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SHORT COMMUNICATION

Colour variation in female common lizards: why we should speak of morphs, a reply to Cote *et al.*

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Abstract

Female common lizards display variation in their ventral colour, ranging from pale yellow to bright orange. In a previous study, we proposed a discrete classification of this variation into three colour classes: yellow, orange and mixed. However, a recent study reported on continuity of reflectance spectra and plastic change in spectrum components in female common lizards, which made the authors question the validity of a discrete classification of colour patterns in this species. Here, we discuss the relevance of discrete, eye-based colour classification for understanding the ecological processes that shape the social structure of common lizard female populations. First, as the mixed colour class is characterized by heterogeneous distribution of colour, we explain that it cannot be reliably described by localized spectrophotometer measurements. On the contrary, the classification of colour into three discrete classes is consistent with the description of three alternative strategies in female common lizards. Then, although we support the ecological importance of colour plasticity in this species, we refute the hypothesis of a condition-dependent signal, which is not supported by experimental data. At last, we explain that colour plasticity, and in particular hormone-mediated plasticity is compatible with genetic inheritance of colour and the evolution of alternative strategies. Indeed, the genetic background and the environment, especially the social environment, are expected to interact adaptively to modulate the expression of colour signals and alternative strategies.

The degree to which colour variation in nature is genetically vs. environmentally determined (e.g. by plasticity) is the key to understanding the evolutionary stability of any alternative reproductive strategies associated with colour (Sinervo, 2001). Recently, Cote *et al.* (2008) reported on plastic changes in colour in the European common lizard, *Lacerta vivipara*, and claimed that their study refuted the discrete nature of colour morphs that we previously reported (Vercken *et al.*, 2007a). Their argumentation relied on two major points: (1) the analysis of female reflectance spectra did not reveal consistent clusters of individuals based on spectrum components; (2) spectrum components were significantly affected by an experimental manipulation of the social environment.

Here, we propose alternative interpretation for their results, consistent with our previous findings that supported the hypothesis of discrete colour morphs reflecting the existence of alternative reproductive strategies in females of this species.

On the continuity of colour variation

Cote *et al.* (2008) performed quantitative analysis of reflectance spectra, which revealed continuous variation

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in spectrum components (hue, chroma and brightness). However, the continuity of reflectance spectra had been previously described (Vercken *et al.*, 2007a), based on a larger and more balanced sample of individuals. Therefore, not only such elements are neither new nor in opposition with our own results (contrary to Cote *et al.* assertions), but also they do not stand as valid argument against the existence of discrete colour morphs.

Indeed, in a recent paper on colour morphs in the oviparous form of the European common lizard from the Pyrenees, Sinervo *et al.* (2007) clearly show that male morphs apparently intermediate in colour are actually resolved as such from the mosaic of two colours that are present on the ventral scales (see Fig. 1, Sinervo *et al.*, 2007). The actual number of mosaic scales and the distribution of one colour vs. the other is a highly variable trait. In oviparous populations, males express orange, yellow and white colours in a very discrete set of six phenotypes, extremely reminiscent of the six discrete colour morphs of the North American side-blotched lizard (*Uta stansburiana*). Heterozygotes in both species present a mixture of two discrete colours that form a mosaic pattern (e.g. blue and orange in *U. stansburiana* distribute in blue and orange stripes on the throat). Therefore, in our work on the European common lizard, we have adopted a system of scoring macrophotographs of the ventral surface to score the discrete morphs of *L. vivipara*, much like the visual scores that have been made of the North American side-blotched lizard. In both species, the actual distribution of one colour vs. the other and the location on the body (e.g. distance from the venter in *L. vivipara*, expression of colour on just the throat vs. body flanks and belly in *U. stansburiana*) are highly variable. We suspect that other loci govern the expression of the distribution of colour in both species.

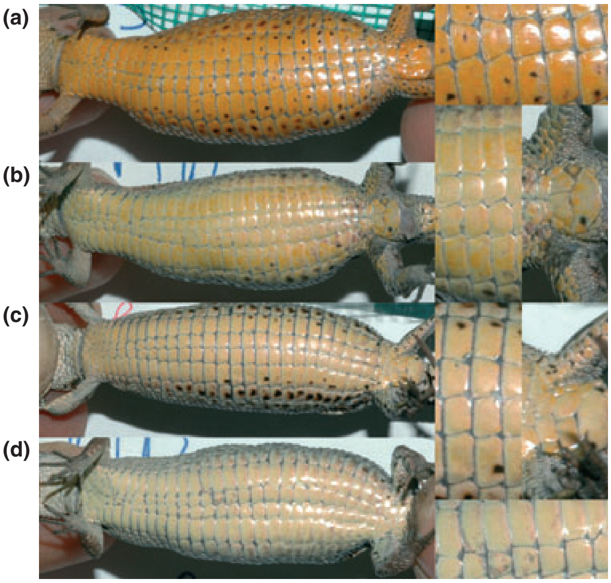

Fig. 1 Photographs of abdomen from female lizards, and macrophotographs of ventral and cloacal scales. (a) Orange female (macro: ventral area uniform for orange). (b) Mixed female (macro: ventral and cloacal area present with a colour mosaic of both discrete orange and yellow colour patches distributed uniformly across both areas). (c) Mixed female with strong spatial heterogeneity in the distribution of yellow and orange (macro: ventral area shows only a few very small orange patches on a largely yellow background but the cloacal area presents as a mosaic of both discrete orange and yellow patches). (d) Yellow female (macro: ventral area uniform for yellow).

This observation on colour mosaics is salient with regard to the ability of a spectrophotometer to actually resolve discrete morphs. Early in our work, it was obvious that the spectrophotometer probe could never be used to simply provide an index of colour that was superior to the human eye, or the analysis of colour macrophotographs by the human eye. Given that the distribution of colour is heterogeneous and highly variable from one individual to another, this represents a huge component of uncontrolled variation in colour that escapes detection with a simple placement of a spectral probe on the ventral surface of the organism. The human eye is a far more discriminating tool that can weight these differences among belly scales, and resolve discrete colour variation. With a simple eye loop, it is possible to find just a few mosaic colour scales in one individual that are concentrated at the cloaca, or resolve the separate mosaic colour scales among a large field alternating yellow and orange scales in another 'mixed' phenotype. The spectrophotometer would classify these two types of individuals as different: the first would be scored erroneously as pure, the second as a perfect mixture of the two only if the probe happened to span the two colour patches. In reality, the expression of such two-colour mosaics of scales ties these two phenotypes together as a mixed phenotype, regardless of the varying fraction of orange and yellow scales, and this is why we did not rely on the spectrophotometer for this determination. We present the representative phenotypes of pure and mixed colours in Fig. 1, where a putative homozygote orange, two heterozygotes orange–yellow and a homozygote yellow are depicted. Careful inspection of colour macrophotographs for the two putative heterozygote phenotypes reveals the existence of individuals with mixed colour phenotypes, whereas such variation is impossible to resolve with a spectrophotometer probe. Placement of a spectrophotometer probe mid-venter would classify them as very different phenotypes, but they share this striking colour mosaic of two discrete colours, albeit extensive in the first female and concentrated at the cloaca in the second female.

Therefore, it is not surprising that the distribution of spectrum components appears continuous, but it does not accurately reflect the reality of colour patterns in this species. In addition, a strong argument in favour of the existence of discrete morphs relied on the fact that colour classes defined visually were correlated with discrete alternative strategies in reproduction (Vercken *et al.*, 2007a). These first results were further supported by a recent study describing colour-based differences in social behaviour (Vercken & Clobert, 2008). In this context, if there are alternative strategies and if ventral colour is indicative of these strategies, then one can refer to colour morphs even if colour variation is somehow continuous.

Based on these considerations, we believe that the most ecologically relevant way to describe colour variation in female common lizards is to consider three discrete morphs: yellow, orange and mixed. The importance of phenotypic plasticity in the expression of colour and the issue of colour determinism are independent issues from the discrete nature of colour patterns, and thus are discussed separately.

On the plasticity of colour

Following a manipulation of the population sex ratio, Cote *et al.* observed changes in female colour determinants, especially hue. Although we agree that environmental influences on colour expression are likely to be important (see discussion below), we have some concerns about the relevance of Cote *et al.*'s experimental design. First, although Cote *et al.* had complete maternal pedigrees, they never tried to estimate either the heritability of colour or the amount of genetic variation in their experimental populations, which could strongly modify the interpretation of their results. Indeed, if the heritability of colour under their mesocosm enclosures were dramatically lower than observed in our free-ranging pedigree (maternal heritability of 0.48, Vercken *et al.*, 2007a), this finding alone would explain why their spectrophotometer measurements of repeatability were so low. In addition, in small mesocosms, genetic variation might be very depleted, given the propensity for inbreeding and drift. These confounding effects could readily be computed from the pedigree. If additive genetic variation in colour loci were depleted, Cote *et al.* would have overestimated the component due to plasticity and environmental effects. Second, in the experiment described therein, the modification of hue was induced by extreme environmental conditions (strongly male-biased sex ratio), outside the range observed in natural populations of common lizard. Thus, although Cote *et al.* demonstrated that there was a potential for plasticity of colour in this species, it is unclear whether this plasticity is actually expressed in natural conditions, or whether it is a pathological response observed under extreme experimental conditions.

Nevertheless, the role of environment in modulating the colour of lizards is quite obvious. Indeed, in a separate study on density manipulations of the populations in nature, Meylan *et al.* (2007) reported on the effect of density in reducing the brightness of female lizards, and another experimental study showed that the measure of chroma was variable through time (E. Vercken, B. Sinervo and J. Clobert, unpublished data). However, these quantitative changes in spectrum components never affected the visual scoring of colour (the same being true for the experiment described in Cote *et al.*), which supports the hypothesis of a stable signal indicative of female strategy. On the other hand, all quantitative spectrum components examined were influenced to some extent by environmental factors (brightness: density, Meylan *et al.*, 2007; hue: population sex ratio, Cote *et al.*, 2008; chroma: annual effects, E. Vercken, J. Clobert and B. Sinervo, unpublished data). Such contrast between stable and variable aspects of colour might indicate that ventral colour is a complex signal that conveys multiple messages. If colour class is indicative of female strategy, and thus is likely to be stable throughout an individual's life, other more quantitative components of colour (such as hue, chroma or brightness) could reflect more variable aspects of female phenotype.

Which phenotype components could be reflected by such quantitative colour variation? There are numerous possibilities, among which reproductive state, motivational state or even age (as described by Cote *et al.*). However, the hypothesis of classical carotenoid-related condition dependence as discussed by Cote *et al.* is unlikely. As carotenoids are involved in both immune defence and coloration, there might be a physiological trade-off between these two functions, and the intensity of carotenoid-based colouration is expected to reflect immune status (Olson & Owens, 1998). Such hypotheses are based on the idea that healthy individuals do not need to spend carotenoids for immune functions and then can allocate more carotenoids to their colouration. By contrast, individuals that suffer from immune challenge should allocate less carotenoid to their colouration and therefore display attenuated colour. Although this theory is well verified in many species (especially birds, Møller *et al.*, 2000), it is poorly supported in the common lizard. Indeed, an earlier study of the effect of carotenoid supplementation on ventral colour in this species failed to demonstrate any influence of the experimental treatment on the redness of colouration (J. Cote, unpublished data), which is in opposition to the predictions of the condition dependence hypothesis. In addition, if this hypothesis were correct, we would expect individuals of higher quality to display redder colouration, i.e. orange females should have higher fitness, and the shift in colour in response to unfavourable environmental conditions (male-biased populations) should be stronger for individuals of lower quality, i.e. for yellow and mixed females. These predictions were not met. First, mixed females, and not orange females, seem to have higher reproductive fitness on average in natural populations (Vercken *et al.*, 2007a). Second, the modification of colour in response to the sex ratio experiment did not depend on the initial colouration of individuals. These results are in opposition with the condition dependence hypothesis, and rather suggest that colour is more likely to reflect condition-independent alternative strategies and other variable aspects of the phenotype unrelated to individual quality.

On genetic vs. environmental determinants of colour and alternative strategies

There is general confusion in the literature regarding the role of genetic vs. environmental determinants of alternative strategy. Colours (and other patterns) provide animals with signals that are used in alternative strategies. We (Vercken *et al.*, 2007a) showed how heritable colour signals were also associated with alternative female strategies for fitness-related traits (progeny survival, clutch sex ratio, etc.). Sinervo *et al.* (2000, 2001) have reported surprisingly parallel findings on colour and strategy in the North American side-blotched lizard. Moreover, Sinervo *et al.* (2007) report on a rock–paper–scissors set of strategies (and ventral colour) in male European common lizards that is very similar to the rock–paper–scissors set of strategies (and throat colour) exhibited by the North American side-blotched lizard (Sinervo & Lively, 1996). Such similarities are not a coincidence because stable games of two players (e.g. *r*- and *K*-strategies to different density conditions) and three-players (e.g. rock–paper–scissors strategies) will assemble via underlying mutations on the endocrine networks (Sinervo & Calsbeek, 2003).

Once such strategies arise, plasticity will evolve to modulate the success of each genotype. Such plasticity can alter the components of a colour signal, and thus the meaning of the colour signal in a social context. For instance, in the North American side-blotched lizard, in which the main colour gene has been crudely mapped to a single location (between two genetic markers), colour can still change plastically depending on salient cues, both in homozygotes and heterozygotes. These plastic changes seem to be mediated by the endocrine system (gonadotropins, Mills *et al.*, 2008; testosterone, Sinervo *et al.*, 2000; Mills *et al.*, 2008), alone or in interaction with other factors. Similar results were found in the tree lizard *Urosaurus ornatus*, where the different plastic responses of genetically based male morphs are mediated by interactions between steroid hormones such as corticosterone and testosterone (Knapp *et al.*, 2003). Interactions between the endocrine system and colour loci thus seem to play a major role in the expression of colour or strategy plasticity.

We suggest that such plasticity will evolve, when it enhances the propagation of either colour allele to the next generation. These plasticity loci will modify the expression of male or female strategies and their associated colour signals through genetic interactions. In European common lizards, we suggest that the plasma carotenoid-binding proteins and enzymes implicated in carotenoids metabolism are likely candidates that will modify the deposition of carotenoids in the dermal scales of male and female lizards. Such colour genes will naturally interact with other components of the endocrine system (discussed by Sinervo & Calsbeek, 2003). Corticosterone in particular could be implicated in the plasticity of colour in common lizards. Indeed, corticosterone plays a major role in life-history plasticity in this species (Meylan *et al.*, 2002, 2004; Meylan & Clobert, 2005; Vercken *et al.*, 2007b), and the experimentally elevated corticosterone plasma level was found to decrease hue in male common lizards (J. Cote, unpublished data). Corticosterone has been shown to modify the survival of female morphs of side-blotched lizards in a context-dependent fashion (Comendant *et al.*, 2003) via the interaction of corticosterone with immune function (Svensson *et al.*, 2002). As female common lizards suffered from high fitness costs in populations with male-biased sex ratio (Cote *et al.*, 2008), such unfavourable environments must have triggered physiological stress responses, which are mediated by corticosteroids in vertebrates. The modification of corticosterone secretion pattern in response to the manipulation of the social environment thus would have affected colour expression in female lizards.

Mendelian expression of colour signals involving either one or two loci (Sinervo *et al.*, 2001, 2006) or many loci, are not at all incompatible with the existence of other loci that might be under selection to modify the expression of the colour signal, depending on social context. Our goal should be to first identify the genetic sources of colour signals with laboratory crosses (Sinervo *et al.*, 2001), gene mapping studies (Sinervo *et al.*, 2006) or deep field pedigrees (Sinervo & Zamudio, 2001; Vercken *et al.*, 2007a). The source of plasticity will be an important tactical component that will evolve to modify the strategic aspects of a major colour gene(s). Uncovering the source of plasticity will, however, require manipulations of both the environmental agents and cues that induce plasticity and manipulations of the endocrine networks that regulate plasticity. Such plasticity will often interact with genetic control of strategy types thereby generating a complex norm of reaction in behaviour, colour signals and life history.

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