

COMPARATIVE TEMPORAL DISCOLOURATION OF DRY MUSEUM SPECIMENS OF MOUNTAIN BLACKEYE (*Chlorocharis emiliae*)

MOHAMAD FIZL SIDQ RAMJI* & MUSTAFA ABDUL RAHMAN

Department of Zoology, Faculty of Resource Science and Technology,
Universiti Malaysia Sarawak 94300, Kota Samarahan, Sarawak

ABSTRACT

Plumage colour studies using museum skins is one of the ways to understand species evolution and plumage polymorphism. The invaluable scientific information on a single historic specimen should be well-presented in the most regarded form of quality skin. However, aging plumage feathers are hypothetically susceptible to colour fading therefore doubting the relevant of using old specimens for plumage colour assessment. We examined the colour changes in 36 museum specimens by conducting series of Munsell colour scoring by comparing recent and old skins of mountain blackeye (*Chlorocharis emiliae*), at Universiti Malaysia Sarawak (UNIMAS) and Sarawak Museum (SM). The year-gap of both sets of specimens ranged from 52 to 75 years. Eight plumage characters included in this study were crown, supercilium, auricular, mantle, rump, breast, belly and vent. The results revealed that the head (crown and auricular) and underparts (breast and vent) tend to fade extensively overtime compared to the upperparts. Therefore the results of this study support the earlier findings that aging plumage feathers are susceptible to colour fading.

Keywords: plumage, museum specimens, colour scoring, fading, mountain blackeye

INTRODUCTION

Over the years, several studies had shown significant evidence of discolouration on museum specimens. Armenta *et al.* (2008) highlighted that museum specimens collected within the 50 years-mark were adequately robust for colour and plumage observations in birds. Conversely, specimens exceeding 50 years since the collection date were more vulnerable to degradation of colour pigments in the feathers. The renewed interest in the study of animal colouration has triggered interest for numerous researches to focus on the evolution of bird plumage colouration (Grande *et al.* 2004). Winker (2000) expressed concern over the importance of preparing archival-quality skins to retain the scientific values and elude colour degrading on aging skins. The latest advancement in animal colour research introduced objective methods such as reflectance spectrophotometer and digital imaging. Although these highly accurate techniques are obvious "winners" in plumage colour measurement, it is beneficial to emphasize on the fundamental concepts in colour. Standard colour charts such as the Munsell colour system are well-established and considerably influential in terms of providing options to users by ensuring simple application, practically and

cost-wise effective. The rationale of choosing this method was essentially to concur with these objective methods. Such findings were described by Grill and Rush (2000) and Bolund (2009). This study attempts to determine the extent of discolouration of mountain blackeye dry skin specimens using standard colour charts.

The mountain blackeye (*Chlorocharis emiliae*) is an excellent model species to illustrate evolution and speciation. Due to its endemic status and only occur on major mountain tops of Borneo, the species had attracted much interest by past and recent researchers, for example diet, behavioural and ecological study by Harrison (1955) and Steinheimer (1999). Generally, mountain blackeye is grouped into the huge family of Zosteropidae (the white eyes) (Mackinnon & Phillips 1993; Smythies 1999). The current taxonomic classification recognized four subspecies of mountain blackeye; *C. e. emiliae* (Sharpe 1888) from Mt. Kinabalu, *C. e. trinitae* (Harrison 1956) from Mt. Trus Madi, *C. e. moultoni* (Chasen & Kloss 1927) from Mt. Murud, Mt. Mulu, Mt. Poi and Tama Abo Range and the fourth subspecies, *C. e. fusciceps* (Mees 1954) from Mt. Maga. (Gawin & Rahman 2005) further validated the variation in mountain blackeye based on genetic and morphological characters.

*Corresponding author: f_sidq@yahoo.com

The definitive feature of mountain blackeye can be characterized by a conspicuous black orbital ring edged with light yellowish green on the supercilium and jugulum of the head (Davison 2001). These tit-like birds varied in terms of plumage colouration of the underparts from a range of olive-greens and yellows (Smythies 1999). Although the plumage complexities were apparent in each subspecies, they were documented as having distinctive plumage features by subjective interpretations of greenness, yellowness and relative darkness of blackish tone (Table 1). Mountain blackeye were also noted to possess monomorphic colouration on both sexes (Mees 1955). In 1955, Mees reviewed on

the plumage colour of *C. emiliae* skins taken by early Sarawak Museum collectors. He speculated that colour degradation was evident on earlier specimens prior to their collection dates. Latest collections by Harrison in 1952 featured the blackest crown followed by slightly browner crown found in specimens collected by Harvard Primate Expedition in 1937. Holotype specimens by Whitehead (1888) displayed distinguished green crown compared to both recent specimens. Thus, Mees (1955) concluded that melanin pigments degraded over time with specimen age.

Table 1: Plumage and body parts colouration in *Cholorocharis emiliae*

Body part	<i>C. e. emiliae</i> (Sharpe 1888)	<i>C. e. trinitae</i> (Harrison 1956)	<i>C. e. moultoni</i> (Chasen & Kloss 1927)	<i>C. e. fusciceps</i> (Mees 1954)
Head				
Crown	Blackish	Crown not dark as	Slightly darker than mantle	Dark sephia (darker than <i>emiliae</i>).
Forehead	Part yellowish/part blackish	<i>fusciceps</i> but similar to <i>emiliae</i>	yellow	
Supercilium	Yellow/dark and ill-defined	Distinct but moderate	Conspicuous broad yellow/yellowish than <i>emiliae</i>	-
Auricular	Dark green cheeks	Yellow cheeks		-
Upperparts				
Mantle	Dark olive green	Olive green	Less green and duller than <i>emiliae</i>	Intermediate between <i>emiliae</i> and <i>moultoni</i> (Banks, 1952)
Rump	Less dark, brighter than mantle	-		
Underparts				
Throat	Pale yellowish green (Davison, 1998)	Pale green to yellowish	Light green	Agrees almost entirely with <i>moultoni</i>

MATERIALS & METHODS

Specimens examined

A total of 36 dry museum skins of mountain blackeye were assessed to compare plumage colouration and the degree of fading overtime. The skins were acquired from two museum depositories namely Museum of Zoology UNIMAS (MZU) and Sarawak Museum (SM). Specimens were categorized as “recent” for 25 MZU fresh specimen sampled within one year period (13 February 2008 to 12 February 2009) and “old” for 11 SM specimen collected between 14 August 1934 and 29 August 1956 (Table 2). The year-gap between both collections ranged from 52 to 75 years, exceeding the 50 years cutoff age for the “old” definition suggested by Armenta *et al.* (2008). It is important to

note that only fine and average skins were selected for the plumage assessment as poorly-graded skins would greatly affect its natural plumage colour. All MZU study skins were prepared based on the guidelines by Proctor & Lynch (1993) and Winker (2000).

Plumage colour assessments

Plumage colours were scored using Munsell Book of Colour (Glossy finish collection; Gretag Macbeth LLC). The Munsell system of colour notation decodes a single colour into three dimensions; Hue (H) specifying the attribute of a colour (red, yellow, green, blue, purple etc.); Value (V) indicating the darkness and lightness of the colour (ranging from 0: very dark to 10: Very Light); Chroma (C) describing the intensity of the colour (ranged from very saturated/weak to very intense/vivid/strong). The

formal notation for describing a particular colour is written as HV/C, for example; 7.5H 6/10 describing a colour with a hue of 7.5 yellow, a moderate value of 6 and a very intense chroma of 10 (Table 3). Plumage observation and colour scoring were standardized by using one person to record data for every session, similar to techniques described by Miskimen (1980), Burley *et al.* (1992), Vanderwerf (2001) and Bostrom & Ritchison (2006). The scoring task was conducted under controlled environment and consistent lighting (ample daylight/cloud-free). This is to avoid illuminant (metamerism) during colour matching (Wood & Wood 1972).

Appropriate Munsell colour chips were matched to the following eight plumage characters; crown, supercilium, auricular, mantle, and rump, breast,

belly and vent. For multiple colouration in plumage region such as on the belly, colour is determined by considering the most dominant colour with the largest surface area. Both MZU and SM specimens were scored on separate sessions with three series of repetitions. The means were obtained from the scores of the overall dataset. Munsell value and chroma were used directly in the analysis, but the scores for hue were initially transformed to a numerical scale (10YR = 0, 2.5Y = 1, 5Y = 2, 7.5 = 3, 10Y = 4). Discriminant function analysis (DFA) was performed for the scored characters by assigning the specimens as grouping variables (specimen holders). Data were analyzed using SYSTAT 12 (2007) statistical software.

Table 2: Detailed listings of MZU and SM specimens

No.	Sample	Sex	Sampling Site	Collection Date	GPS	Elevation (asl; meters)
01	MD01	F	Mt. Murud	29 Oct 2008	N 03° 55.645' E 115° 30.676'	2113
02	MD02	M	Mt. Murud	29 Oct 2008	N 03° 55.645' E 115° 30.676'	2113
03	MD03	F	Mt. Murud	29 Oct 2008	N 03° 55.645' E 115° 30.676'	2113
04	MD04	-	Mt. Murud	31 Oct 2008	N 03° 55.645' E 115° 30.676'	2113
05	ML01	-	Mt. Mulu	6 Feb 2009	N 04° 02.694' E 114° 54.651'	1764
06	ML02	-	Mt. Mulu	6 Feb 2009	N 04° 02.694' E 114° 54.651'	1764
07	ML03	-	Mt. Mulu	6 Feb 2009	N 04° 02.694' E 114° 54.651'	1764
08	ML04	-	Mt. Mulu	6 Feb 2009	N 04° 02.694' E 114° 54.651'	1764
09	ML05	-	Mt. Mulu	9 Feb 2009	N 04° 02.694' E 114° 54.651'	1764
10	KK14	-	Mt. Kinabalu	20 Feb 2008	N 06° 02.676' E 116° 33.795'	2691
11	KK16	-	Mt. Kinabalu	20 Feb 2008	N 06° 02.676' E 116° 33.795'	2691
12	KK12	-	Mt. Kinabalu	20 Feb 2008	N 06° 02.676' E 116° 33.795'	2691
13	KK26	-	Mt. Kinabalu	21 Feb 2008	N 06° 02.676' E 116° 33.795'	2691
14	KK15	-	Mt. Kinabalu	20 Feb 2008	N 06° 02.676' E 116° 33.795'	2691
15	TM01	F	Mt. Trus Madi	11 July 2008	N 05° 33.453' E 116° 30.362'	2368
16	TM02	M	Mt. Trus Madi	10 July 2008	N 05° 33.453' E 116° 30.362'	2368
17	TM03	F	Mt. Trus Madi	10 July 2008	N 05° 33.453' E 116° 30.362'	2368
18	TM04	M	Mt. Trus Madi	11 July 2008	N 05° 33.453' E 116° 30.362'	2368
19	TM05	M	Mt. Trus Madi	10 July 2008	N 05° 33.453' E 116° 30.362'	2368
20	TM06	M	Mt. Trus Madi	10 July 2008	N 05° 33.453' E 116° 30.362'	2368
21	TM07	F	Mt. Trus Madi	11 July 2008	N 05° 33.453' E 116° 30.362'	2368
22	TM08	M	Mt. Trus Madi	11 July 2008	N 05° 33.453' E 116° 30.362'	2368
23	TM09	M	Mt. Trus Madi	11 July 2008	N 05° 33.453' E 116° 30.362'	2368
24	TM10	M	Mt. Trus Madi	10 July 2008	N 05° 33.453' E 116° 30.362'	2368
25	TM11	F	Mt. Trus Madi	10 July 2008	N 05° 33.453' E 116° 30.362'	2368

26	SMMG01	M	Mt. Maga	1 March 1939	4°20'N 115°50'E	-
27	SMMG02	F	Mt. Maga	1 March 1939	4°20'N 115°50'E	-
28	SMML01	F	Mt. Mulu	14 Aug 1934	-	1981
29	SMML02	F	Mt. Mulu	16 June 1935	-	1828 - 2134
30	SMPH01	M	Poi Range	18 Aug 1936	1°30'N 110°E	1219 - 1524
31	SMPH02	F	Poi Range	16 Aug 1936	1°30'N 110°E	1219 - 1524
32	SMTA	M	Tama Abo Range	18 Apr 1936	-	1372
33	SMTM01	M	Mt. Trus Madi	26 Aug 1956	-	2377
34	SMTM02	F	Mt. Trus Madi	29 Aug 1956	-	2377
35	SSMKK01	M	Mt. Kinabalu	6 Feb 1952	-	3353
36	SMKK02	F	Mt. Kinabalu	6 Feb 1952	-	3353

Key : “-” = sex unknown

RESULTS

General plumage description

When comparing old specimens against fresh specimens, the old skins were slightly worn and likely to expose drab colouring on the tip of feathers (grey, dull-brownish look). To some extent, the supercilium and blackeye lores does not exhibit putative textures of well-defined ring and were potentially prone to colour fading. Furthermore, feather arrangements appeared aberrant, pale and less natural. In contrast, the recent skins were appealing through its fresh colouration and perfectly-outlined plumage criteria.

Plumage colour analysis

Based on Figure 1, DFA clearly separate all 36 specimens into two distinct clusters. There was no overlap of hue between both specimen holders. Hue scores of SM specimens ranged from two hue yellow sheets; 2.5Y and 5Y while all MZU specimens consistently scored at 7.5Y. The value and chroma scale for MZU specimens were considerably larger, ranging from 2 to 8 and 2 to 11, respectively.

On the other hand, SM specimens revealed smaller range for value (3 to 7) and chroma (4 to 10). The statistical values are as follows; Hue ($F = 231.64$, $df = 7$, $P = 0$), value ($F = 0.429$, $df = 7$, $P = 0.884$) and chroma ($F = 0.327$, $df = 7$, $P = 0.942$). To make qualitative interpretation of the colour scores simpler, we used a simple colour index to standardize the colour readings (Table 3). The crown area showed drastic decrease in amount of very dark olive-green;

7.5Y 3/3 to 2.5Y 3/4 describing a fading dark/saturated deep yellow to dark/saturated pale yellow. The followings are colour index descriptions for other plumage characters; Supercilium = moderate/very intense deep yellow to pure yellow, Auricular = moderate/very intense deep yellow to moderate/intense pale yellow, Mantle = dark/saturated deep yellow to dark/saturated pure yellow, Rump = dark/moderate deep yellow to dark/moderate pure yellow, Breast = moderate/intense deep yellow to moderate/moderate pale yellow, Belly = moderate/very intense deep yellow to moderate/very intense pure yellow, Vent = light/very intense deep yellow to moderate/very intense pale yellow (Table 4).

Colour-age relationships

Specimen age was determined by calculating the number of years between the specimen collection date and date of scoring. The average age of MZU and SM specimens are 0.95 ± 0.1 years and 66.18 ± 9.08 years respectively, while the average year-gap between both sets of specimens was approximately 65 years. Based on the colour-age linear graphs (Figure 2), specimen age was significantly correlated with hue ($r = -0.995$, $p = 0.000$) while value ($r = -0.206$, $p = 0.227$) and chroma ($r = -0.226$, $p = 0.184$) effects were diminutive against time. Hue dramatically decreased by two steps, from 7.5Y to 2.5Y suggesting degradation of colour pigments overtime on all plumage regions particularly on the crown, auricular, breast and vent.

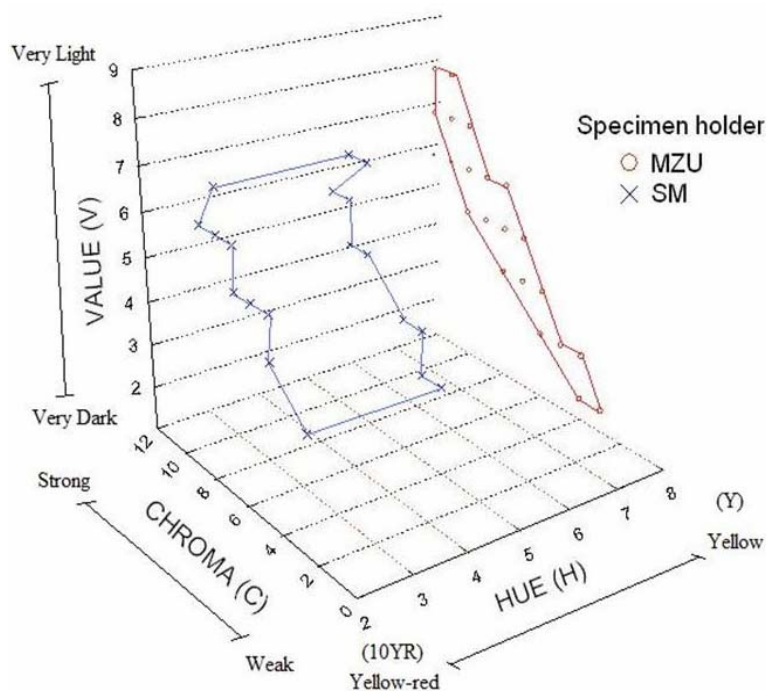


Figure 1. Scatterplot generated clear groupings of MZU and SM specimens as displayed in a modified 3-dimensional colour space.

DISCUSSION

Plumage colouration is strongly correlated with colour pigments. Most bird plumage consists of three primary substances which includes melanins (black, grey, brown), carotenoids (intense red and yellow) and porphyrins (red, brown and vivid green). In relation to mountain black eye, the crown, mantle and rump including flight feathers on the wing and tail are visually dark olive green while the underparts displayed blends of bright and intense yellow/yellowish green. It was suggested that melanin and carotenoid were involved directly in the plumage of mountain blackeye.

This finding supports Proctor & Lynch (1993) which suggested melanins are fundamental and frequent in plumage feathers (usually on the upper parts). Carotenoids were commonly influenced by diet of the species (Price *et al.* 2006). For instance, the diet of mountain blackeye consist mainly of brightly coloured rhododendrons, yellow nectars of *Schima wallichii* and red fleshy fruits of rubus berries. Another type of colour pigment associated with the yellow-green complexity on the belly was probably caused by structural pigments

produced by combinations of yellow carotenoids and black melanins.

The head (crown and auricular) and underparts (breast and vent) faded more compared to the upperparts. The decrease in hue (7.5Y to 2.5Y) on the old specimens was adjacent to 10YR (yellow-red).

This somehow revealed the colouration of yellow with rusty and drab appearance. Several factors potentially attributed to colour fading in these areas were subject to handling, preparation and storage condition of specimens. Bird skins are highly vulnerable to biodeterioration due to activities of organism (Allsopp *et al.* 2004). Grande *et al.* (2004) discussed with details the implication and role of feather-degrading bacteria in museum specimens. The SM specimens were possibly affected by similar threats considering the storage condition of the specimen. The over exposure of the head and underparts to humidity and bacterial infections are presumably characterized by the common practice of laying specimen with the underparts facing upwards. Similar findings were reported by Armenta *et al.* (2008).

Table 3: Munsell hue, value and chroma index description

Hue		Value		Chroma	
S ^a	D	S	D	S	D
10Y/ 0GY	Near Yellow-green (4)	10	Very Light/pure white (VL)	>10	Very Intense/vivid/Strong (VI)
		9		10	
				9	
7.5Y	Deep Yellow (3)	8	Light (L)	8	Intense (I)
		7		7	
5Y	Pure Yellow (2)	6	Moderate (M)	6	Moderate (M)
		5		5	
2.5Y	Pale Yellow (1)	4	Dark (D)	4	Saturated (S)
		3		3	
10YR/ 0Y	Yellow-Red (0)	2	Very Dark/ pure black (VD)	2	Very Saturated/Weak (VS)
		1		1	

S = Score; D = Description

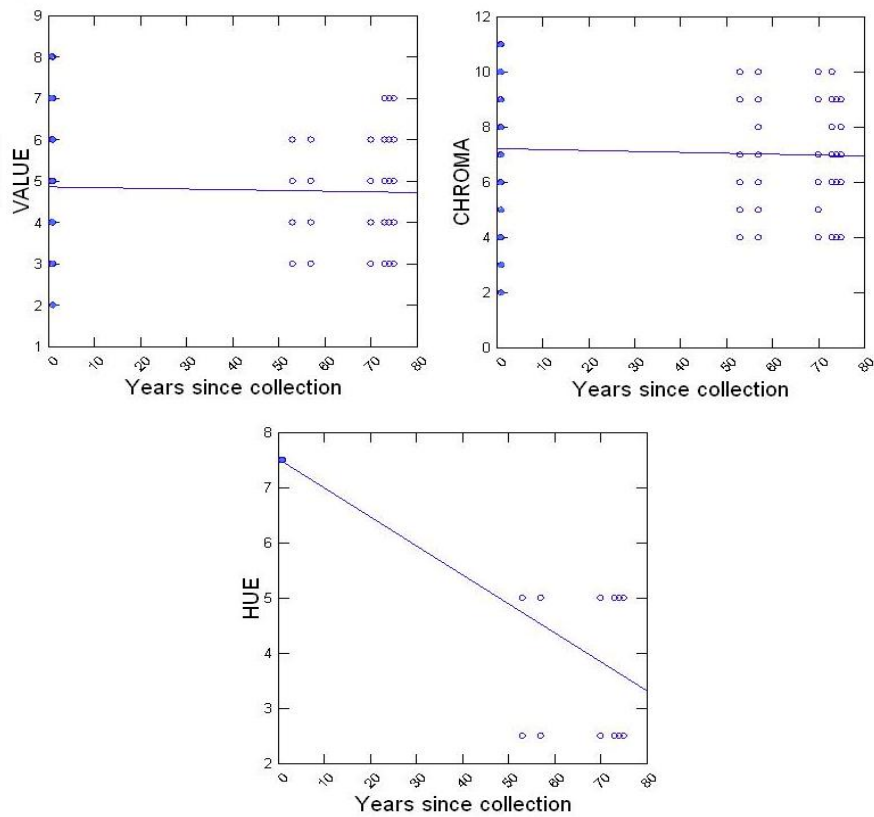


Figure 2. Comparison of hue, value and chroma of both specimens overtime. Closed circles represent MZU specimens while open circles for SM specimens (linear fit shown).

Table 4: Colour scores for each plumage characters were given in mean \pm SD

Plumage Character	Hue		Value		Chroma	
	MZU	SM	MZU	SM	MZU	SM
Crown	7.5Y	2.5Y	2.68 \pm 0.56 (2-4)	3	3.4 \pm 1 (2-6)	4
Supercilium	7.5Y	5Y	6 \pm 0.5 (5-7)	5.73 \pm 0.47 (5-6)	9.76 \pm 1.01 (7-11)	9.78 \pm 0.47 (9-10)
Auricular	7.5Y	2.5Y \pm 0.47 (2.5Y-5Y)	5.32 \pm 0.56 (4-6)	5.45 \pm 0.52 (5-6)	8.64 \pm 1.11 (6-10)	8.36 \pm 1.43 (7-10)
Mantle	7.5Y	5Y	3	3	4	4
Rump	7.5Y	5Y	3.96 \pm 0.45 (3-5)	3.81 \pm 0.4 (3-4)	5.76 \pm 0.44 (5-6)	5.55 \pm 0.52 (5-6)
Breast	7.5Y	2.5Y	4.92 \pm 0.4 (4-6)	4.55 \pm 0.52 (4-5)	7.52 \pm 0.71 (6-9)	6.36 \pm 0.5 (6-7)
Belly	7.5Y	5Y	6.08 \pm 0.76 (4-8)	6.18 \pm 0.75 (5-7)	9.4 \pm 1.15 (7-11)	9 \pm 0.77 (8-10)
Vent	7.5Y	2.5Y \pm 0.52 (2.5Y-5Y)	7.04 \pm 0.73 (6-8)	6.09 \pm 0.3 (6-7)	9.44 \pm 0.91 (7-11)	8.9 \pm 0.7 (8-10)

The results of matching feathers reflect human subjective perception towards plumage colour. It is critical to emphasize on the subjectivity and limitations of human colour system whereby even under controlled parameters such as identical lighting conditions, such as full sunlight or artificial light, the results of colour scoring by different human observers would regularly produced inconsistency and bias colour data (Endler 1990; Stevens 2009). In this study, this was minimized by regularly having only one person and controlled parameters to conduct the colour scoring in three separate sessions, thus improving scoring accuracy. Furthermore, Bennett *et al.* (1994) debated earlier hypothesis of birds perceiving colour equally as human. He stated that birds appeared to be UV sensitive and probably tetra chromatic. Consequently, the introduction of reflectance spectrophotometers persuades more researchers to focus on more objective techniques as it gradually becomes affordable. In support of Stevens *et al.* (2009), the current study assents that colour charts should only be a recommended option for plumage colour assessment if objective methods are not available. However, conventional colour charts are still relevant considering its uncomplicated and practical approach. Hence, we encourage the use of Munsell charts to describe plumage colour of museum skins by providing colour notation associated with subjective colour interpretation on the specimen label. To enhance accuracy, we firmly suggest repetitive test to be incorporated by ascertaining reliability of regular human observers and uniform time measurement of data collection.

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