-box used in experiment 6. According to the statistical analyses (Table S5), the differences are not significant. More details in Table S5.

Species (N = 100)	LCP light stimulus	UP light stimulus
Cetonia aurata	49 (crawling = 49, flying = 0)	51 (crawling = 51, flying = 0)
Potosia cuprea	47 (crawling = 47, flying = 0)	53 (crawling = 53, flying = 0)
Anomala vitis	53 (crawling = 53, flying = 0)	47 (crawling = 47, flying = 0)
Anomala dubia	52 (crawling = 52, flying = 0)	48 (crawling = 48, flying = 0)

motivation of the test beetles to look for mate/conspecifics and/or host plants. This is very improbable, because: (1) Experiment 1 was conducted with *C. aurata* scarabs freshly collected on the preceding day (when actively looking for flowers and mate), thus it is pertinent to suppose that they did not lose their motivation during the experiment. (2) Experiments 2, 3, 5 and 6 were performed in choice-boxes with scarabs collected in the field on the preceding day, and prior to a test the beetles were kept for 6 h in a container without food, furthermore each individual was tested only once. These conditions minimized the possibility that the beetles would lose their motivation to look for mate/conspecifics and/or host plants. (3) Experiment 4 was performed in the field with actively flying, motivated scarabs. (4) In experiments 1–3, 5 and 6 the test beetles frequently displayed flight responses toward the visual stimuli (Tables 1–3), demonstrating their activity and motivation. Furthermore, the test beetles were obviously in mating mood, because in captivity several individuals of all four scarab species mated or performed copulation attempts.

Hence, in two species (*A. vitis*, *A. dubia*) of the subfamily Rutelinae, and in two species (*C. aurata*, *P. cuprea*) of the subfamily Cetoniinae in the family Scarabaeidae (Coleoptera), all possessing left-circularly polarizing exocuticle, we found no evidence for behavioral responses to CP light. Furthermore, there were no sexual differences in the polarization characteristics of exocuticular reflections, and attraction by pheromones seemed more important than vision in finding mate/conspecifics. Since the visual system of scarab beetles shares basic optical and anatomical features [6], our results might be generalized. In the future it would be important to test the circular polarization sensitivity of several other scarab species and whether they are attracted or not to CP light, in order to draw a general conclusion about the circular polarization vision or blindness in scarabs.

Acknowledgments

Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.physbeh.2011.11.020.

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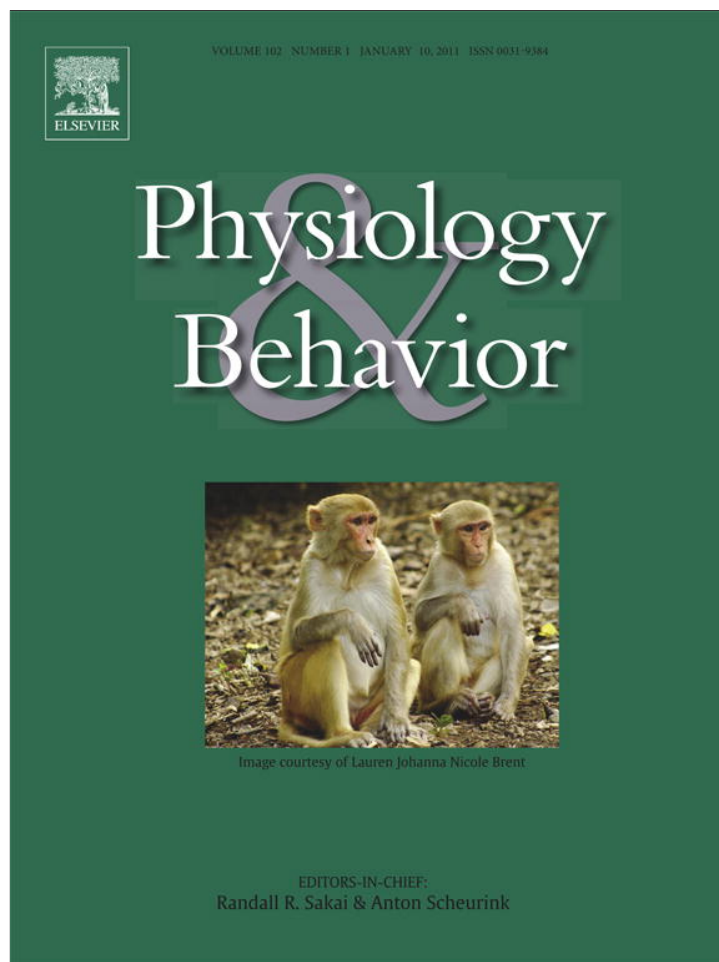
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