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Objective evaluation of colour variation in the sand-burrowing beetle *Chaerodes trachyscelides* White (*Coleoptera: Tenebrionidae*) by instrumental determination of CIELAB values

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The objective evaluation of the different colours of individual sand-burrowing beetles *Chaerodes trachyscelides* White illustrates that instrumental techniques may replace visual assessments and subjective descriptions of colour variation. Colour measurements based on the principles established by the Commission Internationale d'Eclairage (CIE) provide quantitative specifications of colours as their coordinates in the 1976 CIE L*,a*,b* (CIELAB) uniform colour space. The established precision of the method enables rapid and reliable determination of the distribution of colours within collections made for studies on cryptic melanism.

Keywords: Colour measurement, Chaerodes trachyscelides, *CIELAB, cryptic melanism*

INTRODUCTION

There have been many studies of colouration in animals with particular reference to its primary adaptive significance, often in relation to its camouflage effect. Camouflage makes individual animals less conspicuous to predators, so different colours are required in different environments (Poulton, 1890; Cain and Sheppard, 1952; Kettlewell, 1973; Harris, 1988 and see summaries in Endler, 1978; Bishop and Cook,1980; Lees, 1981). In the above studies, colours were assessed subjectively and described by the use of general colour names, and this restricted the precision of the results. However, the limitations of visual observations and ill-defined descriptive terms for colours and colour differences may, in principle, be overcome by the use of instrumental techniques to provide objective specifications of colour values.

In this paper an application of the colour measurement system established by the Commission Internationale d'Eclairage (CIE, 1931) is introduced. The usefulness of a quantitative method providing CIELAB colour specifications is illustrated from samples of the sand burrowing beetle *Chaerodes trachyscelides* White. In a previously reported investigation on cryptic melanism in this species (Harris, 1988), the various colours of the beetles were described subjectively as pale whitish yellow, through shades of brown, to almost black. The animals were grouped into four categories on a subjective appearance scale. The present study aimed to provide an objective evaluation of those colours and their distribution in terms of CIELAB values.

CIE COLOUR SYSTEM

The perceived colour of an object depends on (1) the nature of the illuminating light, (2) its modification by interaction with the object, and (3) the characteristics of the observer's visual response (Billmeyer and Saltzman, 1981). The CIE system defines these conditions as

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follows. (1) The relative spectral power distributions of various illuminants, known as Standard Sources, are specified and are available as published tables (Wyszecki and Styles, 1967). (2) The modification of an illuminant by interaction with an object is measured with a reflectance spectrophotometer having an optical geometry which conforms to CIE recommendations, and provides a visible spectrum expressed as the fractions of incident light intensity reflected in the wavelength range 400-700 nm. (3) The nature of human colour vision has been quantified for the purposes of colour measurement in terms of three colour matching functions $\bar{x}$, $\bar{y}$ and $\bar{z}$. Three are required since colour vision has been found to be trichromatic: a single perceived colour may be regarded as resulting from the effect of three separate stimuli on the visual cortex (Wright, 1969). Their numerical values are available as published tables and are known collectively as a CIE Standard Observer (Wyszecki and Styles, 1967).

Colours are measured in terms of their tristimulus values X,Y and Z by combining the illuminant, reflectance and observer data with three summations, each having the form:

$$\sum E.R.\bar{x} = X$$

At selected intervals in the wavelength range 400-700 nm the relative energy in the chosen Standard Source is multiplied by the fraction reflected and the numerical value of the Standard Observer. A wavelength interval of 10nm, which requires 32 terms in each summation, gives adequate precision for most purposes.

Tristimulus values are inconvenient in some applications since they are not easy to relate to the subjective experience of seeing the colours. In addition, in any study involving comparisons, contrasts or changes, they do not directly enable measurement of the difference between two colours. These concerns can be overcome by using the tristimulus values to calculate the 1976 CIE L*a*b* (CIELAB) colour space values (CIE, 1978; Hunter and Harold, 1987). Colours may then be regarded as existing in a three dimensional space in which each particular colour has a unique location defined in terms of its cartesian coordinates with respect to the axes L*, a* and b*, as shown in Fig. 1.

The L* value of a colour has been recommended by the CIE as the psychometric correlate of the visually perceived colour attribute of "lightness", to which the subjective descriptive terms assigned include the words pale, light, dark, *etc*. The use of polar coordinates enables definition of a hue angle h°= arctan(b*/a*) which is recommended by the CIE as the psychometric correlate of the visually perceived attribute of hue (eg. red, orange, yellow *etc*.). The angular position of some generic hues are shown in the diagram. Colours for which both a* and b* are zero are termed achromatic and would be perceived as grey. The subjective attribute of chroma or "saturation" may be measured in terms of the position of a colour with respect to the achromatic L* axis, in the a*b* plane. This is the length of the line C in the diagram and is termed the CIE 1976 a, b chroma calculated as $[(a^*)^2 + (b^*)^2]^{1/2}$. Thus the use of CIELAB coordinates enables objective specification of the three attributes of a colour by which it is subjectively distinguished (Munsell, 1981).

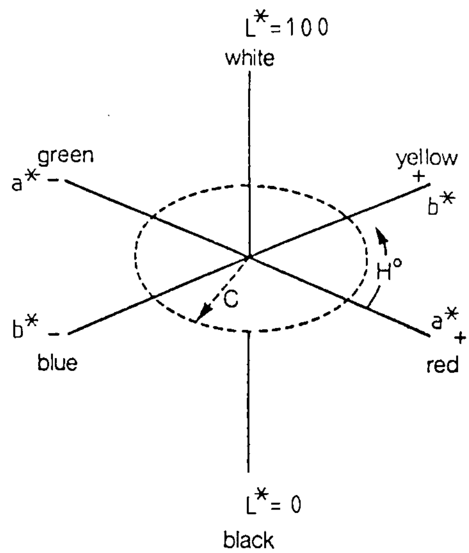

Fig. 1 – CIELAB Colour space diagram. In the text, H° (for hue angle) is written as h°.

An important property of CIELAB space is its uniformity with respect to visually perceived differences between colours. Therefore, it provides a means for measuring the difference between two colours (Hunter and Harold, 1987). This is calculated, using coordinate geometry, as the length of the line joining their coordinate positions. A special case would be the quantitative assessment of differences in only one colour attribute. Thus a set of colours which are subjectively arranged in perceptually equal steps with respect to "lightness" will be numerically equidistant from each other in terms of their L* axial position.

Current instrumentation for scanning reflectance spectophotometry enables colour specification with a precision greater than the human eye can perceive (Chong, 1988). However, this refers to measurements made on uniform flat opaque surfaces for which the equipment has been specifically designed. The precision obtainable in colour measurements on non-ideal substrates depends on the form of the sample, its uniformity and on the experimental method used (Hunter and Harold, 1987). The convex form of *C. trachyscelides* is a non-standard substrate for colour measurement, which required the development of an appropriate experimental method, as described below.

METHODS

Reflectance measurements were made with a LabScan 6000 scanning reflectance spectrocolorimeter (Hunter Associates Inc.) which had 0° illumination, adjustable beam diameter and 45° viewing geometry. This was controlled by an IBM XT microcomputer which also processed the signals by means of a suite of programmes supplied with the instrument. Reflectance spectra were measured at 10 nm intervals from 400-700 nm and their CIELAB coordinates computed for CIE Standard Source D65 and the 1931 CIE 2° Standard Observer.

The specimen holding device consisted of a matt black anodised metal tube 20 mm long and 20 mm outside diameter. The inside diameter was 12 mm at the lower end and narrowed from a point half way up the inside to 5 mm at the top. The lower end could be centrally located in the 17 mm circular optical port of the colour sensor by means of a lip 0.5 mm deep and the same in diameter as the port opening. The upper end of the tube was capped with a piece of black plasticine modelling clay. The experimental procedure consisted of gripping the head of the pin on which each beetle was mounted and, without disturbing the specimen, inserting the pointed end into the tube so that it passed through the plasticine cap and was held by it. The beetle was centrally located and its position adjusted so that the convex dorsal surface just projected out from the lower end of the tube. This was then placed over the open port of the spectrometer and the measurement sequence initiated. The incident light beam was focussed to a 6 mm diameter spot which just illuminated the entire specimen. A short length of the mounting pin projected vertically down from the specimen into the sensor chamber. Its effect was assumed to be similar for all specimens and to be small because of the optical geometry of the sensor.

The experimental precision of the method was examined by repeated measurements on six individual beetles from the collections of *C. trachyscelides* specimens held at the Otago Museum. The specimens were selected visually. The six beetles appeared to represent the full range of colours in the collection and differed subjectively in perceptually equal steps. They were arranged in a numbered sequence from lightest (1) to darkest (6). Each was measured 10 times, and between readings the specimen was removed from the holder and then reinserted.

Samples of beetles collected from beaches at Waitotara, Plimmerton and Rabbit Island had been previously assessed visually for their colour distribution (Harris, 1988). The same beetles were measured and the CIELAB data analysed with the statistics programme Microstat (Ecosoft Inc.), to give L* frequency distributions, in which the measurement intervals selected were based on the values obtained for the reference set of samples, taking into account the experimental precision of the method. These distributions were compared with those previously obtained by subjective analysis.

RESULTS

Table 1 shows the mean CIELAB coordinates of the six representative beetles and the experimental variability. The precision of the method is indicated by the within-sample standard deviations, coefficients of variation and ranges.

The "lightness" of the six specimens as measured by their mean L* values decreased in sequence from 46 to 22.4. The average difference between categories was 4.7. The mean L* standard deviation, CV and range averaged over all specimens were 0.83 and 2.6% and 2.5 respectively. The largest range within any set of L* measurements was ± 1.8 about the mean. This was about twice the largest standard deviation.

Hue angles were calculated from each individual measurement of a* and b* , not their means, so as to obtain a measure of experimental precision for h°. The mean hue angles decreased in sequence but specimens 1-3 differed by less than 4° compared with the overall difference of 18.5°. The within-sample CV for hue angle was lower than for L* in all cases except specimen 6, but in that case there was a larger experimental error due to the low a* and b* values, and this had a proportionately greater effect in the calculations of the within-sample hue angle variability. The average CV was 1.6% and the average range about the means ± 1.5°.

Table 1 – Colour measurements of six representative beetles. Mean CIELAB coordinates from 10 readings.

Specimen	CIELAB values		Sd	CV%	Min.	Max.	Range
1	L*	**45.97**	0.59	1.3	44.97	46.71	1.74
	a*	**6.49**	0.22				
	b*	**23.25**	0.61				
	h°	74.4°	0.18°	0.2	74.1°	74.6°	0.5°
	C	24.1					
2	L*	43.24	1.02	2.3	41.20	44.68	3.48
	a*	8.21	0.26				
	b*	24.33	0.54				
	h°	71.4°	0.45°	0.6	70.8°	72.3°	1.5°
	C	25.7					
3	L*	37.75	0.85	2.3	36.52	38.80	1.28
	a*	6.48	0.14				
	b*	18.68	0.32				
	h°	70.9°	0.43°	0.6	70.5°	71.7°	1.2°
	C	19.8					
4	L*	29.49	1.00	3.4	27.51	31.19	3.68
	a*	5.94	0.14				
	b*	11.64	0.56				
	h°	63°	0.82°	1.3	64.6°	61.6°	3°
	C	11.6					
5	L*	24.63	0.75	3.0	22.92	25.59	2.67
	a*	3.53	0.23				
	b*	6.2	0.72				
	h°	60.3°	1.35°	2.3	62.0°	57.6°	4.4°
	C	6.2					
6	L*	22.39	0.79	3.5	20.98	23.40	2.42
	a*	1.96	0.11				
	b*	2.89	0.29				
	h°	55.9°	2.4°	4.3	59.1°	51.5°	7.5°
	C	3.5					

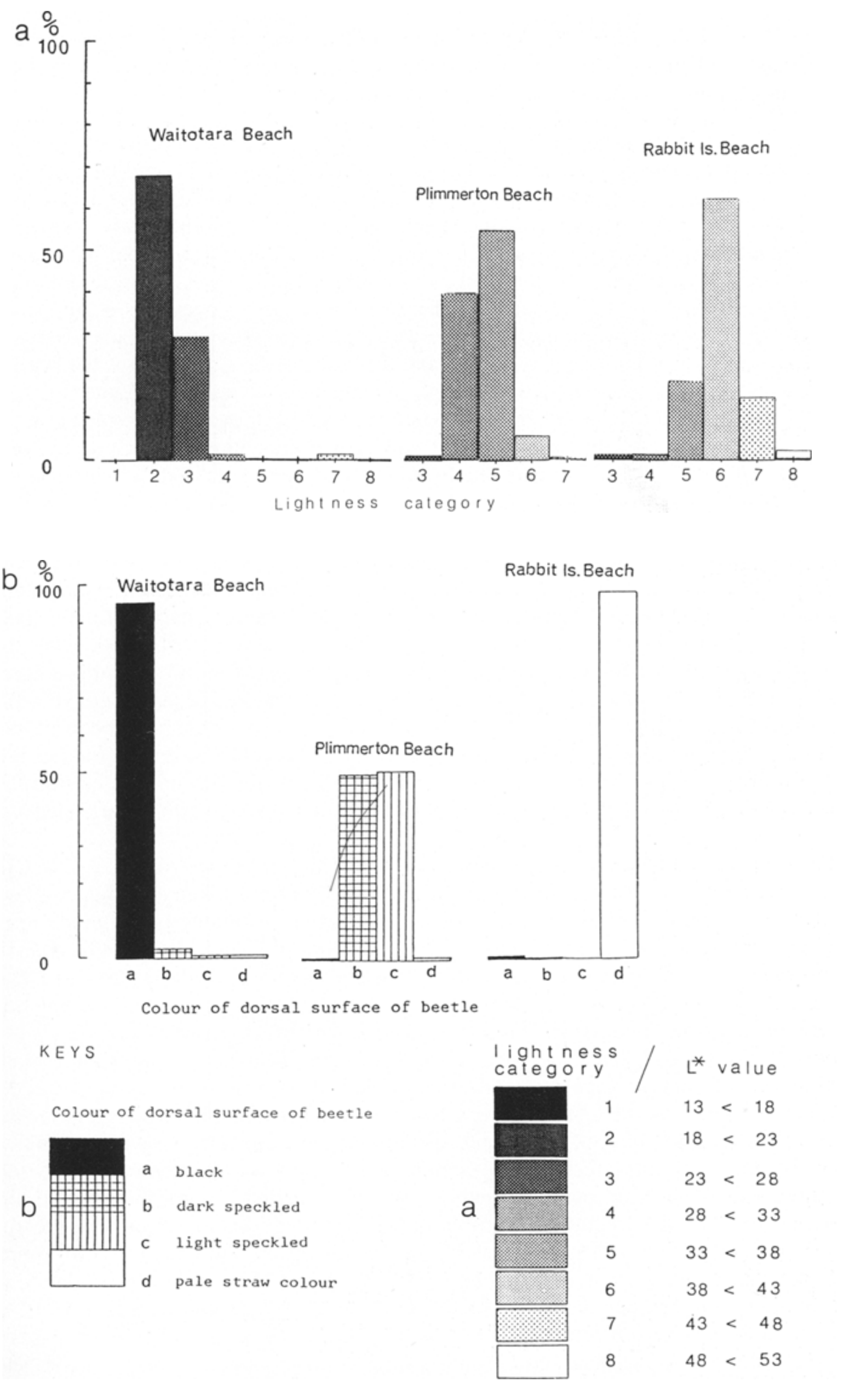

Fig. 2a – Frequency distribution of beetle L* values within intervals from 13>18 (category 1) to 48>53 (category 8). Number of beetles measured: Waitotara, 74; Plimmerton, 195; Rabbit Island, 270
Fig. 2b – Frequency distribution of beetles classified in subjective categories

The CIELAB 1976 a, b chroma values were calculated using only the average a* and b* values. The value of C increased between specimens 1 and 2 before decreasing in sequence with specimen category.

DISCUSSION

The CIELAB coordinates of the representative set of six beetles provided an objective specification of their colour attributes. The descriptive terms which could be applied to the sequence ranged from very pale to almost black. The measured values of L* decreased from 46 to 22.4 in the same sequence as the specimen category and objectively confirmed the selection order subjectively based on the perceived progression of the attribute of "lightness". However, the differences between successive L* values in the sequence were not the same, which demonstrated that the visual judgement of colour differences was accompanied by considerable uncertainty when the objective was to create a sequence of perceptually equal steps. In the present study this was made more difficult by the small size of the beetles and irregularities on the dorsal surface of many specimens, such as the presence of spots or speckles. The instrumental evaluation was not affected by these as it involved an optical averaging with an illuminating beam diameter larger than any within-sample non-uniformities (Hunter and Harold, 1987). The average difference between successive L* values in the set was nearly 5.

The colours of *C. trachyscelides* have been reported as ranging from "pale whitish yellow ... through shades of brown, to completely black", and this was the case for the observed hues of the specmens used in the present study. However the measured hue angles, which are the psychometric correlate of perceived hues, were similar to each other, particularly for specimens 1-3. The observed range of 74.4° to 55.9° corresponds in the CIELAB colour space diagram to perceived hues usually described as "yellow" or "orange-yellow". For those specimens which also have a relatively low L* value the more usual descriptive term would be "brown". The appearance of all specimens would depend on the same basic structural constituents and melanin at different levels and this explains the similarity of the hue angles for specimens 1-3. At relatively high levels of melanin the hue would be subjectively less obvious and the appearance would tend to be perceptually dominated by the visual effect associated with low L* values.

The CIELAB a b chroma values decreased significantly from specimens 2-6. In CIELAB space this represents a shift towards the achromatic L* axis and, taken in conjunction with the trend in L* values, was consistent with the subjective attributes upon which specimen selection was based. Specimen 6, with the lowest values of C and L*, could be described as almost black. The increase in the value of C between specimens 1 and 2 was consistent with the established observation that the subjective chroma range for a hue of intermediate "lightness" is greater than when it is very pale or very dark (Munsell, 1981).

For the beetles collected from beaches at Waitotara, Plimmerton and Rabbit Island, the distributions of colours were assessed using the L* values. In the analysis of frequency a group interval of 5 on the L* scale was selected on the basis that it was close to the average measured difference between the sample specimens, twice the average within-sample range observed, and six times the average standard deviation for the representative set. The measured values included numbers both greater and smaller than those observed with the reference set. For the frequency distributions eight categories were needed, the first for values in the interval 13>18 and the last 48>53. The results are shown in Fig. 2 in comparison with the earlier subjective distributions (Harris, 1988). The obvious similarity of the distributions demonstrated that the objective evaluation of the colours in terms of L* values correctly reflected the subjective colour attribute that was the basis for the earlier assessment. The precision of the measurements enabled finer colour distinctions to be made, and also avoided the uncertainties associated with subjective visual selection. The convenience of the method developed has provided the basis for more detailed investigations on the colours of

Chaerodes trachyscelides in relation to that of the sand they live on. These studies will be reported elsewhere.

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