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Author/s:

Franklin, AM;Rankin, KJ;Ospina Rozo, L;Medina, I;Garcia, JE;Ng, L;Dong, C;Wang, LY;Aulsebrook, AE;Stuart-Fox, D

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Cracks in the mirror hypothesis: high specularity does not reduce detection or predation risk

Amanda M. Franklin¹, Katrina J. Rankin¹, Laura Ospina Roza¹, Iliana Medina¹, Jair E. Garcia², Leslie Ng¹, Caroline Dong¹, Lu-Yi Wang¹, Anne E. Aulsebrook¹, Devi Stuart-Fox¹

Affiliations:

¹ School of BioSciences, The University of Melbourne, Parkville, Victoria, Australia

² Bio-Inspired Digital Sensing Lab, RMIT University, Melbourne, Victoria, Australia

Corresponding author:

Amanda Franklin, am7franklin@gmail.com; +61 3 8344 1978

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Conflict of Interest

The authors declare no conflicts of interest.

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28 AMF, KJR, LOR, IM, JEG, DSF conceived the ideas and designed the methodology; all authors
29 conducted the experiment; AMF, LOR, IM and JEG completed data analysis; AMF wrote the
30 manuscript; all authors contributed critically to drafts and gave final approval for publication.

DR AMANDA M. FRANKLIN (Orcid ID : 0000-0002-8650-8344)

MS LAURA BIBIANA OSPINA-ROZO (Orcid ID : 0000-0002-1904-202X)

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ABSTRACT

1. Some animals, including certain fish, beetles, spiders and Lepidoptera chrysalises, have such shiny or glossy surfaces that they appear almost mirror-like. A compelling but unsubstantiated hypothesis is that a highly specular or mirror-like appearance enhances survival by reflecting the surrounding environment and reducing detectability.
2. We tested this hypothesis by asking human participants to wear a mobile eye-tracking device and locate highly realistic mirror-green and diffuse-green replica beetles against a variety of backgrounds in a natural forest environment. We also tested whether a mirror-like appearance enhances survival to wild predators by monitoring survival of mirror-green and diffuse-green replica beetles in a forested habitat and an open habitat.
3. Human participants showed no difference in the detection probability or detection latency of mirror versus diffuse replica beetles, indicating that mirror-like appearance does not impair prey capture. The field predation experiment found no difference in survival between the mirror and diffuse replica beetles in forested environments. Similarly, there was no difference in survival when beetles were deployed in open habitat where there is no background to reflect, indicating that predators detect and do not actively avoid mirror-like beetles.

4. Our results suggest that a mirror-like appearance does not reduce attack by predators. Instead, highly specular, mirror-like surfaces may have evolved for an alternate visual function or as a secondary consequence of selection for a non-visual function, such as thermoregulation.

KEYWORDS

Coleoptera, field predation, gloss, human vision, protective colouration, specular reflectance

RESUMEN

1. Algunos animales incluyendo peces, escarabajos, arañas y crisálidas de mariposa tienen superficies tan brillantes que pueden parecer prácticamente un espejo. Se cree que dicha apariencia especular es una ventaja para su supervivencia ya que, al reflejar diversos elementos a su alrededor, les permite pasar desapercibidos. Si bien esta hipótesis parece bastante convincente, hasta ahora no había sido puesta a prueba.
2. En este estudio examinamos dicha hipótesis. Registramos el movimiento de los ojos de personas mientras intentaban localizar réplicas realistas de escarabajos verdes, algunas brillantes con apariencia de espejo, otras mate, sin brillo ni reflejos especulares. Estas réplicas se encontraban sobre superficies con distintos colores y contrastes en un bosque nativo. Adicionalmente, monitoreamos la supervivencia de los dos tipos de réplicas de escarabajo (especular y mate), ante depredadores naturales, dentro del bosque y en un área abierta sin cobertura de árboles.
3. Las personas que participaron en el estudio fueron igualmente capaces de detectar réplicas especulares y mate sin mostrar diferencias en probabilidad o latencia. Este resultado sugiere que la apariencia especular no tiene el potencial de impedir la captura de presas brillantes. El experimento en campo con depredadores naturales tampoco mostró diferencias en la supervivencia entre las dos réplicas de escarabajos en un ambiente de bosque. Así mismo, no se encontraron diferencias en supervivencia entre los dos tipos de réplicas de escarabajos en el ambiente abierto, donde no hay elementos que reflejar. Este resultado indica que los depredadores naturales fueron capaces de detectar las réplicas brillantes y el efecto de espejo no les generó aversión.
4. Nuestros resultados sugieren que la apariencia especular de los escarabajos verdes no reduce su probabilidad de ser atacado por depredadores. En cambio, este tipo de superficies brillantes y altamente especulares pudo haber evolucionado como

consecuencia de alguna otra función visual, o incluso no visual como por ejemplo termorregulación.

INTRODUCTION

Some animals have astonishingly shiny surfaces that appear almost mirror-like. A mirror-like appearance has evolved in diverse lineages including some spiders, beetles and pelagic animals, and chrysalises of certain moths and butterflies (Fig. 1)(Neville 1977; Steinbrecht et al. 1985; Seago et al. 2009; Jordan et al. 2012; Feller & Cronin 2014; Whyte & Anderson 2017). Optically, this mirror-like appearance is produced by a high degree of specularity: a very high proportion of light reflected at the specular or mirror angle. If combined with an extremely smooth surface, this may produce a distinct reflected image. We define a mirror-like appearance as having very high specularity such that the surface reflects the chromatic and spatial characteristics of the surrounding environment. This differs from iridescence, which produces a change in hue with different viewing and illumination angles (iridescent surfaces may or may not be mirror-like). A compelling but unsubstantiated hypothesis for the evolution of a mirror-like appearance is that it increases survival of prey by reflecting the surrounding environment, thereby improving the match with the background (Neville 1977; Thomas et al. 2007). Improving chromatic match should be beneficial against all backgrounds and visual predators whereas improving spatial match by reflecting a distinct image should only be beneficial in environments where there are spatial patterns to reflect and against predators whose visual system can resolve those patterns. By reflecting the surrounding environment, a mirror-like appearance could enhance matching against a wider variety of colours than the ones that can be matched by diffuse colouration.

In reality, no natural surface is a perfect mirror but many are highly specular, with reflections that enhance similarity to background colour (Fig. 1). To date, research into the protective function of a mirror-like appearance has largely focussed on silvery fish in the pelagic environment (Denton 1971; Johnsen et al. 2014). The pelagic environment is approximately vertically symmetrical (but see Johnsen et al. 2014 for exceptions) and relatively uniform. The mirror-like structures in the fishes' scales are positioned vertically so that the scales reflect light equivalent to the background from most viewing positions (Denton 1970; Denton 1971), providing camouflage by background matching. The uniform environment and protective benefit may explain why a mirror-like appearance is more common in fish compared to terrestrial animals. Other marine and terrestrial environments are more visually complex than the pelagic environment, and whether

a mirror-like appearance enhances survival by improving background match in different habitats remains untested.

In addition to reducing prey capture via improved background matching, a mirror-like appearance could increase survival compared to a diffuse appearance via other mechanisms. Movement of either the observer or the mirror-like animal will cause reflections on the surface to move and vary in intensity and perhaps colour. This effect could reduce prey capture via impairing object recognition or edge detection, similar to iridescence (Kjernsmo et al. 2018; Kjernsmo et al. 2020). Alternatively, a mirror-like effect may be a form of warning colouration and elicit avoidance by predators. For example, the glossiness of the chemically defended alpine leaf beetle (*Oreina cacaliae*) facilitates avoidance learning by birds (Waldron et al. 2017). However, to our knowledge there are no studies demonstrating that mirror-like animals tend to be distasteful or toxic. Whether a mirror-like appearance improves survival compared to a diffuse appearance via impaired prey capture (due to either improved background matching or impaired object recognition/edge detection) or prey avoidance remains untested. This is likely because of the difficulty of experimentally manipulating specularly independently of other optical properties and of distinguishing between mechanisms that reduce predation, both of which we address in this study.

Human detection experiments provide a powerful means to discriminate reduced prey capture from other mechanisms of protective coloration, such as avoidance due to warning colouration (Rößler et al. 2018). Although human vision clearly differs from that of birds and other predators, there are fundamental similarities in visual processing and several studies have found common features are used for object detection and recognition (Troscianko et al. 2009; Xiao & Cuthill 2016; Barnett et al. 2018). Human detection experiments generally require participants to find camouflaged prey in an image on a computer screen (e.g. Barnett et al. 2018; Troscianko et al. 2018). However, this is ineffective for testing the benefit of a mirror-like appearance in complex natural environments where viewing angle, illumination, movement and the surrounding environment can influence appearance of the mirror-like prey. Here, we use mobile eye-tracking technology to test whether a mirror-like appearance reduces prey capture in a complex terrestrial environment. This technology allows us to test whether a mirror-like appearance reduces the probability of prey capture by a predator observer by precisely recording timing and direction of gaze preceding a detection event (Billington et al. 2020). Here, we asked participants to wear eye-tracking glasses while locating highly realistic mirror-like or diffuse replica beetles in natural forest.

The mirror-like and diffuse replica beetles differed in specularity but were similar chromatically, and resembled scarab beetles that can be found naturally in these environments.

To test whether human detection corresponds to predation by natural predators, we conducted a field predation experiment and documented replica beetle 'survival' over several days. This experiment was conducted in forested habitat and focused on predation attempts by birds because birds are visual predators. We placed replica beetles on a range of backgrounds (stems, branches, leaves) and in a range of orientations (vertically, horizontally). The reflections on the mirror replica surfaces varied based on their location (and the observer position) but are always a sample of the surrounding vegetation, which should improve match with a variety of background colours. The degree of background matching for the diffuse replicas, however, varied substantially across these diverse backgrounds and should be lower than the mirror replicas (see Figure S1 in Supporting Information). Therefore, we predicted that if a mirror-like appearance improves survival by reflecting the surrounding environment, in forested habitats mirror replica beetles would be attacked less than diffuse replica beetles. A mirror-like appearance could enhance survival due to improved background match or another mechanism (impaired recognition or active avoidance). To distinguish these possibilities, we also placed beetles in an open habitat in which mirror camouflage is expected to be ineffective because the reflections of open sky on the convex beetle surface would contrast with the grass background on which the beetles were placed. If the mechanism of protective coloration was background matching, we predicted that in the open habitat mirror replicas would be attacked with higher probability than diffuse replicas; whereas we predicted the opposite pattern if mirror replicas impaired recognition or were actively avoided.

MATERIALS AND METHODS:

Study sites

We conducted the human detection experiment and bird predation experiment concurrently at the Daintree Rainforest Observatory (16° 6' 14" S, 145° 26' 58" E) in October, 2019. In February 2020, we repeated the bird predation experiment at a second site comprising dense woodlands adjacent to the Grampians National Park in south east Australia (37° 12' 43" S, 142° 36' 19" E). At this site, we compared predation in closed forest and open ground. This allowed us to test the prediction that a mirror-like appearance only provides a benefit in habitats where vegetation can be reflected such as forested habitats. The artificial beetles were replicas of locally occurring

Christmas beetle species (*Anoplognathus parvulus*, a mirror-green beetle, and *A. prasinus*, a diffuse-green beetle; Fig. 2). These two species are similar in size (17-22mm long) and colour, and are not chemically defended. Different species of green and/or metallic and mirror-like Christmas beetles are present at both sites during summer months and are usually found on Eucalypt trees eating the leaves. Common bird predators at the Daintree site included butcherbirds, shrike-thrushes and metallic starlings, and at the Grampians site included magpies, cuckooshrikes, and treecreepers.

Replica beetle construction

To determine whether a highly specular surface improves survival compared to a diffuse surface, we compare detection and survival of mirror and diffuse clay replicas of Australian Christmas beetles. To ensure our replicas matched the size and shape of real Christmas beetles, and to incorporate natural variation in these traits, we used ten preserved specimens of two similarly sized *Anoplognathus* species (Supp. Methods; Supp. Fig. S2). These clay beetles were covered in a thin layer of resin to create the ultrasmooth surface required for the mirror-like effect and the diffuse beetles were sanded to remove surface smoothness. All replica beetles were airbrushed with chrome silver (Alcad II Lacquer, USA) followed by 3:1 mix of transparent yellow and transparent green acrylic paint (PL21 and PL20 respectively, SMS, Australia). All replica beetles had a small tether embedded ventrally to secure the model in place in the field.

We compared reflectance characteristics of our diffuse and mirror replica beetles ($n = 3$ each) and between our replica beetles and real Australian Christmas beetles, *A. parvulus* and *A. prasinus* ($n = 1$ each). First, we compared the specularity (i.e. mirror-like effect) of the replica beetles and real beetles by measuring the directional reflectance of all samples following the method described by Gruson et al. (2019) (Supp. Methods). This set of measurements captures the light reflected at the specular angle (i.e. bisector between the light source and collector probe is 0° to the normal) and incremental angular changes away from the specular angle (Supp. Fig. S3). For each measurement, we extracted the maximum reflectance value and plotted these values against the position of the bisector divided by 2 to fit a Gaussian curve consistent with Gruson et al.'s methods. Then we calculated specularity as the inverse of the width of the Gaussian curve (Fig. 2b). The higher the value, the higher the specularity. Both the replica and real mirror-like beetles have very high specularity (specularity = 0.39 and 0.31, respectively) whereas both the replica and real diffuse beetles have low specularity (specularity = 0.18 and 0.09, respectively; Fig. 2b). The magnitude of this difference in specularity between mirror and diffuse beetles is very similar for both replica and

real beetles. Furthermore, a distinct reflected image can be seen in the mirror replica beetles but not the diffuse replica beetles (Supp. Fig. S4). Second, we measured total reflectance over all angles using an integrating sphere (Supp. Methods, Supp. Fig. S5) to compare overall chromatic properties. These spectra were plotted in an avian colour space (based on blue tit spectral sensitivity) and human CIE 1931 colour space (Supp. Methods). All points are tightly clustered together in both human and avian colour space indicating our treatments are likely to be chromatically indistinguishable from each other and appear similar to real Christmas beetles (Supp. Fig. S6, Supp. Fig. S7). Total reflectance is slightly higher for mirror replicas compared to diffuse replicas (Supp. Fig. S5). However, total reflectance is unrelated to perceived brightness of the mirror treatment because the mirror replicas' reflectance is largely determined by the objects being reflected. For example, if the mirror replica is surrounded by dark objects, the reflections of those objects will appear dark. There is also no angular-dependent hue-shift in models (i.e. no iridescence; Supp. Methods; Supp. Table S1). Thus, the difference in appearance between the mirror and diffuse replica beetles is due only to differences in specularity and the optical properties of replica beetles closely match those of real Christmas beetles.

Many camouflage studies estimate background match as the degree of chromatic or achromatic contrast estimated from models of animal colour vision; however, these models implicitly assume that reflectance properties do not change with angle (i.e. are diffuse). Our two treatments (diffuse and mirror-like) have similar values in avian colour space because their reflectance averaged across all angles is similar. The differences between them is due to angle dependent effects disregarded in the visual models. Furthermore, reflectance is measured using a constant light source whereas under natural conditions a mirror-like surface produces a reflected image at the specular angle (i.e. mirror angle) that is context-specific and spatially variable. Therefore, the appearance of the highly specular replica beetles will differ for every placement in the environment. Current widely-used models of animal vision (Vorobyev & Osorio 1998; Vorobyev et al. 2001) suggest that our replica beetles are chromatically similar under laboratory and specific viewing conditions, but do not capture the appearance of mirror beetles in natural conditions and cannot quantify their background match in the field.

Human detection experiment

We tested whether human participants differed in detection success or detection latency for the diffuse and mirror replica beetles. Four different locations in the rainforest were selected, to

account for natural variation in light environments and vegetation (Supp. Fig. S8). At each location, 12 replica beetles (six diffuse, six mirror) were placed within a 2 m x 2 m x 2 m area of forest. To ensure that the placement of the replica beetles was random and did not bias our results, the placement of treatment types was reversed after four participants (i.e. diffuse beetle replaced with mirror beetle and vice versa). A mirror-like appearance would be expected to reduce prey capture against any background, regardless of chromatic match, so we placed beetles on a range of backgrounds, including ones that were chromatically similar (e.g. light green leaves) and highly contrasted (e.g. brown branches) with the replica beetles (Supp. Fig. S7). Replica beetles were visible to the participant in a static position 1 m away. From this distance the target beetles projected an image with size of about 1 degree of visual angle. Targets of this size are about twice as large as the threshold visual acuity predicted from visual acuity modelling (Fülep et al. 2019) and thus relatively easy to detect by a human subject with normal vision.

To enable quantification of success rate, participants were fitted with a portable eye tracking unit (Tobii Pro Glasses 2, Tobii Sweden). The eye tracking unit recorded the position of the participant's left and right eyes during the sampling task with a frequency of 100 Hz. Raw data were subsequently analysed in a laptop computer using Tobii Pro Lab software. Immediately prior to each trial, participants were shown the replica beetles on a plain piece of cardboard, and instructed to find a total of 12 beetles within a time frame of 5 minutes. During each trial, the participant was asked to point to the location of the beetle detected and the experimenter, positioned behind the participant, noted the order, position, and treatment type of successful model detections by the participant. Trials were concluded either after the participant found all 12 replica beetles or the allocated time was lapsed. Eight participants completed trials at each location for a total of 32 experimental subjects. For subsequent analyses, data from two individuals were removed because the quality of the eye tracking data was unreliable.

To test whether there were differences in detection probability between replica beetles, we used a generalised linear mixed model (GLMM) with binomial error, and detection (or not) as a binomial response variable (lme4 package in R, Bates et al. 2015)). The fixed predictors were the type of beetle (diffuse or mirror) and the location (from A to D). Individual ID and position ID were included as random factors to account for multiple detections for each participant and whether some beetle positions were harder to detect than others.

In a second model, we determined whether detection latency differed between beetle treatments by using a mixed effect cox model implemented in R using the coxme package (Therneau 2020). Detection latency was calculated as the duration of time from the start of the experiment until the fixation immediately prior to the participant stating they detected a beetle. A fixation is when the subject has a sustained gaze on the same location for more than 200 ms. Using fixation rather than the participants' verbal cues allows for a precise measurement of detection latency, particularly when beetles were detected in quick succession. In the model, we included a variable indicating whether each beetle was detected or not (i.e. whether the data were censored), and fixed and random predictors were the same as described above. To determine the significance of each factor, we implemented marginal hypothesis tests using the Anova function from the car package (Fox & Weisberg 2019).

Bird predation experiment

At each site, we set up linear transects with ten replica beetles per treatment group, each beetle spaced 2 m apart. In the open habitat replica beetles were tethered to a nail in the ground. In the forest habitat, replica beetles were tethered to a branch at approximately chest height and placed on backgrounds that ranged from chromatically similar (e.g. light green leaves) and highly contrasted (e.g. brown branches) with the replica beetles (Supp. Fig. S6). Thus, the background match of diffuse beetles was variable whereas the background match of the mirror-like beetles should be equal or higher. Predators would be expected to most easily detect diffuse beetles that contrast highly with the background; therefore we would expect higher overall predation on diffuse than mirror-like replicas (Supp. Fig. S1).

Transects were checked for predation daily. Any beetle showing signs of predation was removed and placed in a ziplock plastic bag for scoring. Replica beetles that were attacked or removed were replaced with a new beetle of the same treatment in a different position. In the Daintree, 11 transects were set up for four days in forested habitat. In the Grampians, we initially set up 15 transects in forest and 15 transects in the open for six days to increase predation events. We added an additional five forested transects for three days to further increase sample size. In the Daintree, 220 replica beetles were deployed with 50 replaced due to attack marks or removal by a predator ($N = 270$), and in the Grampians 400 replica beetles were deployed in the forest with 34 replaced ($N = 434$) and 300 in the open habitat with 97 replaced ($N = 397$).

All replica beetles with signs of predation were assessed independently by two people. Replica beetles were scored as either: bird attack, mammal or unknown attack (small ambiguous markings), or completely removed (Supp. Fig. S9). Mammal attacks were likely from small, nocturnal marsupial mice (*Antechinus sp* or *Melomys sp*) that primarily use olfaction rather than vision to locate prey. Any beetles scored as mammal or unknown attack were removed from further analysis. Beetles where the target had disappeared were considered a bird attack because the small marsupial mice could not completely remove tethered replica beetles whereas comparatively large birds such as kookaburras and magpies could potentially do so. We ensured that replica beetles were not removed or disturbed by humans because both sites had no public access.

To investigate whether mirror-like appearance improves survival compared to a diffuse appearance, we assessed survival of replica beetles using a mixed effect cox model. Survival was calculated as number of days until attacked or the total number of days in the field if the beetle was never attacked. A variable indicating whether each data point was censored was included. First, using only the data collected in forest habitats, we tested whether a mirror-like appearance improves survival by including treatment (mirror or diffuse) and field site (Grampians or Daintree) as fixed effects. Second, using the data from the Grampians field site, we tested whether habitat (forest or open) affected survival of mirror or diffuse replica beetles with habitat, treatment and the interaction between the two factors as fixed effects. The interaction term was included to specifically test the prediction that a mirror-like appearance should reduce detection in forest but not open habitat where there is no vegetation to reflect. Both statistical models included transect ID as a random effect. To determine the significance of each factor, we implemented marginal hypothesis tests.

Ethics approval and permits

University of Melbourne Faculty of Science Animal Ethics Approval – 1914947.1, and Human Ethics Approval – 1955499.1. Research was also approved by the Daintree Rainforest Observatory Research Panel.

RESULTS

Human detection experiment

Prey capture variables (detection probability and detection latency) were calculated for 30 individuals (total beetle placements: 360). Overall, 79.7% of the replica beetles were detected and two participants detected all twelve replica beetles. There were no differences in detection between treatments ($\chi^2 < 0.01$, $P = 0.97$; mirror: 142 detected, diffuse: 145 detected; Fig 3a). There were also no differences in detection latency between treatments ($\chi^2 = 0.1$, $P = 0.76$; Fig. 3b) but there were significant differences in latency to detection between the four forested locations ($\chi^2 = 10.8$, $P = 0.01$; Supp. Fig. S10). The effect of the treatment did not depend on the location (model with interaction: $\chi^2 = 1.4$, $P = 0.71$).

Bird predation experiment

Throughout the experiment there were 24 bird predation events in forested habitats and 70 bird predation events in open habitat, equating to a predation rate of 3.4% and 17.6%, respectively. In forest habitat, there were no differences in survival of mirror or diffuse replica beetles ($\chi^2 = 0.6$, $P = 0.44$; Fig. 4a) and there were no differences in survival between field sites ($\chi^2 = 0.3$, $P = 0.58$). Replica beetles were attacked more often in open habitat than forested habitat ($\chi^2 = 32.1$, $P < 0.001$; Fig. 4b), but there was no difference in survival between mirror or diffuse beetles ($\chi^2 = 0.5$, $P = 0.49$; Fig. 4b) and no difference in survival of each treatment between habitats (model with interaction: $\chi^2 = 2.7$, $P = 0.10$; Fig. 4b).

DISCUSSION

Our results indicate that a mirror-like surface does not reduce detectability to humans in a complex natural environment, nor does it improve survival against wild bird predators, compared with a diffuse surface. Human participants were equally likely to find mirror and diffuse beetles and the detection latency for each beetle type was almost identical. This indicates that a mirror-like effect does not reduce prey capture of observers with a relatively high visual acuity (Caves et al. 2018) located 1 m away from the subject. The bird predation experiment showed no difference in survival between mirror and diffuse beetles in either forest or open habitats. If a mirror-like effect improves background matching by reflecting the surrounding environment, we would expect to see greater survival for mirror-like beetles compared to diffuse beetles across a range of background types in forested habitats. Furthermore, the lack of survival difference between beetle types in the open habitat indicates that birds did not avoid the mirror-like beetles. Together, these results

challenge the predictions that a mirror-like effect improves survival of chemically undefended prey by impairing detection, prey capture or acting as warning colouration.

Human participants found a high percentage (~80%) of the replica beetles and this was not influenced by treatment, suggesting that participants were using visual cues other than specularly to locate the beetles. All participants were shown several replica beetles from both treatments prior to commencing the search task, which allowed participants to form a search image. The eye tracking data showed that several participants systematically scanned the area for beetles. This is a cognitive strategy used by humans and animals and is indicative of using a search image to locate a target (Billington et al. 2020). It is likely that shape or the underlying green of the beetles contributed to a search image (Cuthill 2019), allowing participants to locate beetles relatively quickly. It is possible that mirror-like beetles are more difficult to detect than diffuse beetles at greater viewing distances. However, only two participants (out of 30) successfully detected all the beetles, indicating that detection was still reasonably challenging. Furthermore, there was no difference in prey capture rates between diffuse and mirror-like beetles in the bird predation experiment, which would include greater and more variable viewing distances.

Although we found no evidence that a mirror-like appearance confers a survival advantage over a diffuse appearance across a broad range of backgrounds, it is possible that a mirror-like appearance provides a benefit in specific contexts. One possibility is that high specularly only provides a survival advantage above a certain threshold of distinctness of image or for organisms of a certain colour (e.g. silver, gold). Whilst our replica mirror beetles reflected a distinct image, it is possible that greater specularly or differently coloured beetles would experience a survival benefit. Alternatively, a mirror-like appearance may only provide a benefit to prey when moving. Highly specular surfaces, including mirror-like surfaces, can produce intensity flashes as an animal moves. This may create an effect similar to motion dazzle whereby highly contrasting patterns impact the predator's ability to localise prey (Stevens et al. 2011). However, not all mirror-like animals or life stages are mobile, such as the chrysalises of moths and butterflies. Christmas beetles, which are mobile, spend a large proportion of their time relatively still while feeding or resting on *Eucalyptus* leaves. Therefore, an advantage of specular reflectance while moving is unlikely to provide an explanation for a mirror-like appearance in all instances.

The detectability of mirror-like beetles may be influenced by the specularly, or glossiness, of the background, which may increase visual noise and reduce the signal to noise ratio of the target

(Merilaita et al. 2017; Kjærnsmo et al. 2020). Specularity of the background can vary substantially between sites, among locations within sites, and with illumination conditions (Grant 1987; Kjærnsmo et al. 2020; Franklin & Ospina-Rozo 2021). For example, leaves of plants in rainforest, such as the Daintree, tend to be very glossy and much glossier than leaves of plants in drier Eucalypt forest, such as the Grampians. Background specularity should improve camouflage for highly specular and glossy targets more than diffuse targets; therefore, we might expect any survival benefit of the mirror treatment to be greater at the Daintree site than the Grampians site. Survival of mirror relative to diffuse beetles was slightly higher at the Daintree and slightly lower at the Grampians; whether this trend represents a real relationship requires further investigation. In the human eye-tracker experiment, detection latency was significantly longer at one location compared to the other three. However, this is likely to be due to the vegetation arrangement, which could increase visual complexity, and illumination conditions rather than a difference in background specularity. Thus, variation in background specularity is unlikely to explain the lack of support for mirror camouflage in our experiments.

In the bird predation experiment, attack rates were similar to other field predation experiments with both camouflaged and conspicuous unrewarded insect models (attack rates 4-12%; (Merrill et al. 2012; Arenas et al. 2015; Seymoure et al. 2017; Gunnarsson et al. 2018)) and these experiments detected significant treatments effects. Therefore, even though the attack rate was low in the forest habitat, we would expect to detect a biologically significant difference in attack rate between replica beetles, if there was a difference. A food reward associated with insect artificial prey will increase the attack rate (attack rates 20-85%; (Cuthill et al. 2005; Schaefer & Stobbe 2006; Kjærnsmo et al. 2020)) because birds are particularly good at learning the profitability of prey (Halpin et al. 2014; Hämäläinen et al. 2020). However, bird predators may use cues other than the visual cue of interest to locate artificial prey, such as olfactory cues from the reward or the presence of the investigator.

We detected higher attack rates in open habitats than forested habitats, which may be due to differences in prey conspicuousness or predator composition between habitats (Noske et al. 2008; McMillan & Irschick 2010; Bateman et al. 2017). Greater structural habitat complexity can make prey harder to locate by providing more refuges or increasing visual complexity, resulting in lower predation rates (Rohner & Krebs 1996; Grabowski et al. 2008; Xiao & Cuthill 2016). In our study, the open habitat is essentially two-dimensional and consists only of grasses whereas the forested habitat is three dimensional with various species of trees and shrubs. The greater

complexity of the forested habitat likely makes the replica beetles harder to detect (Cain et al. 2019). Predator composition could also vary between open and forested habitats. However, the open habitat was immediately adjacent to the forested habitat, and it is likely that the birds foraging in the open are a subset of those in the forest.

Our experiments investigated whether a mirror-like appearance improves survival compared to a diffuse appearance in a complex natural environment. We isolated the effect of high specularly from other optical effects and differentiated impaired prey capture due to improved background matching from other mechanisms that may reduce predation. Results of both experiments were consistent and challenge the hypothesis that high specularly improves survival compared to diffuse appearance (Thayer 1909; Neville 1977; Thomas et al. 2007). It is possible that high specularly provides protection by other mechanisms, such as mimicking other specular materials that are not of interest to predators. For example, it has been proposed that mirror-like chrysalises or leaf beetles may mimic water droplets (Neville 1977), but this remains to be tested. Alternatively, a mirror-like appearance may be a consequence of a non-visual function. Mirrors are produced by extremely smooth and ordered materials. A highly ordered, smooth structure may play a role in another function of the exoskeleton, such as water or dust repellency, physical protection, thermoregulation or a barrier to disease (Vincent & Wegst 2004). For example, structures that create a mirror-like appearance can substantially increase reflectivity (Feller et al. 2017), which may reduce heat gain in hot climates. Beetle elytra with high reflectivity have slower heating rates than elytra with low reflectivity (LOR & L-YW, unpublished data). This suggests that a mirror-like appearance may provide a thermal benefit if structures that produce a mirror-like appearance also substantially increase reflectivity. Our results suggest that the mirror-like effect produced by high specularly does not improve survival via impairing prey capture or active avoidance but has an alternate visual or non-visual function.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article.

FIGURES



Figure 1: Selection of arthropods with mirror-like appearance. From top left: *Anoplognathus aureus*, an Australian Christmas beetle (Photo: Alan Henderson); *Cetonia aurata*, a European rose chafer (Photo: Nigel Voaden); *Chrysina chrysargyrea*, a Central American jewel scarab beetle (Photo: David Laurencio); *Charidotella sexpunctata*, golden tortoise beetle found in the Americas (Photo: Andreas Kay); chrysalis of *Mechanitis polymnia*, the orange-spotted tiger clearwing butterfly from South America (Photo: Rubens Luciano); *Thwaitesia* sp. mirror spider from Madagascar (Photo: Nicky Bay).

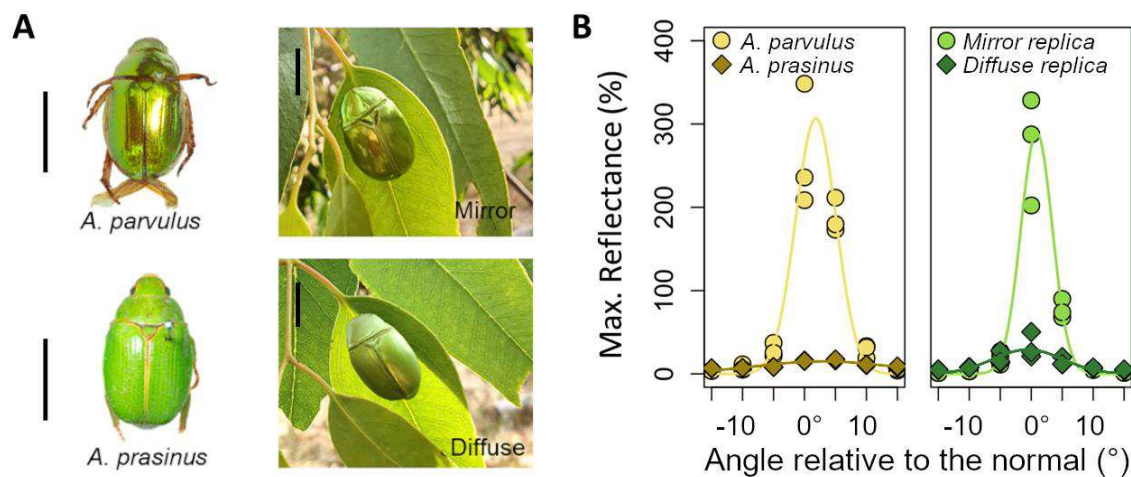


Figure 2: Optical properties of model beetles and Christmas beetles. **A.** Christmas beetles (left) and our beetle replicas (right). Scale bars 10 mm. **B.** Maximum reflectance of spectral curves measured at the specular/mirror angle (bisector between light source and collection probes is 0° to normal) and at different angles away from the specular angle. The mirror beetles and replicas (circles) show a large drop in maximum reflectance away from the specular angle (i.e. high specularity) whereas the

diffuse beetles and replicas (diamonds) show minimal angle dependent change in maximum reflectance (i.e. low specularity).

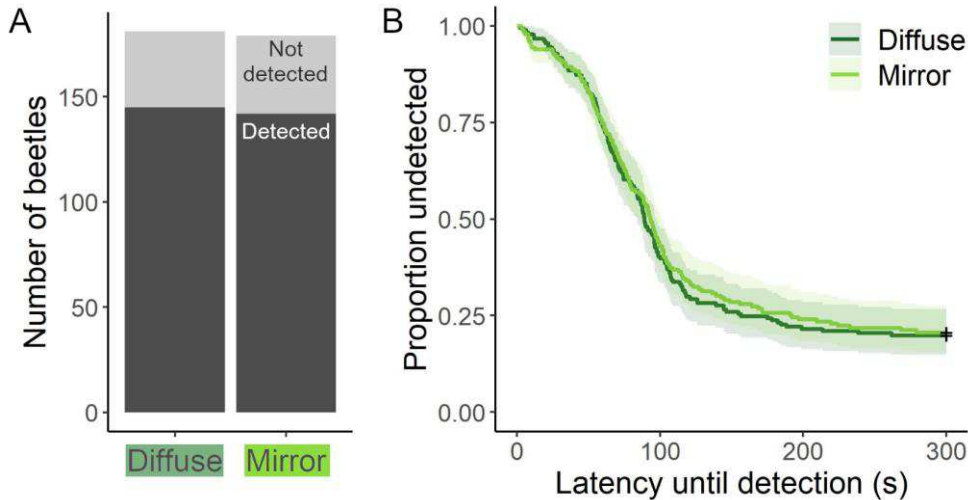


Figure 3. Results from the human detection experiment. A. Number of beetles detected for each type of replica beetle. **B.** Detection latency of replica beetles for each treatment type. Crosses indicate censored data and shading indicates 95% confidence intervals (n = 360, 30 experimental subjects).

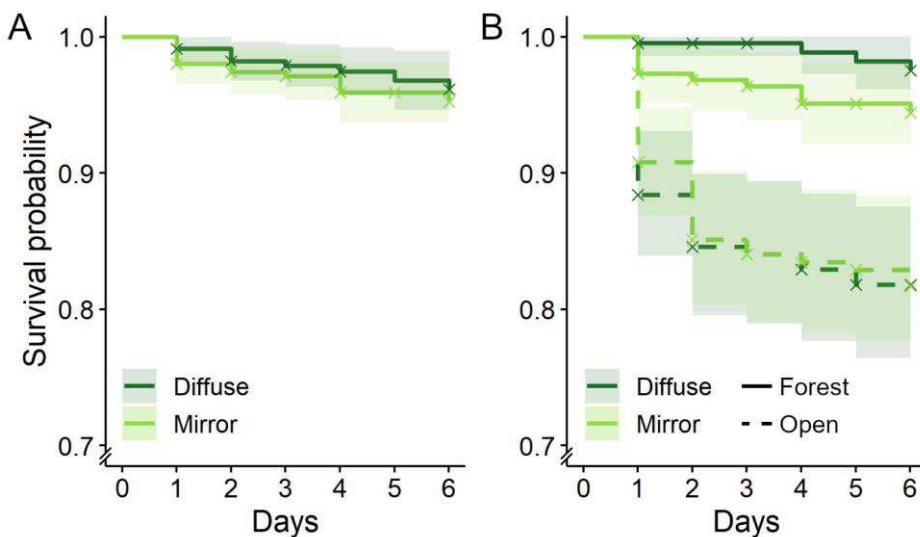
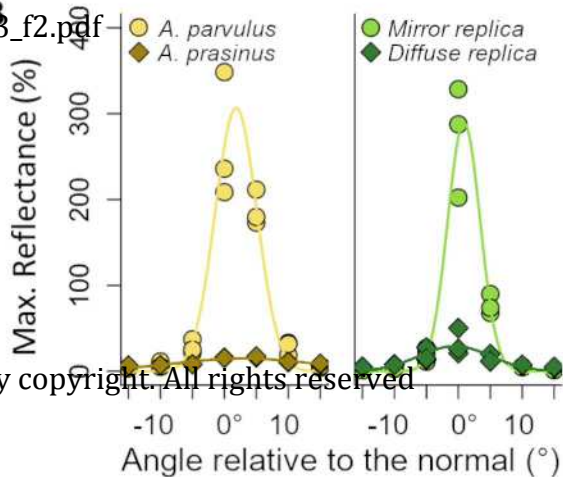


Figure 4: Results from the bird predation experiment. A. Survival of replica beetles in forest habitats (Grampians and Daintree data combined; n = 704). **B.** Survival of replica beetles in forest

648 (solid; n = 434) and open (dashed; n = 397) habitats at the Grampians field site. Crosses indicate
649 censored data and shading indicates 95% confidence intervals.



fec_13963_f1.tif

A**B**

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A

Number of beetles

150
100
50
0

Diffuse

Mirror

Not
detected

Detected

B

fec_13963_f3.pdf

Proportion undetected

0.00

0.25

0.50

0.75

1.00

Diffuse
Mirror

0

100

200

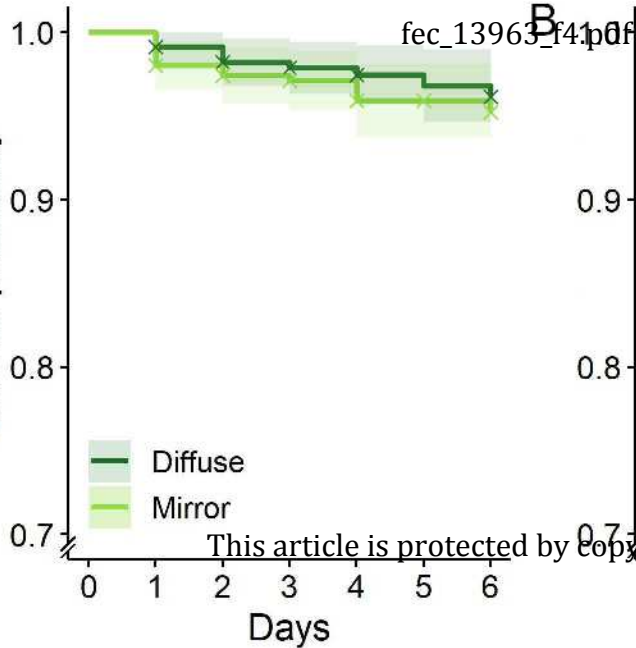
300

Detection latency (s)

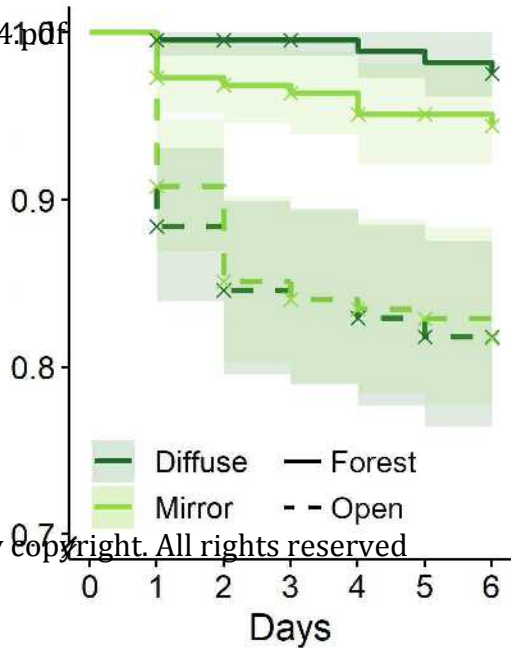
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A

Survival probability



B



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