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The numerical encoding of scale morphology highly improves photographic identification in lizards

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Abstract. Photographic identification is a promising marking technique alternative to the toe-clipping, but is time consuming, particularly when a large number of individuals is involved. For this reason several authors had frequently preferred the toe-clipping. In this study we analysed the black spot pattern of ventral scales of wall lizards (*Podarcis muralis*) and we showed that photographic identification is an effective method for recognizing individuals, and the error of this technique is much less than that of the toe-clipping arising from natural toe loss. Moreover, the numerically encoding of the black spot pattern may radically reduce the time needed to compare the pictures of large samples of individuals, solving one of the more important obstacle against the use of photographic identification.

Keywords. Individual marking, photographic identification, non invasive marking technique, toe-clipping.

INTRODUCTION

Many types of ecological studies need the unique identification of individuals, which is usually achieved by marking. General methods used for vertebrates such as ringing, tattooing, and banding are difficult to use with reptiles because of their anatomy and skin shedding (Spellerberg and Prestt, 1978; Dunham et al., 1994). Up to now the most common method of marking lizards and skinks used has been the toe-clipping, in which a unique combination of digits is removed from each individual (Ferner, 1979; Hero, 1989; Waichman, 1992). Recently, the effectiveness of this method has been questioned, since the base assumption that toe-clipping does not influence the survival or behaviours has been shown to be frequently violated (Parris and McCarthy, 2001; McCarthy and Parris, 2004). Indeed, the toe-clipping may cause inflammation, infection of feet and limbs, reduced mobility, increased mortality (Bustard, 1968; 1971; Clarke, 1972; Humphries, 1979; Golay

and Durrer, 1994; Lemckert, 1996; Reaser and Dexter, 1996; Williamson and Bull, 1996; Davies and Ovaska, 2001; Bloch and Irschick, 2004), potentially affecting the quality of the data collected during field researches (McCarthy and Parris, 2004; Bell and Pledger, 2005). Moreover, natural toe loss is common enough in some species of skinks and lizards to potentially cause difficulties with possible misidentifications of individuals marked by toe-clipping (Rand, 1965; Schoener and Schoener, 1980; Middelburg and Strijbosch, 1988; Hudson, 1996). By contrast, several other studies have found no negative effects of toe-clipping (Huey et al., 1990; Dodd, 1993; Van Gelder and Strijbosch, 1996; Hudson, 1996; Williamson and Bull, 1996; Ott and Scott, 1999; Paulissen and Meyer, 2000; Borges-Landaez and Shine, 2003), suggesting that the effects of this technique may vary among species and must be assessed accordingly (Funk et al., 2005).

Irrespectively to the negative effects on individuals, there are also ethical and conservation implications that lead to consider toe-clipping with caution as a marking technique (McCarthy and Parris, 2004).

In this scenario, several non-invasive marking methods alternative to the toe-clipping have been proposed, such the small passive integrated trasponders (PIT, Elbin and Burger, 1994), the visible implant elastomers (VIE, Penney et al., 2001), or the freeze-branding (Spellerberg and Prestt, 1978).

The photographic identification is an emergent technique with a promising future for marking lizards, since it is completely harmless, cheap, and allows in theory long time identification of individuals (Fox, 1975; Gosá, 1987; Elbing and Rykena, 1996; Schmidt-Loske, 1996; Steinicke et al., 2000; Perera and Perez-Mellado, 2004). This approach bases on the identification of regular and individually specific patterns of colour spots or scale shape within well identified body regions of individuals, which do not change over time despite skin moults. For example, the pattern of head scales of the *Lacerta bilineata* is unique within individual, and do not vary over time (Fox, 1975; Elbing and Rykena, 1996), while the scale pattern of the first four rows of the ventrals is suitable for recognizing individual of six species of lacertids (*Lacerta agilis, L. bilineata, L. viridis, L. perspicillata, Zootoca vivipara* and *Podarcis muralis*) (Steinicke et al., 2000; Perera and Perez-Mellado, 2004). Colour spot patterns have been also used to individually identify lizards (Schmidt-Loske, 1996), but spot shape may vary with reproductive condition or age, being less useful for long-term identification (Henle et al., 1997).

Although regular patterns of colour spots and scale shape may supply a useful way to individually recognize lizards, photographic identification is a time consuming technique, particularly when a large number of individuals is involved, since the number of paired-comparisons for each picture increases exponentially according to the sample size. For this reason, the method must be improved to reduce the time and/or the number of comparisons required for identification.

The common wall lizard *P. muralis* is a good model for testing the effectiveness of photographic identification since it has easily recognizable individual scale shape patterns (Schmidt-Loske, 1996; Steinicke et al., 2000) and shows black spot patterns within ventral scales that are highly variable, particularly among males. In this study we therefore tested the suitability of this ventral pattern of black spots to be used for identifying males, and we proposed a new method for numerically coding the spot pattern in order to minimize the time needed to identify a given individual.

MATERIALS AND METHODS

During spring-summer 2004 and 2005 we overall made 235 captures and recaptures of male common wall lizards in an historical garden of Cesano Maderno (Northern Italy, 45°38'N – 9°07'E): 41 (35 individuals) were made during the first year and 194 (42 individuals) in the second one. In both years, all lizards were individually marked on the back by a unique combination of coloured inks, photographed ventrally using a Nikon Coolpix 4300 (resolution 2048 × 1536 pixels), and released. We obtained 6 recaptures (3 individuals) in 2004 and 152 recaptures (33 individuals) in 2005 correctly recognized on the basis of the colour marks on the back; all recaptured lizards were photographed.

The ventral pattern of black spots of each lizard was numerically encoded using the following procedure: the shapes of the black spots of the 4 scales of the first 10 ventral rows (overall 40 scales) were classified from 0 to 63 according to the code reported in figure 1, which resumes all possible shapes from an unspotted scale (code = 0) to a completely black scale (code = 63). By this procedure each individual (as well as each picture) was univocally paired to a numerical string of 40 features that could be easily compared with the strings of all other lizards using a worksheet software, such as Microsoft Excel.

We referred to the "code distance" (CD) as the number of differences between the correspondent features of two codes, which therefore varied between 0 (i.e. the codes are the same) and 40 (i.e. all the paired-features of the two codes are different). In order to assess a CD threshold to ascertain if two codes belong to the same lizard or not, the pictures of 10 different males were encoded twice by the same observer and significant differences between the mean CD of each male with all others (CD_{among}) and the CD of each male with its replicate (CD_{within}) were assessed by a one-way ANOVA. Then, we used a kernel estimation (bandwidth = 8.40) to compute the probability density curve of the CDs and we computed the CD threshold from the graphic.

Three main sources of error may arise in analysing the pictures: encoding errors, seasonal changes of black spot's shape, and variability among observers. The first source of error was assessed by verifying that a picture was paired to a unique and repeatable code: the same observer replicated the measures of the CD_{among} and CD_{within} of the previous analysis with a one-day interval, and we evaluated the repeatability of the CD measures (Lessels and Boag, 1987). In order to analyse the second source of error, we assessed the repeatability of the pictures by encoding two different pictures from 10 males with one-day interval between two successive analysis of the same picture; the observer was the same as previously. Finally, in order to assess the effects of the third source of error (variability among the observers), three observers computed the CD_{among} and CD_{within} of the same 10 different males, and significant differences among observers were checked using a mixed analysis of variance where CDs were included as the dependent variable, the observer was included as random factor while the type of comparison (among or within individuals) was included as factors; the effect of the interaction between observer and type of comparison was also incorporated into the model.

In order to quantify the percentage of error (number of misclassification/number of individuals) of this marking procedure, we compared the numerical codes of all recaptures of colour marked males with the numerical codes of all first captures using a worksheet in Excel and we considered as true identifications all pairs of individuals differing less o equal to the CD-threshold. For this analysis we used the sample of 42 first captures and 152 recaptures collected during 2005, and consequently we performed 6384 paired-comparisons.

Finally, we applied the same procedure to recognize between-year recaptures, comparing all the 35 first captures of males in 2004 with all 194 captures (first captures and recaptures) in 2005; in this case the sample involved 6790 paired comparisons. For this analysis we lacked independent validation (we did not intentionally use toe-clipping, and painting with coloured inks do not persist over successive years), so all pairs of pictures whose codes differed less or equal to the CD-threshold were visually compared using the scale shape patterns (Schmidt-Loske, 1996; Steinicke et al., 2000). All statistics

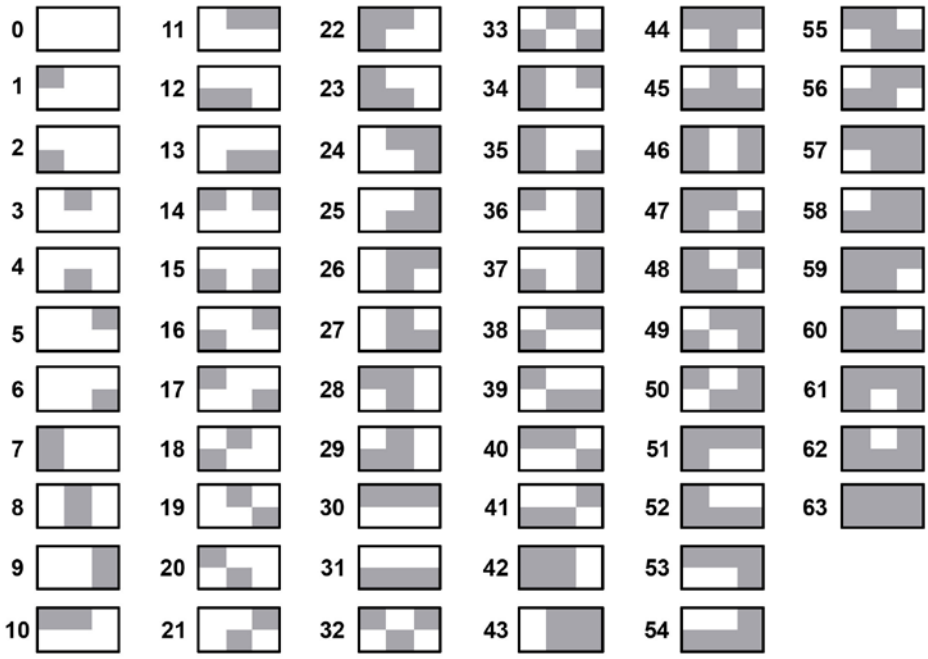

Fig. 1. Numerical codes used for encoding of the black spot pattern of ventral scales of wall lizards.

were two-tailed, and were performed with SPSS 12.0. When necessary, normal distribution and homogeneity of variances was verified. Unless otherwise stated, values reported are means ± SE.

RESULTS

The CD among different males was on average 35.8 ± 0.6, while the CD between two replicates of the picture of the same individual was 2.9 ± 0.5; this difference was highly significant ($F_{1,18}$ = 1762, $P < 0.0001$) and resulted in a CD-threshold of 19 differences (Fig. 2). The code of a single picture was highly repeatable (r = 0.98), as well as the pictures of the same individual (r = 0.92). These results suggested that the code coupled with the black spot pattern of the ventral scales univocally identified each male, was unaffected by the quality of the picture, and was univocally recognizable in different pictures of the same individual.

The effect of the observer encoding the ventral pattern of black spots was also negligible (Fig. 3), since the CD_{among} was larger than the CD_{within} for all observers ($F_{1,2}$ = 470, P = 0.002), and both -among and -within CDs did not differ among them ($F_{2,2}$ = 2.68, P = 0.27). However, the interaction between the observer and the type of comparison was near to the significant threshold ($F_{2,60}$ = 2.92, P = 0.06), suggesting that the third observer valued the CD_{within} a little higher than that measured by other people, the CDs_{among} matching perfectly (Fig. 3).

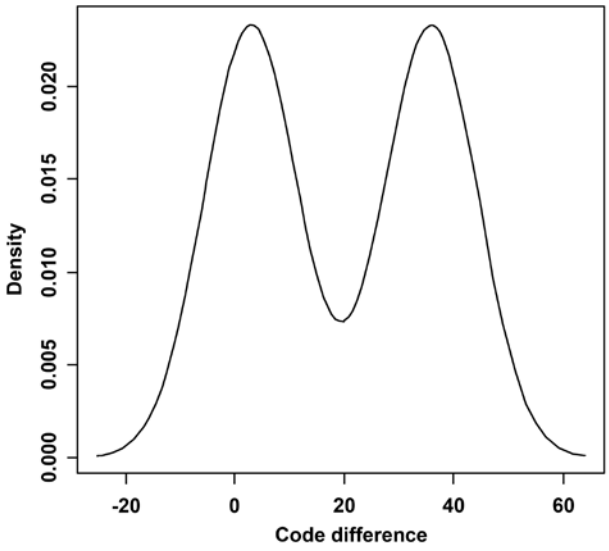

Fig. 2. Probability density distribution (kernel procedure) of the CDs in the sample of 10 males of wall lizards (see Methods for details).

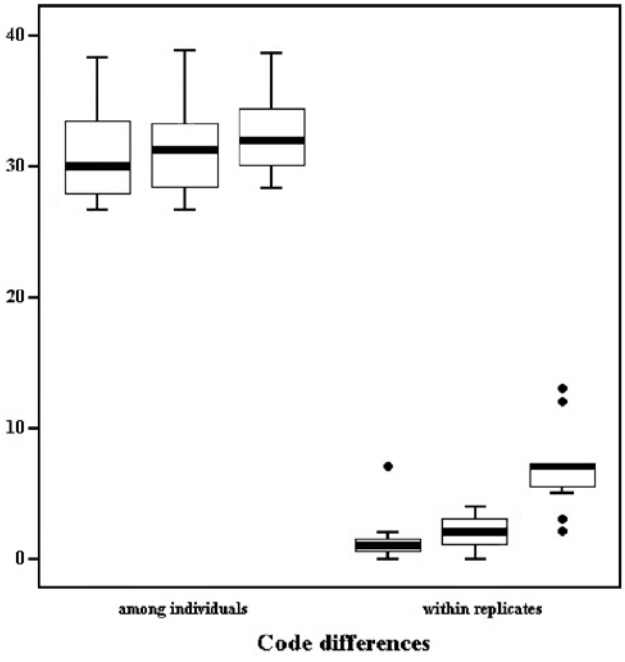

Fig. 3. CDs among individuals and within the replicates of the same individual measured by three different observers.

The 96.7% (147 out of 152) of the colour-marked recaptures and 97.0% of the recaptured individuals (32 out of 33 males) were correctly identified basing on the code of the ventral spot pattern.

Basing on our procedure (CD less or equal to 19), 11 males out of the 35 captures during 2004 were identified as recaptures in 2005, and the visual comparisons of the shape pattern of the first four rows of the ventrals confirmed the identification in 7 cases (64%). All misclassifications had CDs varying between 18 and 19, and raised from the higher number of completely unspotted scales that exceeded 50% in all individuals (i.e. the mean number of zeros in the codes of these four misclassified males was on average 22 ± 0.7).

DISCUSSION

This study confirms that photographic identification is a useful marking technique for lizards (Steinicke et al., 2000; Perera and Perez-Mellado, 2004), and can be considered an effective alternative to the toe-clipping in ventrally pigmented lizards. Indeed, we showed that the mismatching of photographic identification of wall lizards basing on the black ventral spot pattern was very low and much less than the error intrinsic to the toe-clipping technique arising from natural toe loss: in our study we failed the recognition of only 4 out 152 recaptures (3.3%) and one out 33 individuals (3.0%). By contrast, Hudson (1996) in his study on 12 Australian skink species showed that 19% of females (83 out 445) naturally lost toes, and in some populations this feature increased to more than 30%. Ontogenic changes of pigmentation occurring in this species (Gosà, 1987; Henle et al., 1997) did not significantly affected the encoding procedure within a single breeding season, but might reduce it applicability in long term studies. However, the effects of black spot changes might be easily controlled by reducing the time intervals between two successive pictures.

The photographic identification technique described in this study allowed also the effective recognition of individuals over successive years, despite the fact that 26% of between-year recaptures resulting from our procedure (4 out of 11) were not confirmed by the direct comparisons of pictures. Indeed, all these misclassifications involved males having more than 50% of ventral scales that completely lacked black spots, and an high proportion of zeros obviously increases the probability for given code of matching the codes of other individuals. Moreover, in all these cases the CD was very close to the CD threshold for true recognition, suggesting that this nuisance may be easily removed by increasing the number of scale rows to determine.

The most important limit to the application of the photographic identification up to now has been the large amount of time needed to compare the pictures of a given sample, which raises exponentially as the number of individuals involved increases. This objection was the main factor that leaded several authors to prefer the toe-clipping as marking technique because it had been considered the most economical and practical method for long term studies among all other current methods (i.e. Ott and Scott, 1999). In this study we show that the translation of the ventral spot pattern of male wall lizards into a simple numeric code dramatically reduces the time needed to compare a large sample

of pictures. Indeed, we would have performed more than 6000 paired-comparisons for identifying recaptures in the sample of pictures collected during 2005 without this encoding procedure. The time required to encode a picture was less than one minute, while the comparison of codes using a worksheet software is practically instantaneous. This leads us to obtain a complete identification of all individuals of the sample in less than one day.

This procedure may be generalized, and other kind of colour spots or scale shape pattern of lizard species may be numeric encoded to immediately identify individuals or, at least, to easily find a very restricted sub-sample of pictures to be visually compared.

However, in long term monitoring programs that involve large samples of individuals photographic identification remains a marking technique that does not allow immediate recognition of individuals at the time of their capture, but only after a process of analysis of images. Despite this, photographic identification is undoubtedly the less invasive technique available today, and would be preferred for all researches involving the measure of physiological variables, such hormone levels, that greatly varied in response to both stress and injuries.

In conclusion, the numeric encoding of individual pattern associated with digital cameras and image processing software radically reduce time consuming of photographic identification, which can be considered a fully alternative method to toe-clipping.

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