

Does colour impact attention towards 2D images in geckos?

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Abstract

Animals are exposed to different visual stimuli that influence how they perceive and interact with their environment. Visual information such as shape and colour can help the animal detect, discriminate and make appropriate behavioural decisions for mate selection, communication, camouflage, and foraging. In all major vertebrate groups, it has been shown that certain species can discriminate and prefer certain colours and that colours may increase the response to a stimulus. However, because colour is often studied together with other potentially confounding factors, it is still unclear to what extent colour discrimination plays a crucial role in the perception of and attention towards biologically relevant and irrelevant stimuli. To address these questions in reptiles, we assessed the response of three gecko species *Correlophus ciliatus*, *Eublepharis macularius*, and *Phelsuma laticauda* to familiar and novel 2D images in colour or grayscale. We found that while all species responded more often to the novel than to the familiar images, colour information did not influence object discrimination. We also found that the duration of interaction with images was significantly longer for the diurnal species, *P. laticauda*, than for the two nocturnal species, but this was independent from colouration. Finally, no differences among sexes were observed within or across species. Our results indicate that geckos discriminate between 2D images of different content independent of colouration, suggesting that colouration does not increase detectability or intensity of the response. These results are essential for uncovering which visual stimuli produce a response in animals and furthering our understanding of how animals use colouration and colour vision.

Keywords: 2D images, Behaviour, Familiar object, Habituation, Image perception, Novel object, Reptile, Vision

Short running title: 2D stimulus perception in geckos

2 Introduction

3 Animals are confronted with a multitude of visual stimuli that affect their interactions with the
4 environment (Dall *et al.*, 2005). Although a stimulus with a single component (e.g., animal shape) may be
5 sufficient to elicit a response, many species and some specific functions may integrate two or more
6 components (e.g., shape and colour) to increase the detectability of the stimulus by the receiver (Grether,
7 Kolluru & Nersissian, 2004) and the magnitude of the response (Stevens, 2013). Colour variation and colour
8 vision are among the most studied visual stimuli for their role in adaptation, as they are used for
9 communication, including mate choice, sexual selection, and intrasexual competition, foraging, camouflage
10 and background matching (e.g., Hubbard *et al.*, 2010; Olsson, Stuart-Fox & Ballena, 2013; Cuthill *et al.*,
11 2017; Zambre & Thaker, 2017; Caro & Mallarino, 2020). Representatives of all major vertebrate groups
12 have been shown to be able to detect colour and colour contrast and even show preference for specific
13 colours (e.g., Sherwin & Glen, 2003; Roth & Kelber, 2004; Hansen, Beer & Müller, 2006; Luchiari &
14 Pironhen, 2008; Svádová *et al.*, 2009; Passos, Mello & Young, 2014; Maia *et al.*, 2017; Yovanovich *et al.*,
15 2017; Smithers *et al.*, 2018), indicating that colouration alone can elicit a response in animals. Furthermore,
16 colouration in combination with scent, sound or motion may modulate the detection of the stimulus (for
17 review see Hubbard *et al.*, 2010; Olsson *et al.*, 2013; Cuthill *et al.*, 2017; Caro & Mallarino 2020). Yet, the
18 impact of colouration alone on perception of biologically relevant vs irrelevant (unfamiliar) stimuli, and
19 the magnitude of that impact is still relatively unclear, especially for non-human vertebrates (e.g., Delorme,
20 Richard & Fabre-Thorpe, 2000; Tanaka, Weiskopf & Williams, 2001; Delorme, Richard & Fabre-Thorpe,
21 2010; Klomp *et al.*, 2017; Schwedhelm, Baldauf & Treue, 2020). Such research will further our knowledge
22 on how animals use colouration and colour vision, especially depending if they are familiar or not with the
23 observed stimulus.

24 In reptiles, research on visual stimuli has generally focused on the role of colouration in an
25 ecological and evolutionary context (but see Wilkinson, Mueller-Paul & Huber, 2013; Frohnwieser *et al.*,
26 2017), especially in combination with other stimuli such as movement, scent, or shape information (e.g.,
27 LeBas & Marshall, 2000; Klomp *et al.*, 2017; Ossip-Drahos *et al.*, 2018; Kabir, Radhika & Thaker, 2019;
28 Dollion *et al.*, 2020). It is therefore difficult to tease apart the role that colour alone plays in the process of
29 object discrimination, especially in the context of biologically relevant vs irrelevant images. For example,
30 Frohnwieser *et al.* (2017) found that colour information was not necessary for lizards (*Pogona vitticeps*) to
31 perceive an object shaped like a lizard as a lizard. To our knowledge this is the only work that has
32 investigated this issue and did so using only one tested species. Due to the lack of similar studies also on
33 other lizard species, it remains unclear is the influence that colouration has on 2D object perception when
34 other confounding factors are removed (see Kleiber *et al.*, 2021 for a similar approach in fish). Here, study
35 how three species of geckos (order Squamata) –*Phelsuma laticauda*, *Correlophus ciliatus* and *Eublepharis*

36 *macularius* – interact with images with differing colour content. Specifically, we use an object familiar to
37 the geckos (an image of a conspecific gecko) and a randomly chosen unfamiliar object (an image of a car),
38 both in colour and grayscale. We then record whether or not the geckos interact with the coloured and
39 grayscale images and measure the duration of each interaction when occurring. The three test species
40 differed in their habitat use (terrestrial vs. arboreal), main time of activity (nocturnal vs. diurnal), origin
41 (captive bred vs. wild caught), body colour, and colour pattern, which may influence how they respond to
42 colour information.

43 Geckos have largely been overlooked among reptiles in studies focusing on responses to visual
44 stimuli (but see Kabir *et al.*, 2019), possibly because most, but not all (Gamble *et al.*, 2015), genera in this
45 group are nocturnal. All geckos evolved from diurnal lizards and have retained eyes comprised of cone-
46 derived photoreceptors that are used for colour vision even in low light conditions (Röll, 2000; Roth &
47 Kelber, 2004; Pinto, Nielsen & Gamble, 2019). Geckos also possess tetrachromatic colour vision with cones
48 sensitive to ultraviolet (UV), blue, and green light (Röll, 2000; Roth & Kelber, 2004). Colour and colour
49 pattern variation is extremely prominent both within and between species (Allen *et al.*, 2020), including
50 sexual dimorphism and sexual dichromatism (Lukas & Frynta, 2002; Marcellini, 1977), suggesting that
51 these characters could be used in communication among individuals and species, and for sexual selection.
52 However, despite the large variation in colour and colour pattern observed in geckos and the diverse biology
53 of these animals, little is known about how colour information is perceived and, to our knowledge, on how
54 colour is used to improve perception.

55 Although all species used in this work can see colour, we expect the brightly coloured diurnal
56 species (*P. laticauda*) to rely more on colour information – potentially used in communication among
57 conspecifics – and therefore show greater responses to coloured images than nocturnal species.
58 Furthermore, we also anticipate a dissimilar response between the gecko images shown in their natural vs.
59 grayscale colour, as the gecko is both familiar and biologically relevant. The use of the unfamiliar image
60 (car) allows us to examine impact of colour on an object that was not biologically relevant. As such, we do
61 not expect to find any difference between the coloured vs. grayscale images of the unfamiliar object. The
62 data collected in this work will improve our understanding of how colour is used as a visual stimulus in
63 geckos, with implications for their foraging, mating, and communication.

64

65 **Materials and Methods**

66 ***Ethical note***

67 All capture, handling and experimental protocols were approved by the University of South
68 Alabama IACUC committee (protocol #993866). Experiments were carried out to minimize stress and
69 disturbance to animals and in accordance with relevant guidelines and regulations.

70

71 ***Experimental Set-Up and Testing Procedure***

72 Seven to eight geckos per species were used in experiments (Table 1). Tests were run in an arena
73 (30.5 cm x 61 cm x 20.3 cm) constructed of clear plastic and silicone. In order to test how geckos interact
74 with coloured visual stimuli, we used printed images that were presented only on one side of the testing
75 arena. All experiments were recorded using two cameras (Supplementary Material Fig. S1). To test if
76 geckos interact differently with a coloured vs. non-coloured object, we presented pictures of each object in
77 their natural colour or in grayscale. Grayscale was used as an alternative to natural colour to eliminate the
78 hue and saturation and leave just the brightness and intensity of each pixel in a given image (see
79 Supplementary Material for details). We therefore refer to colour in this work as to the chromatic
80 components of colouration, since brightness was not modified. To understand if colour impacts object
81 perception, especially if biologically relevant vs irrelevant, we selected two distinct objects: (1) an image
82 of each gecko species and (2) an image of a car. A car was chosen as a random unfamiliar irrelevant object
83 as most likely geckos have never seen it before (for an example of the use of randomly selected objects
84 never seen by the tested species see also Kleiber *et al.*, 2021). For each object type (here called image type),
85 two different images of the same type (e.g., two individual geckos) were used to ensure that the responses
86 obtained were not due to the specific image shown but rather the type of image itself (Supplementary
87 Material Fig. S2). Within a single week, geckos were shown only one image type in both colour and
88 grayscale and each trial lasted for 10 minutes for each image (Supplementary Material Fig. S3). In our
89 experimental set up, we tested for differences in responses based on species, sex, image colour, image type,
90 different images of the same type, image order based on colour, as well as habituation or memory to the
91 experimental setup (Supplementary Material Table S1). Complete details on the captivity conditions,
92 facilities, and experimental set up can be found in Supplementary Material.

93

94 ***Data Collection and Statistical Analyses***

95 We considered geckos to be interacting with an image (called “response” here) if they looked at,
96 touched, or licked the images during each experiment using the following definitions: *touching* – when a
97 gecko’s tongue (licking) or snout was touching the image; *looking* – when a gecko’s head and eyes were
98 directed directly towards the image. Both behaviours, *touching* and *looking*, were considered as “response”.
99 Gecko were considered *not looking* (defined as absence of a response) when a gecko had faced the image
100 and was aware of it but after that its head and part of its body are not directed towards the image so that it
101 is not looking at it (Supplementary Material Fig. S4, see also Supplementary Material for additional details
102 on these categories and using a more conservative approach on what we considered a “response”).

103 We analysed the influence of each variable and their interactions on the presence/absence of a
104 response to the image and on the proportion of time of response (defined here as *PLT*) only for geckos that
105 saw the images. Presence or absence of response ($PLT > 0$ vs. $PLT = 0$, respectively) were coded as geckos
106 that saw the image, moved towards it, touched it, or licked it ($PLT > 0$; *touching* and *looking*), versus geckos
107 that saw the image but turned their head away and ignored it ($PLT = 0$; *not looking*, see detailed definition
108 above and in Supplementary Material). Analyses were run in R (version 3.5.3, R Core Team, 2017) using
109 the “*glmer*” function from the package “*lme4*” (Bates, Maechler & Bolker, 2012). To study the influence
110 of each explanatory variable on *PLT*, we used a generalized linear mixed effects model, with the variables
111 of interest as fixed effects and individual ID as a random variable for random effects. Individuals were
112 considered as a random factor because the same individuals were used throughout multiple experiments
113 (i.e., different image type, two images of the same type, and colour versus non-colour images) and because
114 we were interested in inter-individual variation in response. We tested the effects of each explanatory
115 variable by performing model comparison using chi-square tests (Supplementary Material Table S1 for
116 description of each variable). Wald tests were then used to test whether the coefficients were significantly
117 different from zero in the coefficient table of the fitted mixed effects models. Tuckey’s method was used
118 to adjust the p-values for multiple comparisons.

119 We first tested for variation between response ($PLT > 0$) versus non-response ($PLT = 0$) by fitting a
120 mixed effects logistic regression model with binomial distribution. Successively, we use a generalized
121 linear mixed effects model to compare the mean *PLTs* with data only for $PLT > 0$ to see which factors
122 affected the response of the geckos. Different distributions were checked for $PLT > 0$ data. The Gamma
123 distribution was used in the mixed effects models as it fits the $PLT > 0$ data better than the normal, log-
124 normal and Poisson distributions. For each dataset, we first fitted a full model with all first-order terms of
125 all covariates, and then selected the best model using backward methods. Analyses were re-run after
126 removing non-significant effects in order to make sure that results were the same irrespective of the order
127 of effect removal (results are not reported here because analyses were always consistent). Interaction effects
128 were evaluated among covariates of interest – i.e., colour and species, species and image type, colour and
129 image type, image type and image order, day and image type, sex and colour, sex and image type.
130 Interaction effects were tested one at the time. The R package “*effects*” (Fox, 2003; Fox & Weisberg, 2018)
131 was used to gain insights into the differences among different levels of the factors of interests. A full
132 description of the experimental procedure and variables tested are described in Supplementary Material.
133 Analyses were also repeated using “*touching*” data only (touching vs. all the rest of the responses and then
134 only for $touching > 0$, considering for how long the animal touched the image).

135

136 **Results**

137 **3.1 Within species variation in PLT**

138 For *E. macularius*, 128 videos were collected; in 7.8% of these videos, the geckos never saw the
139 image shown; of the remaining videos, in 11.9% the geckos saw the images and did not respond ($PLT=0$)
140 and in 88.1% they responded ($PLT>0$). For *C. ciliatus*, 124 videos were collected; in 28.2% of these videos,
141 the geckos never saw the image shown; of the remaining videos, in 24.7% the geckos saw the image and
142 did not respond ($PLT=0$) and in 75.3% the geckos responded ($PLT>0$). Lastly, for *P. laticauda*, 97 videos
143 were collected; in 10.3% of these videos the geckos never saw the image shown; of the remaining videos,
144 in 24.1% the geckos saw the images and did not respond ($PLT=0$) and in 75.9% they responded ($PLT>0$).
145 Overall, across all the species, we obtained more responses ($PLT>0$) than non-responses ($PLT=0$) to the
146 images. Detailed summary statistics for PLT measures can be found in Supplementary Materials Table S2.
147 $Touching>0$ corresponds to 106 entries in total, representing 44.73% of the data. Frequency and distribution
148 of PLT on log transformed and raw data for each species can be found in Supplementary Material Fig. S5.

149

150 **3.2 Non-response ($PLT=0$) vs. response ($PLT>0$) analysis**

151 Overall, geckos had a $PLT=0$ (non-response) in 57 out of 294 (19.4%) experiments in which the
152 geckos saw the images. Although *E. macularius* interacted more with images than the other two species,
153 differences were not significant among species ($p>0.05$). Table 2 shows the influence of each variable from
154 a fitted generalized linear effects model. Geckos reacted differently most prominently based on image type
155 ($p=0.003$), but also image order ($p=0.010$), test room ($p=0.037$) and experimental day ($p=0.041$) (Table 2a).
156 Gecko did not respond differently to coloured versus grayscale images (Table 2).

157 We found that geckos responded significantly less to images of geckos than cars ($p=0.037$) –
158 independently of colouration –, to the second image than to the first ($p=0.010$), to the second test room than
159 to the first ($p=0.037$), and during the second day of the experiment than to the first ($p=0.041$) (Table 2b).
160 We found no differences in responses to the two different images with the same content ($p=0.307$ for the
161 car images and $p=0.604$ for the gecko images; Table 2b). When looking at the interaction among variables,
162 we observed that, depending on the image type (gecko or car), animals responded differently to the image
163 order ($p=0.007$); post-hoc pairwise comparisons indicate that animals responded significantly less to the
164 second image compared to the first image in a single test day when the image is a gecko ($p=0.002$), but not
165 when the image is a car ($p=0.85$). We found no significant interaction among all the other tested variables
166 ($p>0.05$). Although a different response was observed between the two experimental rooms, there were no
167 significant interactions with other variables, and thus the pattern of responses did not differ between rooms.
168 When we analysed data for $touching>0$ only, we found that only the image order had a marginally
169 significant effect ($p=0.04$).

170

171 **3.3 Influence of factors on duration of the response (PLT>0)**

172 In addition to analysing the variables affecting the presence or absence of a response, we also tested
173 which variable(s) influenced the proportion of time geckos spent reacting to the images ($PLT > 0$; Table 3).
174 The generalized linear mixed effects model using a gamma distribution indicates that species responded
175 differently ($p < 0.001$). None of the other variables – including colour information – influenced how long a
176 gecko responded to images (Table 3a). When looking at the influence of multiple levels within each
177 variable, we found that the diurnal species, *P. laticauda*, spent more time interacting with the images than
178 the two nocturnal species ($p < 0.001$ in both cases; Table 3b). No significant interaction effects were
179 observed ($p > 0.05$). When we analysed data for *touching* only, *P. laticauda* also spent significantly more
180 time *touching* the images than the other two species ($p = 0.002$).

181

182 **Discussion**

183 Our study focused on understanding whether colour information impacts the perception of a
184 biologically relevant and familiar object vs. a biologically irrelevant and novel object using 2D images.
185 Previous research has demonstrated that some lizard species perceive videos of conspecifics as conspecifics
186 (e.g., Macedonia, Evans & Losos, 1994; Ord *et al.*, 2002, Frohnwieser *et al.*, 2017), suggesting that the
187 ability to recognize two-dimensional images as real objects might be widespread across lizards. We
188 therefore expected geckos to perceive and interact with the 2D images shown to them. We also expected
189 that geckos would interact more often and longer with the coloured image than with the one shown in
190 grayscale for the familiar object (an image of the gecko), because of its biological significance, but we did
191 not expect to find a difference for the novel unfamiliar object (an image of the car).

192 Our results revealed that the geckos showed different responses depending on the image content –
193 car or gecko (Table 2). Geckos responded more frequently to the novel images (car) than to an image
194 of a conspecific gecko, independently of colouration (Table 2). To our knowledge, this is the first study
195 that has examined the effect of novel images on the behaviour of geckos, but studies in mammals, fish, and
196 other reptiles have found similar results, where more time was spent interacting with novel objects (e.g.,
197 Antunes & Biala, 2012; Lucon-Xiccato & Dadda, 2016; Nagabaskaran *et al.*, submitted). The lower
198 response observed for the gecko image in our study may be due to the image lacking other sensory stimuli
199 associated with a “real” object (e.g., movement, pheromones, visual displays, three-dimensional
200 orientation, UV reflectance) and commonly used in individual or species recognition (d’Eath, 1998; Bovet
201 & Vauclair, 2000; Ord *et al.*, 2002; Mason & Parker, 2010).

202 In our study, geckos did not rely on chromatic information to perceive and discriminate between
203 images, even though these animals are known to be tetrachromatic (Röll, 2000; Roth & Kelber, 2004). In
204 fact, we observed that while geckos discriminate the type of image, they did not react differently if this was

205 in colour or grayscale, independently of the biological relevance of the image (Table 2). The observed lack
206 of support for a role of colour information as a visual stimulus was surprising, since the importance of
207 colour vision and coloured traits in lizards are well established (e.g., LeBas & Marshall, 2000; Klomp *et*
208 *al.*, 2017; Ossip-Drahos *et al.*, 2018; Kabir *et al.*, 2019; Allen *et al.*, 2020; Dollion *et al.*, 2020). It is to note
209 that in our work we tested for chromatic differences in colouration (hue and saturation) and not achromatic
210 ones (brightness), which may be an important component of colouration. However, the role of colouration
211 in improving object perception is not clear (e.g., Delorme *et al.*, 2000, Tanaka *et al.*, 2001), and our findings
212 support the only other paper that has investigated this in reptiles (Frohnwieser *et al.*, 2017). Colour may act
213 in combination with shape information, or shape may be the main driver of discrimination, suggesting a
214 more limited role of colouration in object perception (Tanaka *et al.*, 2001; though see Frohnwieser *et al.*,
215 2017). Our results seem to suggest that geckos discriminate between the two 2D stimuli independently of
216 colour information, therefore shape differences may be more important for the perception of the object than
217 colouration. Shape is considered to be important in conspecific recognition and there is evidence to suggest
218 that other species can discriminate between conspecifics and objects that are shaped similarly to them
219 (Johnson & Horn, 1988; Palmer, Calvé & Adamo, 2006). Although in our work shape seems to be more
220 important than colour for object perception, the use of two-dimensional printed images might not
221 adequately reflect some properties related to colour vision, such as pigmentation or structure of the colour
222 or reflectance in the UV spectrum of the real object that is being tested (e.g., D'Eath, 1998). In our work,
223 the lack of increased response to coloured images, especially for the gecko images, may also be due to the
224 fact that colour may be used in combination with other sensory stimuli such as movement, UV reflectance,
225 or scent, which are known to play a big role in response to conspecifics in lizards (e.g., LaDage *et al.*, 2006,
226 Kabir *et al.*, 2019; though see Frohnwieser *et al.*, 2017).

227 We found that within the same testing day, geckos reacted less to the second image of a gecko than
228 the first, regardless of whether the colour/grayscale images were shown first. This suggests that geckos
229 might become habituated to an image if the image type has social significance (e.g., another gecko). The
230 same habituation was not observed when looking at the car image during the same day. However, when
231 looking at response to the same image type over two consecutive days, we observed a lower response during
232 the second day of testing independent of the image content (Table 2). Nevertheless, a lower response was
233 not observed when another image of the same image type was shown after six weeks (Table 2). This
234 suggests that the lizards habituated to the stimulus types after short-term exposure and did so more rapidly
235 for the gecko than the car. However, this habituation effect was not observed after a more substantial gap.
236 This fits with what we would expect to see from work with mammals and birds (Shettleworth, 2009) and
237 suggests that this sort of paradigm could be a useful approach to testing learning and retention in this group.

238 The visual components that are important to an animal after noticing an object might be different
239 from the factors that initially drew their attention to the object, and positive responses were therefore
240 analysed separately. We found that when analysing only the positive responses ($PLT > 0$), there was an
241 overall lack of distinction in time spent interacting with distinct image types, which suggests that image
242 content may be more important for distinguishing between image types, but other aspects of a stimulus may
243 be important for prolonged interaction. When analysing only $PLT > 0$, we found that *P. laticauda* responded
244 significantly more than the other two species (Table 3). This distinction could be due to different responses
245 to visual stimuli between nocturnal and diurnal species. Although nocturnal species, such as *C. ciliatus* and
246 *E. macularius* are able to see colour in dim light (Kelber & Roth, 2006), it is currently not known what they
247 primarily use vision for. Previous studies on diurnal geckos have found that they can perceive and
248 discriminate between different colours and coloured features of conspecifics (Ellingson, Fleishman &
249 Loew, 1995; Hansen *et al.*, 2006; Minnaar *et al.*, 2013), suggesting that these species may rely on vision
250 more than nocturnal ones. Future studies including other diurnal and nocturnal species could further test
251 this hypothesis.

252 Our work supports the capacity of geckos to perceive and discriminate among images with different
253 content, and that colour information may be less important in improving this discrimination in the absence
254 of other sensory stimuli. Our study also suggests that while all the three tested species respond to visual
255 stimuli, diurnal geckos such as *P. laticauda* may rely more on vision for object processing than nocturnal
256 species. Future studies should aim to further investigate the role of colouration for object perception and
257 discrimination in combination with other sensory information and the influence of acuity and sensitivity to
258 different colours and colour components in geckos, as this will have implications on how colour is used in
259 these animals.

260

261 **Data Availability**

262 Data will be provided as supplementary material after manuscript acceptance

263

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Author Contributions

YC, NK, AW, MK, SG, conceived the project and designed the experiments. NK and MR performed the experiments. BW, JC, and YC performed the statistical analyses. YC and NK wrote the manuscript, SG and AW provided critical revision, and JC, MK, BW provided comments. YC obtained funding for this project. All authors have read and approved the final manuscript.

Competing Interests

The authors declare no competing interests.

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Supplementary Material Figure Legend:

Figure S1. Experimental set up showing the testing arena, the placement of the image within the arena, and the location of the video-cameras outside of the experimental arena (*a* and *b* images). Testing arena shown in the images did not have the three sides covered with white paper (see Methods section).

Figure S2. Images of the geckos and cars used for each species and for each dataset. For each image type, the two image numbers are shown.

Figure S3. Protocol used for habituation and experimental set up.

Figure S4. Example of behaviours analysed from the experiments. A) *Touching*: When a gecko's tongue or the snout was touching the image. B) *Looking*: When a gecko's head and eyes were directed directly towards the image. C) *Not Looking*: When a gecko's head is not directed at the image (see Supplementary Information for additional details).

Figure S5: Frequency (top) and distribution (bottom) of PLT on log transformed (top) and raw (bottom) data for each species.

Table 1: Characteristics of species used in the experiment (IUCN, 2018). Image credit: Tony Gamble.




Species	 <p data-bbox="378 527 688 594">Leopard Gecko (<i>Eublaphariius macularius</i>)</p>	 <p data-bbox="764 527 1027 594">Crested Gecko (<i>Correlophus ciliatus</i>)</p>	 <p data-bbox="1127 527 1390 594">Gold Dust Day Gecko (<i>Phelsuma laticauda</i>)</p>
Sample Size	Male: 4 Female: 4	Males: 4 Females: 4	Males: 2 Females: 5
Habitat	Terrestrial	Arboreal	Arboreal
Diel Activity	Nocturnal	Nocturnal	Diurnal
Captive Bred animals in our study	Yes	Yes	No

Table 2: Summary output from a mixed effects logistic regression model on the reaction of geckos to images. **a)** Chi-square tests on effects **b)** Wald tests on parameters. The results show state 2 in comparison to state 1. A negative estimate signifies that the state 2 is less likely to respond than state 1 (e.g., a gecko is less likely to respond to Image (Gecko) (state 2) when compared against Image (Car) (state 1). Significant p-values (<0.05) are highlighted in bold.

a)

Variable	Chisq.	df	p value
Species	4.02	2	0.134
Colour	0.68	1	0.410
Image	8.92	1	0.003
Sex	2.59	1	0.108
Image Order	6.61	1	0.010
Handler	0.75	1	0.387
Test Room	4.33	1	0.037
Test Day	4.18	1	0.041
Image: Image Number	1.43	2	0.488

b)

State 1	State 2	Estimate	Std. Error	z value	p value
Species (<i>C. ciliatus</i>)	Species (<i>E. macularius</i>)	1.368	0.719	1.90	0.057
Species (<i>C. ciliatus</i>)	Species (<i>P. laticauda</i>)	0.264	0.700	0.38	0.706
Species (<i>E. macularius</i>)	Species (<i>P. laticauda</i>)	-1.104	0.717	1.54	0.124
Colour (N)	Colour (Y)	0.280	0.340	0.82	0.410
Image (Car)	Image (Gecko)	-1.051	0.503	-2.09	0.037
Sex (Female)	Sex (Male)	-0.932	0.579	-1.61	0.108
Image Order (1)	Image Order (2)	-0.904	0.352	-2.57	0.010
Handler (1)	Handler (2)	-0.434	0.502	-0.86	0.387
Test Room (1)	Test Room (2)	-0.767	0.369	-2.08	0.037
Test Day (1)	Test Day (2)	-0.716	0.350	-2.04	0.041
ImageGecko:ImNum 1	ImageGecko:ImNum 2	0.308	0.593	0.52	0.604
ImageCar:ImNum 1	ImageCar:ImNum 2	0.557	0.545	1.02	0.307

Table 3. Summary output of the generalized linear mixed effects model based only on the animals that responded to the image (PLT>0). **a)** Chi-square tests on effects. **b)** Wald tests on parameters. The results show state 2 in comparison to state 1. A negative estimate signifies that the state 2 is less likely to respond than state 1 (e.g., a gecko is more likely to respond if they are sex (Male) (state 2) when compared to Sex (Female) (state 1)). Significant p-values (<0.05) are highlighted in bold.

a)

Variable	Chisq.	df	p value
Species	25.44	2	<0.001
Colour	0.02	1	0.90
Image	0.03	1	0.87
Sex	3.84	1	0.05
Image Order	2.32	1	0.13
Handler	0.91	1	0.34
Test Room	0.16	1	0.69
Test Day	0.03	1	0.85
Image: Image Number	3.75	2	0.15

b)

State 1	State 2	Estimate	Std. Error	t value	p value
Species (<i>C. ciliatus</i>)	Species (<i>E. macularius</i>)	0.999	2.220	0.45	0.652
Species (<i>C. ciliatus</i>)	Species (<i>P. laticauda</i>)	15.371	3.199	4.81	<<0.001
Species (<i>E. macularius</i>)	Species (<i>P. laticauda</i>)	14.372	3.105	4.63	<<0.001
Colour (N)	Colour (Y)	-1.65	1.270	-0.13	0.897
Image (Car)	Image (Gecko)	1.381	2.239	0.62	0.5375
Sex (Female)	Sex (Male)	4.151	2.119	1.96	0.050
Image Order (1)	Image Order (2)	2.004	1.317	1.52	0.128
Handler (1)	Handler (2)	1.737	1.820	0.95	0.340
Test Room (1)	Test Room (2)	0.545	1.350	0.40	0.686
Test Day (1)	Test Day (2)	-0.242	1.305	-0.19	0.853
ImageCar:ImNum 1	ImageCar:ImNum 2	-1.415	1.942	-0.73	0.466
ImageGecko:ImNum 1	ImageGecko:ImNum 2	-3.935	2.337	-1.68	0.092