

# CONCEPT PAPER OPEN ACCESS

# Not everything is blue or brown: Quantification of ocular coloration in psychological research beyond dichotomous categorizations

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#### **ABSTRACT**

The notion that phenomenologically observable differences in the human eye are correlated with behavioral tendencies (other than gaze-following) has been addressed poorly in the psychological literature. Most notably, the proposed correlations are based on an arbitrary categorization in discrete categories of the continuous variability across various traits that could be contributing to individual eye morphologies. We review the relevant literature and assume a view of human eyes as sign stimuli, identifying the relative contrast between the iridal and scleral areas as the main contributor to the strength of the signal. Based on this view, we present a new method for the precise quantification of the relative luminosity of the iris (RLI) and briefly discuss its potential applications in psychological research.

#### ARTICLE HISTORY

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color; eyes; iris; methods; sign stimulus; variable

# Introduction

The importance of eye as signals in vertebrates is well attested in the ethological literature. Indeed, it has been assumed that in most animals the perception of eye gaze (i.e., the establishment of eye contact) elicits arousal and reactions of flight and/or attack.<sup>2,14</sup> It has been hypothesized that this widespread behavior has been selected for and perpetuated in the evolutionary history of many species because of the high likelihood that if an animal is being looked at, it is potentially another animal's prey<sup>15</sup>—the hypothesis gains support from studies on plovers,<sup>32</sup> iguanas,<sup>5</sup> chickens,<sup>37</sup> or snakes,<sup>6</sup> as all these species react defensively when being stared at. Additional evidence from eyespots in insects to intimidate potential predators lends support to this hypothesis. 40 This pattern seems to prevail in primates and, with parametric differences according to the species,8,39 eye contact has been consistently associated with threat displays.<sup>15</sup>

Given the importance of eyes in social signaling, <sup>10</sup> it is plausible to think that small changes in its physiology will escalate to phenomenologically perceivable changes in social interaction. In fact, Tomasello et al. <sup>40</sup> set out to test the hypothesis that it is at least partly the peculiar physiology of the human eye (relative to other great apes) that enables top-down common ground in immediately co-

present perceptual environments,<sup>41</sup> which is deemed essential for processes of cultural transmission and ratcheting. It is thus intuitively plausible to think that small changes in the physiology of the eye will result in significant changes in gaze-mediated behaviors. Conversely, it is also possible that changes in behavior - such as actively using eyes as a cue to infer attentional states - could have resulted in changes in eye physiology.

However, the notion that phenomenologically observable differences in the human eye are correlated with behavioral tendencies other than gaze-following<sup>40,27,29</sup> has been addressed rather sparsely and poorly in the psychological literature.<sup>26,9</sup> What little attention has been given to the issue, has focused on iridal color and its perceived psychological effects (but see also<sup>31</sup> on scleral color as a cue for health;<sup>16</sup> for the perceived effects of brightness of sclera, and pupil diameter in attractiveness;<sup>30</sup> for the influence of the limbal ring in perceived attractiveness).

This article will focus on revisiting the methodologies used in the second kind of study, related to iridal, rather than scleral color, and its perceived psychological effects. Probably the first to address the role of eye color in interaction was Worthy, 44 who hypothesized that 4ark-eyed animals, human and nonhuman, specialize in behaviors that require sensitivity, speed and reactivity, (while)

light-eyed animals, human and nonhuman, specialize in behaviors that require hesitation, inhibition and self-paced responses." The proposal that differences in iridal color correlate with behavioral or psychological tendencies is controversial for many reasons, perhaps the most compromised being the apparent assumption that similar phenotypes arise from similar genotypes across species (see also Bradley et al. 45 for a rebuttal of such assumption).21 Still, this proposal engendered a number of studies that were dubious due to a variety of methodological issues factors rendering their replication difficult or impossible (cf. 33 for a review. 4,35 One of the most important issues relates to the categorization in discrete categories of the continuous variability across various relevant traits contributing to individual eye colorations.

More specifically, previous research has categorized eyes exclusively, according to their hue as being either "blue" or "brown"; 1,3,6,7,11,12,16,17,20,22,23,34,36,42,43 Kocnar et al.<sup>23,25,38</sup> or according to their luminance as being either "light" or "dark"; Bassett & Dabbs, 2001. This presents us with two insurmountable problems - first, it is effectively impossible to compare results from studies using different categorization systems (i.e., hue, and luminance). Secondly, the classifications were subjective which, given the great deal of variation in color perception, makes it impossible to establish reliable comparisons. This problem is further accrued by the influence of the conceptual matrixes imposed by different mother tongues.<sup>28</sup> Thus, even though we admit that the intuitive layman understanding of eye color is in itself an interesting object of study - as the fact that it is perceived as meaningful by a population suggests that it complies with a signaling function in that community - we deem it necessary to develop tools that allow for a greater and more precise comparability with scientific purposes.

In this article, we present a method that partly circumvents both problems. We do this by focusing not on the color of the iris, but on the contrast between the iris and the surrounding scleral area (highest contrast, or HC, in.<sup>29</sup> The underlying assumption for this shift in attention from iridal color to contrast between iris and sclera is that, if there is an intrinsic effect of the perception of different ocular morphologies, it is likely to be due to their contribution to the conspicuity of the gazing signal. As such, a higher contrast between the iridial and scleral areas will result in a more conspicuous signal, regardless of the hue. This entails conceiving of our conspicuous eye morphology;<sup>24</sup> but see also<sup>27,29</sup> as having evolved to comply with the functions of a typical sign stimulus, or releaser in ethological terms, <sup>18</sup> pp. 41–42). To make our measurements reliable across studies, we quantified the HC with an image analysis tool instead of using purely subjective ratings. Lastly, we tested our

method against subjective ratings with a self-report survey. We report the results of this study and conclude that our method can be used in subsequent investigations of the purported influences of iridal color in social interaction in a much more reliable way than purely subjective, or intersubjective ratings.

## **Methods**

# **Participants**

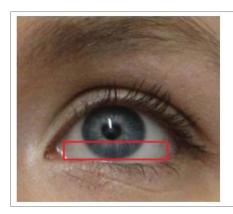
We used social media to recruit 29 males and 55 females (mean age 30,04 y  $\pm$  s.d. 8.64 years, range 21-61 for males; mean age 28.37 y  $\pm$  s.d. 6.12 years, range 21-57 for females) from 24 countries in Europe, North and South America, and Asia.

## Stimuli

All the stimuli were collected from voluntary participants at the HUME lab in Masaryk University, Brno. The photographs were taken in controlled conditions with a Canon EOS 600D, so that all the sources of lighting remained constant across stimuli - 5184×3456 pixels, 72 dpi, exposure time 1/125 sec., ISO-3200, focal length 135mm. The photographs were cropped so that participants filling the questionnaire would see only the eye and some of the surrounding skin. Stimuli representing eyes with strong make up were also excluded. Because we were interested in the conspicuity of the gazing signal, we obtained a measurement of the luminance of the iris relative to the luminance of the sclera—this is unlike the method presented by Perea<sup>29</sup> that obtained an absolute measurement of the difference in luminance between the sclera and iris (highest contrast, or HC). The output of our relative measurement was a percentage indicating how luminous the iris was in relation to the sclera, which was always assumed to be 100% luminous. In practice, this meant that the higher the percentage we obtained, the lighter the iris under scrutiny. The darkest stimulus were within the range of 15–25%, whereas the lightest were in the range of 55-65% Three researchers used this method independently, getting to a divergence in the rating of relative iris luminance (RIL) for each stimuli of not over 2%. An analysis of intercoder reliability showed a Cronbach's  $\alpha$  of 0.999, indicating significantly reliable results across researchers. Stimuli were

Table 1. Categorization of eyes according to their score in Relative Iris Luminance (RIL).

Relative Iris Luminance	15–25%	25–35%	35–45%	45–55%	55-65%	
Name	Darkest	Darker	Medium	Lighter	Lightest	
Category	1–2	3–4	5–6	7–8	9–10	



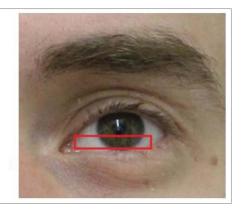


Figure 1. Selecting the region of analysis in ImageJ. Note that the rectangle excludes both skin around the eye as well as pupil, and avoids sclera in the center region, where it touches the bottom fringe of the iris.

grouped in five categories, according to the range of luminance in which they fell (see Table 1). The two more representative stimuli of each group (i.e., closest to 20%, for the darkest category, 30% for the second darkest, etc.) were selected to be used in the questionnaire. Items in the questionnaire were randomized to prevent order effects.

For the assessment of relative luminance we used ImageJ<sup>1</sup>, a freely accessible picture analysis software in the public domain. This tool allows the user to measure degrees of brightness across a selected area of pixels, evaluating their gray value. In order to compare the relative luminance between sclera and iris, the region of analysis has to be selected by hand. As illustrated on Fig. 1, the selected rectangular region captures the color of the eye across its widest part. The rectangular selection must not expand beyond the sclera in its width, and is also limited in its height to avoid distortion of the measurement: while the upper line barely touches the pupil (typically the darkest part of the eye), the bottom line ends at the lowest point of pupil's circle, avoiding the sclera (typically the lightest part of the eye). Once the region has been properly selected, it is possible to display the plot profile (a histogram of gray values across the selected region). The software will display a single average gray value for each column of pixels in the selected rectangle (see Fig. 2). The highest and the lowest of these values are then used to calculate the difference (HC), which is the basis of our RIL value. For example, the first eye in Fig. 1 had a highest value of 140 and a lowest value of 73. The output after calculating the RIL would be around 52%, meaning that the iris is a bit more than half the brightness of the surrounding sclera. The second eye in Fig. 2 had a highest value of 158, and a lowest value of 57, bearing a RIL of around 36%, meaning that the iris around a third of the brightness of the surrounding sclera.

### **Procedure**

In the questionnaire, participants were asked to rate ten images of eyes according to which color they thought they were (free response), and to how light they were in a scale of 10 (1 being the darkest, and 10 the lightest). They were also asked to describe the color of the iris with a free text response, even though this was effectively only a confounder. All participants accessed the questionnaire through a link they received, so we did not control for the time when the questionnaire was filled, neither for the device that they used to complete it. After completing the questionnaire, participants had to fill demographic data.

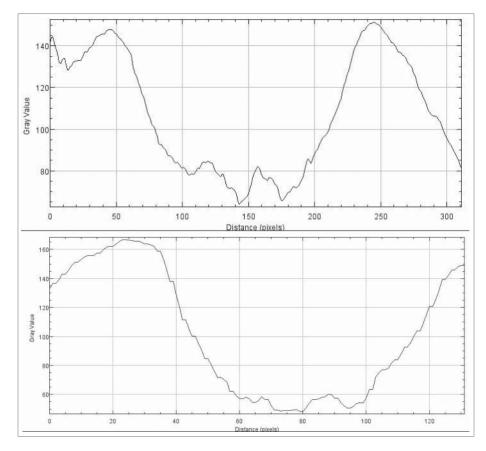
# **Analyses**

We used the data to test the ideas a) "is our method reliable?" and b) "is there a considerable divergence between our quantitative method for the measurement of relative iris luminance, and the ratings of our participants?" To test our first idea, we first ran a Cronbach's  $\alpha$ test of reliability for the ratings from the three independent researchers, which bore a result of 0.999, corresponding to an extremely high level of internal consistency. When any of the two independent raters was eliminated from the analyses, the score was still very high (0.998 and 0.996). In order to answer our second idea, we first re-scaled the average RIL scores between the three researchers for each eye into a scale from 1-10, like the one our participants used. Then, we ran a Pearson's test of correlation (r = -0.090, n = 10, p = 0.805).

#### **Conclusion and Discussion**

Our method allowed all three researchers to independently extract very consistent quantitative scores for each of our stimuli. In contrast, what the correlation index

<sup>&</sup>lt;sup>1</sup>https://imagej.nih.gov/ij/



**Figure 2.** Output graphs of the "plot profile" function in ImageJ on the images of the eyes in figure 1 above, in the same order. The vertical axis shows the average gray value (from 0 to 255) of each column of pixels, whereas the horizontal represents the distance (in pixels). The highest peaks correspond to the sclera, and the lowest values represent the iris. Note that the lightest value varies slightly across pictures, hence the convenience of establish a relative, rather than absolute, value for iris luminance.

indicates is that the average subjective ratings vary in a different way than the objective lightness between the 10 stimuli. That is an indicator that the subjective ratings do not represent objective lightness in a reliable way. A look at the standard deviation and ranges for the subjective ratings (see Table 2) shows that this method is clearly much less consistent than the one we propose. Furthermore the ranges of each stimuli should suffice to demonstrate that relying on a single subjective measurement (as is presumably often the case, with the researcher being the only evaluator) is very risky - a risk that is accrued by potential confirmation biases. Based on our results we conclude that our method has the potential to reliably establish comparisons of eye coloration across individuals, beyond the precision offered by subjective methods.

However, our method does not capture differences in hue. Even though we point at RIL as the main modulator of the strength of the signal in eye contact, it is possible that eye color mediates this effect for example through cultural connotations. Whether this is the case, and the way in which RIL and hue could interact, are topics worth exploring in the future. For now, the view we present should be complementary - or at least should not necessarily be in opposition - to one in which eye color variation arises primarily through sexual selection, or as a biomarker of ethnical in- and outgroups. A number of well-established methods can be used to determine hue as phenomenologically perceived by humans (rather than as purely physical phenomena), such as the Natural Color System.

Table 2. Summary of the descriptive statistics. The sets of scores compared in the statistical analysis of correlation is highlighted in gray.

	EYE1	EYE2	EYE3	EYE4	EYE5	EYE6	EYE7	EYE8	EYE9	EYE10
Range of subjective ratings Mode of subjective ratings	1–9	3–9 6	3–9 8	3–10 8	2–10 9	2–10 7	2–10	1–10 7	1–9	1–9
Mean of subjective ratings	3.52	5.79	6.29	7.49	8.46	7.45	6.67	5.66	5.11	3.51
Re-scaled RIL score SD of subjective ratings	1–2 1.65	3–4 1.51	5–6 1.59	7–8 1.59	9–10 1.78	9–10 1.69	7–8 1.72	5–6 2.00	3–4 2.06	1–2 1.72



The applications in research are many. Most prominently, our method offers a much more refined labeling tool than the commonly used dichotomies "blue/brown" or "light/dark" as the RIL scores capture much more variability in contrast between iridal and scleral areas. This affords a heretofore unprecedented control of eye color as an independent variable in correlational and empirical studies. Furthermore, our method promises a precise way to manipulate stimuli in studies empirically investigating the effects of eye contact, and the influence of RIL in said social stimulus - if the human eye has evolved to function as a sign stimulus, or releaser in ethological terms, <sup>18</sup> pp. 41–42), then it follows that establishing eye contact with an eye displaying a lower RIL (i.e., darker iris) should elicit more arousal than establishing eye contact with an eye that displays a higher RIL (i.e., lighter iris). This idea as well as the thresholds of RIL that could effectively elicit varying degrees of arousal in observers, demand further investigation.

# Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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