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# Evolution of coloration: a study of the palette of vertebrates

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# THÈSE POUR OBTENIR LE GRADE DE DOCTEUR DE L'UNIVERSITÉ DE MONTPELLIER

En Ecologie et Biodiversité

École doctorale Gaia

Unité de recherche : Centre d'Ecologie Fonctionnelle et Evolutive (CEFE – UMR 5175)

## Evolution of coloration: a study of the palette of vertebrates

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Le 4 décembre 2020

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# **GENERAL INTRODUCTION**



The astonishing diversity of coloration observed in nature has fascinated biologists for centuries (Darwin 1871 Poulton 1890; Mayr 1972; Caro and Mallarino 2020). This diversity is the results of the many selective forces acting together, and sometimes in opposite directions, on species coloration. Such diverse selective pressures arose because of the importance of the visual channel for inter- and intraspecific interactions, which lead, in turn, to an effect of coloration on both the survival and the reproduction of individuals (Hill and McGraw 2006; Cuthill et al. 2017). In addition, the multivariate nature of coloration provides several axes of variation along which evolutionary diversification can take place (e.g. hue, brightness, saturation, but also pattern, figure 1). All these characteristics make coloration an interesting trait to understand the fine details of the processes that shape the evolution of phenotypes. However, before going further into the subtlety of coloration evolution, we need to establish more precisely its nature.

## **What is coloration?**

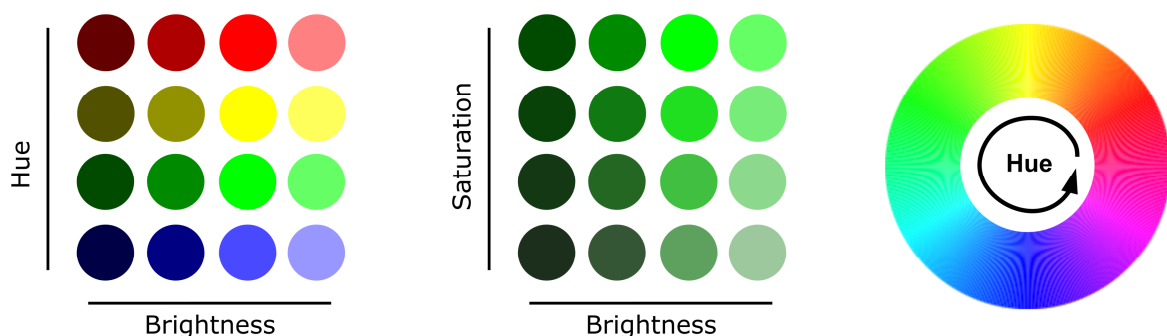
Although this question seems simple at first glance, coloration is actually difficult to define. The coloration that we perceive is the result of an interaction between three parameters: the physical properties of the object of interest, the characteristics of the light illuminating the object and the characteristics of the organism that perceive the object. Among these three parameters, the first two influence the spectral reflectance of the object, which can be measured objectively by appropriate devices, while the third one affects the perception we have of coloration (which is subject-dependent, as are, consequently, most of our usual verbal categories on this subject, e. g. “this is green” has no objective meaning although many observers may have a consensus on which objects should be called green).

The coloration of an object will depend on how it reflects the incoming light. In animals, change of reflection are obtained through two mechanisms: (i) by the accumulation of pigment and (ii) by the presence of microstructures in the skin/feather/scales. Pigments are molecules that absorb the incoming light at some particular wavelengths while other wavelengths are reflected, thus producing “pigmentary colors”. For instance, carotenoids are organic pigments that absorb the short wavelengths and reflect the long wavelengths, giving the yellow, orange or red colorations observed in a wide range of taxa (Gray 1996; Macedonia et al. 2000; Sefc et al. 2014; Galván et al. 2016). Although the two main categories of pigments found in animals are carotenoids and melanin, other types also exist (e.g. psittacofulvins, Hudon and Brush 1992). Change of reflectance can also be achieved

through interferences of the incoming light with microscopical structures, producing “structural colors”. Contrary to pigment, the interference of the incoming light modifies the reflectance without changing the absorption of the tissue at a particular wavelength. Structural colorations usually produce blue and green colorations, and are also at the origins of the iridescence (Prum and Torres 2004; Bagnara et al. 2007). Both pigmentary and structural colors are frequently found in vertebrates, where they can be used separately, or together, to produce the coloration of a patch (Shawkey and Hill 2005; Saenko et al. 2013).

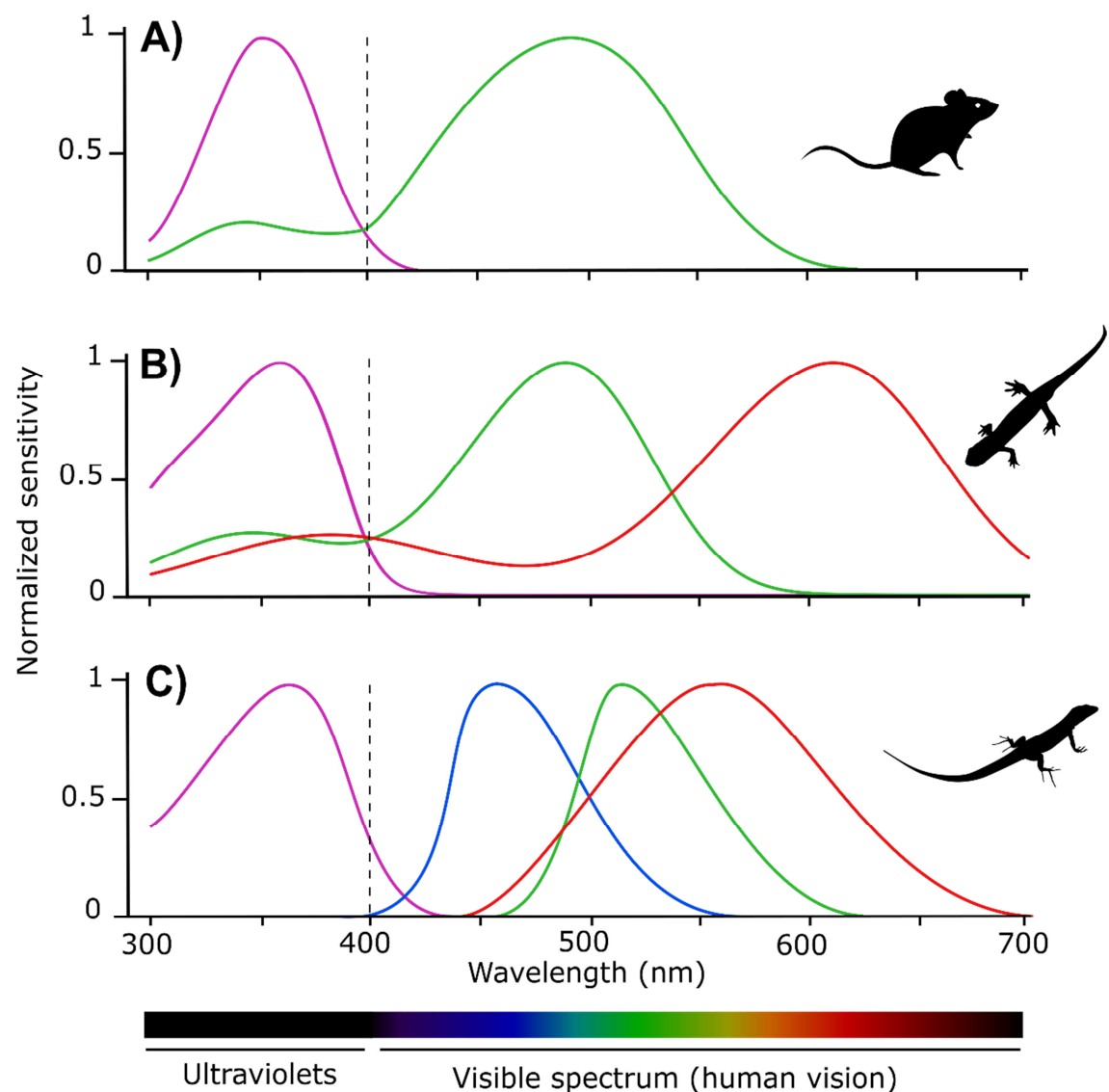
The spectral reflectance of an object depends of a second parameter: the characteristics of the light illuminating the object. Indeed, the light reflected by an object depends directly of the light that illuminates the object. For instance, in the ocean, the ambient light a few meters below the surface is shifted toward blue (long wavelengths) because water absorbs the long wavelengths. In such conditions, the coloration of an object will always be blue whatever its physical properties; objects that would look red in atmospheric light turn to simply very dark under the ocean. The quality of the light is also important in the terrestrial ecosystem, as the ambient light changes depending on time of the day, weather conditions and vegetation (Endler 1993a).

Although spectral reflectance provides useful information about the coloration of an object, it does not encompass all the dimensions of coloration. Indeed, the perception of coloration also depends on the visual and cognitive system of the viewer. Visual systems differ among organisms due to variation in the number and sensibility of photoreceptors (e.g. Figure 2), the absorption of ocular oil droplets and of the ocular media of transmission. These factors have a strong impact on the perception of colors by the viewer.



**Figure 1.** Coloration can be decomposed in three variables: brightness (light vs dark), hue (‘blue’, ‘red’, ‘yellow’), and saturation (intensity of the coloration). It should be noted that saturation and brightness can be quantified on a linear scale (black to white and unsaturated to saturated colors, while hue is a circular variable. This characteristic makes its quantification much more challenging.

Depending on the visual system, the same spectral reflectance can be perceived as two completely different colorations, or inversely two different spectra can be perceived as similar (Glover and Whitney 2010). This is particularly the case when the sensibility of photoreceptors for a species does not encompass all wavelengths (e.g. human cannot perceive ultraviolet coloration). The perception of a coloration is also influenced by the cognitive system of the viewer. Studies on the subject showed that the processing of visual information by the brain could significantly alter the perception of a color. For instance, the human brain “corrects” the visual information it receives to ensure that the perceived color



**Figure 2.** Example of photoreceptor spectral sensitivities in a dichromat specie (A. *Mus musculus*, Sun et al. 1997), a trichromat species (B. *Lissotriton vulgaris*, Korenyak and Govardovskii 2013) and tetrachromat specie (C. *Podarcis muralis*, Martin et al. (2015)). All these species perceive the ultraviolet wavelengths, but they differ in the perception of the long wavelengths.



remains relatively constant under varying illumination conditions (color constancy, Beck 1972). However, because this mechanism is not always accurate, it may lead in some cases to the inverse pattern: the coloration we perceive may be strongly dependent of the elements surrounding the object (Witzel and Gegenfurtner 2018). It should be noted that most of the studies investigating these questions were made on humans, and it remains largely unknown if these mechanisms are shared by other species.

Since coloration is not as simple to define as it appears, quantifying its variation has been a methodological problem for biologists for a long time. Ideally, the three parameters that influence coloration (physical properties of the tissue, characteristics of the illuminant and visual characteristics of the viewer) should be taken into account. However, the methods developed so far were only able to account for some, but not all, aspects of the three parameters (Box 1).

## **Selection and the evolution of coloration**

Properly measuring coloration is the first, but not the only, challenge when it comes to study coloration evolution. Understanding the selective pressures influencing the evolution of coloration is particularly tough due to three main characteristics: (i) the diversity of functions for which coloration has been selected, (ii) the opposition between the selective pressures acting simultaneously on coloration and (iii) the influence of perception on the quantification of the selective pressures.

### **a) The functions of coloration**

For the sake of simplicity, I will refer to the evolution of coloration “for” a function, although I’m well aware that coloration did not evolve for anything but was selected across time because it provides a better fitness, and that this better fitness came from its effectiveness in communication or in hiding its wearer.

Coloration is involved in a multitude of functions in animal. In this section, the term coloration will concern at the same time the coloration and the pattern, because these two components are often simultaneously selected. In such cases, separating coloration and pattern is meaningless (what is the function of a tiger coloration if we do not consider the black stripes?). However, coloration and pattern do not always co-evolve and they can provide different information (see below).

### Box 1: One coloration but multiple measures

The first, and simplest, method used to quantify coloration is coding. Coding is the measure of coloration in discrete categories by an observer (e.g. a score of melanism, from no melanism to completely black). This method has been frequently applied in the past (Kingsolver 1985; Margalida et al. 2008), but is now rarely used, at the exception of few coloration traits like dichromatism (Negro et al. 2018) or polymorphism (Hugall and Stuart-Fox 2012). It presents several limitations: it only gives semi-quantitative information about coloration, and it is subjective, because based on human vision. Hence, it cannot be used to assess UV coloration nor account for variation of photoreceptors tuning.

Secondly, there are methods based on measurements of reflectance spectra. Reflectance spectra are measured from 300 to 700nm and thus include information in both UV and visible ranges. Reflectance spectra provide a measure of the different components of coloration: the hue, the brightness and the saturation (Figure 1). Those components can be extracted directly from the spectra (as we used in manuscript A), or they can be estimated with vision models, which combine the information of reflectance spectra with illuminant data and information about viewer's photoreceptors sensibility. The measures provided by vision models account for the three parameters influencing the perception of coloration (although some aspects of the viewer's cognitive and visual systems are still ignored, like the visual acuity). Nonetheless, these methods have one main limitation: they only provide information about the coloration at a specific spot, and will be of little use when it comes to the spatial arrangement of colors (i.e. coloration patterns).

A third way to measure coloration is to use standardized photography. If taken with the appropriate material, the RGB values of standardized photographs can become accurate measures of brightness, hue and saturation for both the visible and UV spectrum (Troscianko and Stevens 2015). In addition, The RGB values can also be transformed to match the colors perceived by species with other photoreceptor tuning (Verhoeven et al. 2018; van den Berg et al. 2020). Thus, like spectrometric measures, standardized photographs can account for all parameters influencing coloration. It should be noted that, contrary to reflectance spectra, standardized photographs allow the analysis of both colors and patterns (Le Poul et al. 2014; Rönkä et al. 2018), although pattern analysis are mostly adapted to organism that have a standardized pose (e.g. butterflies) and that can be easily separated from their background.

One of the main category of functions of coloration is to respond to environmental constraints. Coloration can be used to regulate the temperature or to resist to ultraviolet radiation. For instance, in reptiles and amphibians, species living in altitude often show darker colorations to facilitate warm-up (Clusella-Trullas et al. 2007; Alho et al. 2010).

Secondly, coloration can be used for crypsis (i.e. to avoid detection by other animals). Crypsis is the most common anti-predator strategy found in nature. Many methods have been developed by organism to avoid detection, such as the resemblance with an inorganic object (masquerade, Skelhorn et al. 2010), body transparency (Barnett et al. 2020), the matching of the species coloration with background coloration (Rosenblum 2006; Stevens et al. 2017), or the utilization of contrasted coloration, in association with specific patters, to break up the outline of the body (disruptive coloration, Barlow 1972; McElroy 2016). It should be noted that crypsis is not only used for the prey to hide, it also allows predators to approach their prey without being noticed.

Coloration can be used as a signal to communicate between species. One of the most striking example of inter-specific color communication is the aposematic colors, where the coloration of a prey evolved to warn predators about prey's unprofitability (Poulton 1890). These colorations are often conspicuous to provide an unmistakable and easy to learn signal for predators (Roper and Wistow 1986; Svádová et al. 2009). This type of communication is frequently observed in nature, as aposematic coloration is found in all major clades of vertebrates (e.g. mammals: Caro 2009; Amphibia: Saporito et al. 2007; Squamata: Brodie 1993). Such signals are not necessarily honest, and many unprotected species mimic the coloration of venomous model (Batesian mimicry, Bates 1862).

Coloration can also influence social relationships between individuals of the same species. Some colorations indicate the immunocompetence and the health status of the individual (e.g. some carotenoids coloration, Saks et al. 2003) and are thus considered as honest indicators of individual's quality. Because of this characteristic, coloration can mediate the competition for resources or territories, and determine the dominance relationship (Siefferman and Hill 2005; Rémy et al. 2010). This function may be very common in nature, and is one of the most likely explanations (with also male mating choice), for the existence of female ornaments in birds (Tobias et al. 2012).

Lastly, coloration can play a role in species reproduction. Coloration may evolve to signal species identity, in order to avoid interspecific interactions and hybridization (Uy et al. 2009; Klomp et al. 2017). Furthermore, because coloration can be an honest signal of individual quality, it often influences the mating choice and the intrasexual competition

(Seehausen and Schluter 2004; Kraaijeveld et al. 2007; Pérez i de Lanuza et al. 2017). Contrary to the other functions, sexual colorations are not only species-specific but also sex-specific (e.g. Figure 3). This function is thus responsible for the sexual dichromatism observed in a wide range of taxa (Pérez i de Lanuza et al. 2013; Shultz and Burns 2017).

It should be noted that pattern, like coloration, have an importance when it comes to mating choice. For instance, in guppies (*Poecilia reticulata*), females prefer males with a high variation in local contrast (Sibeaux et al. 2019).



**Figure 3.** Four species of bellbirds in the genus *Procnias* living in tropical forests. Sexual coloration seems to have evolved only in males, while cryptic coloration evolved in females. Drawing by Chris Rose, from the 2020 book Handbook of the Birds of the World Alive by D. Snow.

Understanding for which function the coloration has been selected offers a valuable insight into the selective pressures that shape the evolution of a color patch. However, this information is often unknown in non-model species, because it requires costly experimental tests. The function can often be deduced from the type of coloration. Generally, colorations used for crypsis are dull, in order to match the background, while coloration involved in communication have to be conspicuous to be effective. Yet, dull coloration has been found to be aposematic in some species (Wüster et al. 2004), and bright coloration has been found to be cryptic in others (Mochida et al. 2015; Rajabizadeh et al. 2015). Thus, it can be difficult to identify without doubt the function, and thus the selective pressure, acting on a color patch.

### **b) Opposition between selective forces**

The coloration of a species usually plays a role in several functions (e.g. sexual signal and crypsis). However, the different functions do not all select for similar degrees of conspicuousness: color selected for crypsis are often dull, and those selected for communication are often conspicuous. Due to this opposition, some functions are more likely to co-evolve than others for a given patch. For instance, dorsal aposematic coloration are frequently found to influence both intraspecific communication and predator deterrence (Figure 4, Rojas et al. 2018). On the other hand, some functions, like crypsis and sexual signal, seem to coexist rarely on the same patch. In such situation, the opposition between selective pressures can be resolved in three ways.

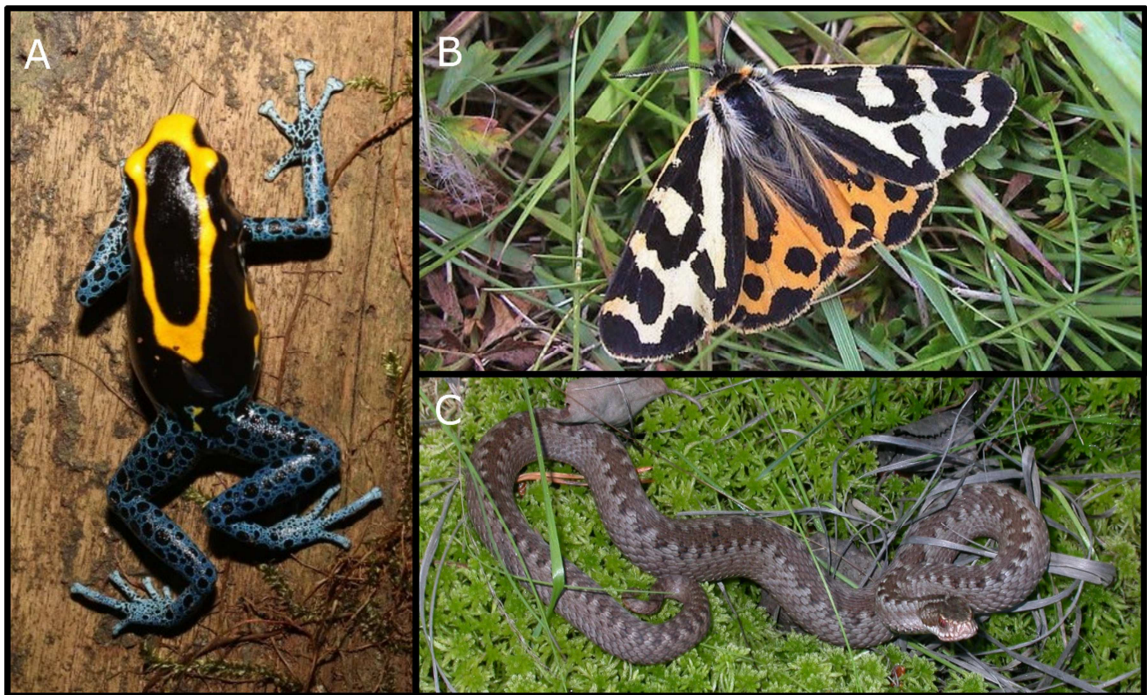
Firstly, colorations with different functions can be located on different patches. Colorations selected for crypsis are often located in body parts visible at distance, because they are under strong selective pressure from predators. Conversely, colorations used for intraspecific communication (either sexual or social colorations) are on body parts concealed from predators and visible from the point of view of the interacting congener. This spatial arrangement is frequently observed in vertebrates (Stuart-Fox and Ord 2004; Gomez and Théry 2007). Some species perfected this strategy by developing a specialized organ that allows displaying the signal only at a particular moment and hiding it from predators the rest of the time (e.g. the dewlap in the *Anolis* genus).

The opposition between opposing selective pressures can also be solved by a differential evolution of the components of the same color patch. These different components could be the hue, the saturation and the brightness or the spectral reflectance at different wavelengths. For instance, in birds, it has been hypothesized that colorations in



the UV are used as private channel for intraspecific communication, while coloration in the visible wavelengths are used for camouflage (Guilford and Harvey 1998, but see Stevens and Cuthill 2007). Such private communication is possible because the predators responsible of the selective pressure for crypsis, mammals and raptors, have a bad perception of UV coloration (Jacobs 2009; Lind et al. 2013, 2014; but see Douglas and Jeffery 2014). Private signals are not restricted to UV and can also concern any wavelength as long it is not perceived by one actor (e.g. long wavelength, Brandley et al. 2016).

Finally, coloration functions can be distance dependent. In this case, the pattern may, at the same time, break body shape of an animal at distance, and be an aposematic signal at short range (Figure 4, Wüster et al. 2004; Barnett and Cuthill 2014; Barnett et al. 2018). Although this phenomenon was first described a long time ago, it is still not fully understood, essentially because it relies on the visual acuity of the viewer, a vision parameter that just begins to be taken into account in studies of coloration evolution (see also discussion).



**Figure 4.** Exemple of species showing coloration involve in multiple function. A. *Dendrobates tinctorius*, its yellow coloration is aposematic and is used by females for mating choice. Photo by Jan Sobotnik. B. *Arctia plantaginis*, the dark spot on the hind wing are aposematic and play a role in thermoregulation. Photo by Niger stone. C. *Vipera berus*, the zigzag pattern is cryptic at long range but becomes aposematic at short range. Photo by Pierre-Andre Crochet.

Overall, the multifunctional nature of color patches remains a subject underexplored until now. Nonetheless, it becomes clearer and clearer, as evidence are accumulating, that the simplistic view that the evolution of a color patch is driven by one main selective pressure is often inaccurate.

### **c) Evolution of coloration: a matter of perception**

The fact that the evolution of a coloration depends on the perception of this coloration by other organisms makes it much more difficult to understand the direction of the selective pressures. Perception depends on two main factors: the vision system of the viewer and the environmental conditions (see previous section; we will put aside the cognitive system of the viewer, as its influence on the evolution of coloration remains almost unaddressed). Because of differences in visual systems, the direction of a selective pressure that appears to be clear at first sight may be in reality difficult to estimate in the wild. For instance, in the case where predation selects for an increased crypsis, several predators with different vision systems may create simultaneous selective pressures in different directions. This hypothesis, although never formally demonstrated, is supported by several studies that found a selection for different cryptic colorations depending on the predators present (Blanco and Bertelotti 2002; Touchon and Warkentin 2008; Willink et al. 2014). Because visual systems not only differ between species, but may also vary between sexes within the same species (Arikawa et al. 2005; McCulloch et al. 2017), it is possible that the direction of the selective pressure differs even at the intraspecific level. In such conditions, it can be extremely difficult to assess accurately the strength and the direction of the selective pressures acting on coloration.

Environmental conditions have an effect on the perception of coloration, and as such, can influence its evolution. This idea was first developed in the sensory drive hypothesis, which states that the signal and the visual systems of the viewer should coevolve with the local environment, in order to maintain signal transmission (Endler 1992, 1993*b*). However, the effect of environment is not restricted to signals (i.e. a trait that evolved for communication functions), and it concerns any coloration as soon as its evolution involves a viewer (as it is the case for crypsis, sexual signal, interspecific communication...). Different aspects of the environment influence the perception of coloration, including habitat light level and spectral quality (Marchetti 1993; Leal and Fleishman 2004; Johnsen et al. 2006) and the extent of visual noise in the background (Ord

et al. 2007; Uetz et al. 2011), and it may be difficult to account for all of them when studying coloration evolution.

### **Genetic and non-genetic bases of variations in coloration**

The diversity of colorations observed in nature reflects the variety of genetic architectures coding and regulating coloration. Some colorations are controlled by few genes only, while other are determined by tens to hundreds of independent genes (Bennett and Lamoreux 2003). Coloration can also be controlled by supergenes, which are an assemblage of loci that do not recombine and evolve like one big gene. These structures were repeatedly found to control discrete changes of coloration (Lamichhaney et al. 2015; Jay et al. 2018; Merritt et al. 2020) and may thus be a common architecture for color polymorphism.

The variation of coloration found between species can have two origins. First, it can be caused by mutations. Mutations can occur in the coding sequence responsible of the pigment/microstructure. It could be by a unique mutation on a large effect locus (Uy et al. 2016; Cooke et al. 2017), or multiple mutations on small effect loci (Rodríguez et al. 2020), depending on the genetic architecture responsible of coloration. Mutation could affect the regulatory region (Durbin et al. 2003; Imsland et al. 2016). Such mutations have been hypothesized to be responsible for changes in pigment-based patterns, while mutation on coding sequence were predicted to affect the coloration itself (Orteu and Jiggins 2020). However, this hypothesis remains to be tested at large scale, as several counter examples have been described. For instance, in a species of lizard (*Podarcis muralis*) the variation of ventral coloration, which varies from white to red, results from a change in the regulatory sequences (Andrade et al. 2019). Secondly, color change can emerge through introgression. Introgression is responsible of the apparition of new morphs in several vertebrates species (Anderson et al. 2009; Tuttle et al. 2016; Giska et al. 2019). The effect of introgression can be especially important in case of structural coloration. Indeed, an example of hybrid speciation have shown that hybridization can results in mixing the way to produce structural colors, leading to a unique coloration that differs from both parent species (Barrera-Guzmán et al. 2017).

Some of the genes controlling coloration can be conserved across long time-scales (e.g. melanocortin-1 receptor and agouti signaling protein are found in birds, fish, reptile and mammals; Theron et al. 2001; Rosenblum et al. 2004; Gross et al. 2009; Kingsley et al. 2009). However, these genes frequently mutate, and a similar coloration can be the result of



different mutations in distantly related species (Rosenblum et al. 2010) as well as within the same species (Bastide et al. 2016; Uy et al. 2016). It is thus difficult to make any generality about the genetic of coloration. This subject is still at its beginning and, except for few model species, we know relatively little on the genetic architecture underlying coloration variations in the wild (San-Jose and Roulin 2017).

It should be noted that changes of coloration are not always genetic, and may instead be driven by environmental cues. Such difference of expression depending on the environment is called plasticity. One of the most striking examples for coloration plasticity can be found in chameleons, where body coloration can vary in few second to communicate with congeners or other species (Ligon and McGraw 2016). However, plastic colorations are not restricted to conspicuous examples, and they actually concern a wide variety of taxa. Plastic change of melanism is frequently observed in ectotherms in order to facilitate thermoregulation (Clusella-Trullas et al. 2007). Plasticity can also be used for camouflage (Polo-Cavia and Gomez-Mestre 2017). In addition, many social and sexual coloration are expected to be plastic, so that they provide a honest and up to date signal of the individual's dominance or health statue (Price 2006). For instance, in birds, molting allows the plumage to reflect every years the current health statue of its wearer (McQueen et al. 2019). Plasticity is thus a fundamental notion for several functions of coloration.

## **Interplay between coloration and species diversification**

Multiple traits correlate with species diversification, (e.g. body size: Rabosky et al. 2013; microhabitat: Bars-Clozel et al. 2017) and coloration is no exception. This link could have four origins.

First, speciation can be triggered by sensory drive, and thus be a by-product of the adaptation of sexual signals to different environmental conditions. In such case, the divergence of sexual colorations, photoreceptor sensibility and mating preference leads to speciation for populations living in different environments (Boughman 2002). Speciation by sensory drive was described in a wide variety of taxa, from fishes to invertebrates, living in both terrestrial and aquatic ecosystems, although the support for this theory mainly comes from studies occurring in the latter (Cummings and Endler 2018).

Secondly, speciation should be increased in polymorphic species – species with several distinct heritable morphs co-occurring within a single population – due to a process called morphic speciation (West-Eberhard 1986). This process predicts that, in polymorphic

specie with specialized morphs, if a population lost a morph during the colonization of a new environment, the remaining morphs should rapidly diverge. The reason of this divergence lies in the release of genetic or competitive constraints acting on the remaining morphs with the disappearance of the focal morph. This hypothesis is described and discussed more extensively in manuscript E. It is supported by a few studies on birds and lizards (Corl et al. 2010, 2012; Hugall and Stuart-Fox 2012).

Thirdly, speciation may be favored if coloration is under disruptive selection. For instance, Batesian mimicry may facilitate the apparition of pre and post-mating barriers if the mimic and the model differ in distribution range (Pfennig et al. 2015). Indeed, selection for mimicry leads to the evolution of aposematic coloration in the mimic in sympatry, but selection for crypsis favors non-aposematic coloration in allopatry. This difference of selective regime selects against immigrant or hybrid individual, and may lead eventually to a reduction of gene flow between sympatric and allopatric populations. This last point however was never confirmed, and more studies are needed to investigate this subject.

Finally, correlation between speciation and coloration can appear through indirect causality. Aposematic coloration are often associated with chemical defenses, which are predicted by the escape-and-radiate scenario to increase the rates of diversification (Ehrlich and Raven 1964; Thompson 1989). The hypothesis predicts that chemical defenses release the selective pressure of predators, opening up new ecological opportunities and ultimately increasing speciation rates. The reduction of predation may also speed up the evolution of sexual signals, as they are no longer constrained by selection for crypsis. In agreement with the escape-and-radiate hypothesis, two studies on amphibians found higher speciation rates in venomous aposematic species (Santos et al. 2014; Arbuckle and Speed 2015).

### **Question and objectives**

The aim of this thesis is to improve our understanding of coloration evolution. More precisely, I investigated the role of different selective pressures in shaping coloration evolution, and the effect of coloration evolution on species diversification. It is clear that these subjects are quite diverse, and that not all their aspects can be explored in one thesis (nor in one life). In addition, there is already a solid background in these subjects, because coloration was one of the first and most studied traits when it comes to evolution (e.g. Darwin 1871; Wallace 1877; Poulton 1890). However, if numerous studies have been made at the species level, some questions have yet to be addressed at larger scales. During my

PhD, I used multi-taxa comparative analyses applied to different species coming from three major clades of vertebrates, Aves, Amphibia and Squamata, to unravel general patterns on these issues.

Firstly, I studied the effect of various selective pressures on the intraspecific variation of whole-body coloration (MS 1). Specifically, I investigated how selection for interspecific recognition and local- and large-scale environmental factors shape the variation of coloration observed in two species of newts at continental scale. In the second chapter, I investigated the evolution of a defensive strategy, the mimicry. First, I developed a new method, based on Artificial Neural Networks, to estimate the phenotypic resemblance between organisms, and I applied this method to the detection of mimicry in Western Palearctic Snakes (MS 2). This study stimulated a more theoretical discussion on the role of signaling and non-signaling components of phenotype on the evolution of defensive mimicry (MS 3). In the third chapter, I highlighted the relative roles of natural and sexual selection in the evolution of vertebrate coloration (MS 4). I compared the phenotypic disparity and evolution rates between sexes and body parts assumed to be mainly involved in survival *versus* sexual selection. I performed this analysis on three different clades of vertebrates. In the last chapter, I studied the link between coloration and diversification process (MS 5). I tested if the presence of sexual coloration and of color polymorphism was associated with increased speciation in Lacertids.

## References

- Alho, J. S., G. Herczeg, F. Söderman, A. Laurila, K. Jönsson, and J. Merilä. 2010. Increasing melanism along a latitudinal gradient in a widespread amphibian: local adaptation, ontogenic or environmental plasticity? *BMC Evolutionary Biology* 10:317.
- Anderson, T. M., B. M. VonHoldt, S. I. Candille, M. Musiani, C. Greco, D. R. Stahler, D. W. Smith, et al. 2009. Molecular and evolutionary history of melanism in Norths American gray wolves. *Science* 323:1339–1343.
- Andrade, P., C. Pinho, G. P. i. de Lanuza, S. Afonso, J. Brejcha, C. J. Rubin, O. Wallerman, et al. 2019. Regulatory changes in pterin and carotenoid genes underlie balanced color polymorphisms in the wall lizard. *Proceedings of the National Academy of Sciences of the United States of America* 116:5633–5642.
- Arbuckle, K., and M. P. Speed. 2015. Antipredator defenses predict diversification rates. *Proceedings of the National Academy of Sciences of the United States of America* 112:13597–13602.
- Arikawa, K., M. Wakakuwa, X. Qiu, M. Kurasawa, and D. G. Stavenga. 2005. Sexual dimorphism of short-wavelength photoreceptors in the small white butterfly, *Pieris rapae crucivora*. *Journal of Neuroscience* 25:5935–5942.
- Bagnara, J. T., P. J. Fernandez, and R. Fujii. 2007. On the blue coloration of vertebrates. *Pigment Cell Research* 20:14–26.
- Barlow, G. W. 1972. The Attitude of Fish Eye-Lines in Relation to Body Shape and to Stripes and Bars. *Copeia* 1972:4.
- Barnett, J. B., and I. C. Cuthill. 2014. Distance-dependent defensive coloration. *Current Biology* 24:R1157–R1158.
- Barnett, J. B., C. Michalis, H. M. Anderson, B. L. McEwen, J. Yeager, J. N. Pruitt, N. E. Scott-Samuel, et al. 2020. Imperfect transparency and camouflage in glass frogs. *Proceedings of the National Academy of Sciences* 201919417.
- Barnett, J. B., C. Michalis, N. E. Scott-Samuel, and I. C. Cuthill. 2018. Distance-dependent defensive coloration in the poison frog *Dendrobates tinctorius*, Dendrobatidae. *Proceedings of the National Academy of Sciences of the United States of America* 115:6416–6421.
- Barrera-Guzmán, A. O., A. Aleixo, M. D. Shawkey, and J. T. Weir. 2017. Hybrid speciation leads to novel male secondary sexual ornamentation of an Amazonian bird. *Proceedings of the National Academy of Sciences of the United States of America* 115:E218–E225.
- Bars-Closel, M., T. Kohlsdorf, D. S. Moen, and J. J. Wiens. 2017. Diversification rates are more strongly related to microhabitat than climate in squamate reptiles (lizards and snakes). *Evolution* 71:2243–2261.
- Bastide, H., J. D. Lange, J. B. Lack, A. Yassin, and J. E. Pool. 2016. A variable genetic architecture of melanic evolution in *Drosophila melanogaster*. *Genetics* 204:1307–1319.
- Bates, H. W. 1862. Contributions to an Insect Fauna of the Amazon Valley. Lepidoptera: Heliconidae. *Transactions of the Linnean Society of London* 23:495–566.
- Beck, J. 1972. Surface color perception. (Cornell University Press, ed.). NY.
- Bennett, D. C., and M. L. Lamoreux. 2003. The color loci of mice - A genetic century. *Pigment Cell Research*. John Wiley & Sons, Ltd.
- Blanco, G., and M. Bertelotti. 2002. Differential predation by mammals and birds: Implications for egg-colour polymorphism in a nomadic breeding seabird. *Biological Journal of the*

- Linnean Society 75:137–146.
- Boughman, J. W. 2002. How sensory drive can promote speciation. *Trends in Ecology and Evolution*. Elsevier Current Trends.
- Brandley, N., M. Johnson, and S. Johnsen. 2016. Aposematic signals in North American black widows are more conspicuous to predators than to prey. *Behavioral Ecology* 27:1104–1112.
- Brodie, E. D. 1993. Differential avoidance of coral snake banded patterns by free-ranging avian predators in Costa Rica. *Evolution* 47:227–235.
- Caro, T. 2009. Contrasting coloration in terrestrial mammals. *Philosophical Transactions of the Royal Society B: Biological Sciences*. Royal Society.
- Caro, T., and R. Mallarino. 2020. *Coloration in Mammals*. Trends in Ecology and Evolution. Elsevier Ltd.
- Clusella-Trullas, S., J. H. van Wyk, and J. R. Spotila. 2007. Thermal melanism in ectotherms. *Journal of Thermal Biology* 32:235–245.
- Cooke, T. F., C. R. Fischer, P. Wu, T. X. Jiang, K. T. Xie, J. Kuo, E. Doctorov, et al. 2017. Genetic Mapping and Biochemical Basis of Yellow Feather Pigmentation in Budgerigars. *Cell* 171:427–439.e21.
- Corl, A., A. R. Davis, S. R. Kuchta, and B. Sinervo. 2010. Selective loss of polymorphic mating types is associated with rapid phenotypic evolution during morphic speciation. *Proceedings of the National Academy of Sciences of the United States of America* 107:4254–4259.
- Corl, A., L. T. Lancaster, and B. Sinervo. 2012. Rapid formation of reproductive isolation between two populations of side-blotched lizards, *Uta stansburiana*. *Copeia* 593–602.
- Cummings, M. E., and J. A. Endler. 2018. 25 Years of sensory drive: The evidence and its watery bias. *Current Zoology* 64:471–484.
- Cuthill, I. C., W. L. Allen, K. Arbuckle, B. Caspers, G. Chaplin, M. E. Hauber, G. E. Hill, et al. 2017. The biology of color. *Science*. American Association for the Advancement of Science.
- Darwin, C. 1871. *The descent of man, and selection in relation to sex*. (John Murray, ed.). London, UK.
- Douglas, R. H., and G. Jeffery. 2014. The spectral transmission of ocular media suggests ultraviolet sensitivity is widespread among mammals. *Proceedings of the Royal Society B: Biological Sciences* 281:20132995–20132995.
- Durbin, M. L., K. E. Lundy, P. L. Morrell, C. L. Torres-Martinez, and M. T. Clegg. 2003. Genes that determine flower color: The role of regulatory changes in the evolution of phenotypic adaptations. *Molecular Phylogenetics and Evolution* 29:507–518.
- Ehrlich, P. R., and P. H. Raven. 1964. *Butterflies and Plants: A Study in Coevolution*. *Evolution* 18:586.
- Endler, J. A. 1992. Signals, signal conditions, and the direction of evolution. *American Naturalist* 139.
- Endler, J. A. 1993a. *The Color of Light in Forests and Its Implications*. *Ecological Monographs* 63:1–27.
- Endler, J. A. 1993b. Some general comments on the evolution and design of animal communication systems. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* 340:215–225.
- Galván, I., J. Garrido-Fernández, J. Ríos, A. Pérez-Gálvez, B. Rodríguez-Herrera, and J. J. Negro. 2016. Tropical bat as mammalian model for skin carotenoid metabolism. *Proceedings of the*

- National Academy of Sciences of the United States of America 113:10932–10937.
- Giska, I., L. Farelo, J. Pimenta, F. A. Seixas, M. S. Ferreira, J. P. Marques, I. Miranda, et al. 2019. Introgression drives repeated evolution of winter coat color polymorphism in hares. *Proceedings of the National Academy of Sciences of the United States of America* 116:24150–24156.
- Glover, B. J., and H. M. Whitney. 2010. Structural colour and iridescence in plants: The poorly studied relations of pigment colour. *Annals of Botany* 105:505–511.
- Gomez, D., and M. Théry. 2007. Simultaneous crypsis and conspicuousness in color patterns: comparative analysis of a neotropical rainforest bird community. *The American naturalist* 169:S42–S61.
- Gray, D. A. 1996. Carotenoids and sexual dichromatism in north American passerine birds. *American Naturalist* 148:453–480.
- Gross, J. B., R. Borowsky, and C. J. Tabin. 2009. A Novel Role for Mc1r in the Parallel Evolution of Depigmentation in Independent Populations of the Cavefish *Astyanax mexicanus*. (G. S. Barsh, ed.) *PLoS Genetics* 5:e1000326.
- Guilford, T., and P. H. Harvey. 1998. The purple patch. *Nature*. Nature Publishing Group.
- Hill, G. E., and K. J. McGraw. 2006. Bird coloration Volume 2: function and evolution. Harvard University Press.
- Hudon, J., and A. H. Brush. 1992. Identification of carotenoid pigments in birds. *Methods in Enzymology* 213:312–321.
- Hugall, A. F., and D. Stuart-Fox. 2012. Accelerated speciation in colour-polymorphic birds. *Nature* 485:631–634.
- Imsland, F., K. McGowan, C. J. Rubin, C. Henegar, E. Sundström, J. Berglund, D. Schwochow, et al. 2016. Regulatory mutations in TBX3 disrupt asymmetric hair pigmentation that underlies Dun camouflage color in horses. *Nature Genetics* 48:152–158.
- Jacobs, G. H. 2009. Evolution of colour vision in mammals. *Philosophical Transactions of the Royal Society B: Biological Sciences* 364:2957–2967.
- Jay, P., A. Whibley, L. Frézal, M. Á. Rodríguez de Cara, R. W. Nowell, J. Mallet, K. K. Dasmahapatra, et al. 2018. Supergene Evolution Triggered by the Introgression of a Chromosomal Inversion. *Current Biology* 28:1839–1845.e3.
- Johnsen, S., A. Kelber, E. Warrant, A. M. Sweeney, E. A. Widder, R. L. Lee, and J. Hernández-Andrés. 2006. Crepuscular and nocturnal illumination and its effects on color perception by the nocturnal hawkmoth *Deilephila elpenor*. *Journal of Experimental Biology* 209:789–800.
- Kingsley, E. P., M. Manceau, C. D. Wiley, and H. E. Hoekstra. 2009. Melanism in *Peromyscus* Is Caused by Independent Mutations in *Agouti*. (J. O. Borevitz, ed.) *PLoS ONE* 4:e6435.
- Kingsolver, J. G. 1985. Thermoregulatory significance of wing melanization in *Pieris* butterflies (Lepidoptera: Pieridae): physics, posture, and pattern. *Oecologia* 66:546–553.
- Klomp, D. A., D. Stuart-Fox, E. J. Cassidy, N. Ahmad, and T. J. Ord. 2017. Color pattern facilitates species recognition but not signal detection: A field test using robots. *Behavioral Ecology* 28:597–606.
- Korenyak, D. A., and V. I. Govardovskii. 2013. Photoreceptors and visual pigments in three species of newts. *Journal of Evolutionary Biochemistry and Physiology* 49:399–407.
- Kraaijeveld, K., F. J. L. Kraaijeveld-Smit, and J. Komdeur. 2007. The evolution of mutual ornamentation. *Animal Behaviour*. Academic Press.

- Lamichhaney, S., G. Fan, F. Widemo, U. Gunnarsson, D. S. Thalmann, M. P. Hoepfner, S. Kerje, et al. 2015. Structural genomic changes underlie alternative reproductive strategies in the ruff (*Philomachus pugnax*). *Nature Genetics* 48:84–88.
- Le Poul, Y., A. Whibley, M. Chouteau, F. Prunier, V. Llaurens, and M. Joron. 2014. Evolution of dominance mechanisms at a butterfly mimicry supergene. *Nature Communications* 5:1–8.
- Leal, M., and L. J. Fleishman. 2004. Differences in Visual Signal Design and Detectability between Allopatric Populations of *Anolis* Lizards. *American Naturalist* 163:26–39.
- Ligon, R. A., and K. J. McGraw. 2016. Social costs enforce honesty of a dynamic signal of motivation. *Proceedings of the Royal Society B: Biological Sciences* 283:20161873.
- Lind, O., M. Mitkus, P. Olsson, and A. Kelber. 2013. Ultraviolet sensitivity and colour vision in raptor foraging. *The Journal of experimental biology* 216:1819–26.
- Lind, O., M. Mitkus, P. Olsson, and A. Kelber. 2014. Ultraviolet vision in birds: the importance of transparent eye media. *Proceedings of the Royal Society B: Biological Sciences* 281:20132209–20132209.
- Macedonia, J. M., S. James, L. W. Wittle, and D. L. Clark. 2000. Skin pigments and coloration in the Jamaican radiation of *Anolis* lizards. *Journal of Herpetology* 34:99–109.
- Marchetti, K. 1993. Dark habitats and bright birds illustrate the role of the environment in species divergence. *Nature* 362:149–152.
- Margalida, A., J. J. Negro, and I. Galván. 2008. Melanin-based color variation in the Bearded Vulture suggests a thermoregulatory function. *Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology* 149:87–91.
- Martin, M., J. F. Le Galliard, S. Meylan, and E. R. Loew. 2015. The importance of ultraviolet and near-infrared sensitivity for visual discrimination in two species of lacertid lizards. *Journal of Experimental Biology* 218:458–465.
- Mayr, E. 1972. Sexual selection and natural selection. Pages 87–104 in *Sexual selection and the descent of man: The darwinian pivot*.
- McCulloch, K. J., F. Yuan, Y. Zhen, M. L. Aardema, G. Smith, J. Llorente-Bousquets, P. Andolfatto, et al. 2017. Sexual Dimorphism and Retinal Mosaic Diversification following the Evolution of a Violet Receptor in Butterflies. *Molecular biology and evolution* 34:2271–2284.
- McElroy, M. T. 2016. Teasing apart crypsis and aposematism - evidence that disruptive coloration reduces predation on a noxious toad. *Biological Journal of the Linnean Society* 117:285–294.
- McQueen, A., B. Kempnaers, J. Dale, M. Valcu, Z. T. Emery, C. J. Dey, A. Peters, et al. 2019. Evolutionary drivers of seasonal plumage colours: colour change by moult correlates with sexual selection, predation risk and seasonality across passerines. (R. Norris, ed.) *Ecology Letters* 22:1838–1849.
- Merritt, J. R., K. E. Grogan, W. M. Zinzow-Kramer, D. Sun, E. A. Ortlund, S. V. Yi, and D. L. Maney. 2020. A supergene-linked estrogen receptor drives alternative phenotypes in a polymorphic songbird. *Proceedings of the National Academy of Sciences* 202011347.
- Mochida, K., W. Y. Zhang, and M. Toda. 2015. The function of body coloration of the hai coral snake *Sinomicrurus japonicus boettgeri*. *Zoological Studies* 54:33–39.
- Negro, J. J., E. Figueroa-Luque, and I. Galván. 2018. Melanin-based sexual dichromatism in the Western Palearctic avifauna implies darker males and lighter females. *Journal of Avian Biology* 49:jav-01657.
- Ord, T. J., R. A. Peters, B. Clucas, and J. A. Stamps. 2007. Lizards speed up visual displays in noisy



- motion habitats. *Proceedings of the Royal Society B: Biological Sciences* 274:1057–1062.
- Orteu, A., and C. D. Jiggins. 2020. The genomics of coloration provides insights into adaptive evolution. *Nature Reviews Genetics*. Nature Research.
- Pérez i de Lanuza, G., M. A. Carretero, and E. Font. 2017. Intensity of male-male competition predicts morph diversity in a color polymorphic lizard. *Evolution* 71:1832–1840.
- Pérez i de Lanuza, G., E. Font, and J. L. Monterde. 2013. Using visual modelling to study the evolution of lizard coloration: Sexual selection drives the evolution of sexual dichromatism in lacertids. *Journal of Evolutionary Biology* 26:1826–1835.
- Pfennig, D. W., C. K. Akcali, and D. W. Kikuchi. 2015. Batesian mimicry promotes pre- and postmating isolation in a snake mimicry complex. *Evolution* 69:1085–1090.
- Polo-Cavia, N., and I. Gomez-Mestre. 2017. Pigmentation plasticity enhances crypsis in larval newts: associated metabolic cost and background choice behaviour. *Scientific Reports* 7:39739.
- Poulton, E. 1890. *The Colours of Animals: Their Meaning and Use, Especially Considered in the Case of Insects*. (D. Appleton and Co., ed.). New York.
- Price, T. D. 2006. Phenotypic plasticity, sexual selection and the evolution of colour patterns. *The Journal of experimental biology* 209:2368–76.
- Prum, R. O., and R. H. Torres. 2004. Structural colouration of mammalian skin: Convergent evolution of coherently scattering dermal collagen arrays. *Journal of Experimental Biology* 207:2157–2172.
- Rabosky, D. L., F. Santini, J. Eastman, S. A. Smith, B. Sidlauskas, J. Chang, and M. E. Alfaro. 2013. Rates of speciation and morphological evolution are correlated across the largest vertebrate radiation. *Nature Communications* 4:1–8.
- Rajabizadeh, M., D. Adriaens, M. Kaboli, J. Sarafraz, and M. Ahmadi. 2015. Dorsal colour pattern variation in Eurasian mountain vipers (genus *Montivipera*): A trade-off between thermoregulation and crypsis. *Zoologischer Anzeiger* 257:1–9.
- Rémy, A., A. Grégoire, P. Perret, and C. Doutrelant. 2010. Mediating male-male interactions: The role of the UV blue crest coloration in blue tits. *Behavioral Ecology and Sociobiology* 64:1839–1847.
- Rodríguez, A., N. I. Mundy, R. Ibáñez, and H. Pröhl. 2020. Being red, blue and green: The genetic basis of coloration differences in the strawberry poison frog (*Oophaga pumilio*). *BMC Genomics* 21:301.
- Rojas, B., E. Burdfield-Steel, C. De Pasqual, S. Gordon, L. Hernández, J. Mappes, O. Nokelainen, et al. 2018. Multimodal Aposematic Signals and Their Emerging Role in Mate Attraction. *Frontiers in Ecology and Evolution* 6:93.
- Rönkä, K., J. Mappes, C. Michalis, R. Kiviö, J. Salokannas, and B. Rojas. 2018. Can multiple-model mimicry explain warning signal polymorphism in the wood tiger moth, *Arctia plantaginis* (Lepidoptera: Erebidae)? *Biological Journal of the Linnean Society* 124:237–260.
- Roper, T. J., and R. Wistow. 1986. Aposematic Colouration and Avoidance Learning in Chicks. *The Quarterly Journal of Experimental Psychology Section B* 38:141–149.
- Rosenblum, E. B. 2006. Convergent evolution and divergent selection: lizards at the White Sands ecotone. *The American naturalist* 167:1–15.
- Rosenblum, E. B., H. E. Hoekstra, and M. W. Nachman. 2004. Adaptive reptile color variation and the evolution of the *Mc1r* gene. *Evolution* 58:1794–1808.
- Rosenblum, E. B., H. Römpler, T. Schöneberg, and H. E. Hoekstra. 2010. Molecular and functional



- basis of phenotypic convergence in white lizards at White Sands. *Proceedings of the National Academy of Sciences of the United States of America* 107:2113–2117.
- Saenko, S. V., J. Teyssier, D. van der Marel, and M. C. Milinkovitch. 2013. Precise colocalization of interacting structural and pigmentary elements generates extensive color pattern variation in *Phelsuma* lizards. *BMC Biology* 11:105.
- Saks, L., I. Ots, and P. Hõrak. 2003. Carotenoid-based plumage coloration of male greenfinches reflects health and immunocompetence. *Oecologia* 134:301–307.
- San-Jose, L. M., and A. Roulin. 2017. Genomics of coloration in natural animal populations. *Philosophical Transactions of the Royal Society B: Biological Sciences*. Royal Society Publishing.
- Santos, J. C., M. Baquero, C. Barrio-Amorós, L. A. Coloma, L. K. Erdtmann, A. P. Lima, and D. C. Cannatella. 2014. Aposematism increases acoustic diversification and speciation in poison frogs. *Proceedings of the Royal Society B: Biological Sciences* 281:20141761.
- Saporito, R. A., R. Zuercher, M. Roberts, K. G. Gerow, and M. A. Donnelly. 2007. Experimental evidence for aposematism in the dendrobatid poison frog *Oophaga pumilio*. *Copeia* 1006–1011.
- Seehausen, O., and D. Schluter. 2004. Male–male competition and nuptial–colour displacement as a diversifying force in Lake Victoria cichlid fishes. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 271:1345–1353.
- Sefc, K. M., A. C. Brown, and E. D. Clotfelter. 2014. Carotenoid-based coloration in cichlid fishes. *Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology*. Elsevier Inc.
- Shawkey, M. D., and G. E. Hill. 2005. Carotenoids need structural colours to shine. *Biology Letters* 1:121–124.
- Shultz, A. J., and K. J. Burns. 2017. The role of sexual and natural selection in shaping patterns of sexual dichromatism in the largest family of songbirds (Aves: Thraupidae). *Evolution* 71:1061–1074.
- Sibaux, A., G. L. Cole, and J. A. Endler. 2019. The relative importance of local and global visual contrast in mate choice. *Animal Behaviour* 154:143–159.
- Siefferman, L., and G. E. Hill. 2005. UV-blue structural coloration and competition for nestboxes in male eastern bluebirds. *Animal Behaviour* 69:67–72.
- Skelhorn, J., H. M. Rowland, M. P. Speed, and G. D. Ruxton. 2010. Masquerade: Camouflage without crypsis. *Science*. American Association for the Advancement of Science.
- Stevens, M., and I. C. Cuthill. 2007. Hidden Messages: Are Ultraviolet Signals a Special Channel in Avian Communication? *BioScience* 57:501–507.
- Stevens, M., J. Troscianko, J. K. Wilson-Aggarwal, and C. N. Spottiswoode. 2017. Improvement of individual camouflage through background choice in ground-nesting birds. *Nature Ecology and Evolution* 1:1325–1333.
- Stuart–Fox, D. M., and T. J. Ord. 2004. Sexual selection, natural selection and the evolution of dimorphic coloration and ornamentation in agamid lizards. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 271:2249–2255.
- Sun, H., J. P. Macke, and J. Nathans. 1997. Mechanisms of spectral tuning in the mouse green cone pigment. *Proceedings of the National Academy of Sciences of the United States of America* 94:8860–8865.
- Svádová, K., A. Exnerová, P. Štys, E. Landová, J. Valenta, A. Fučíková, and R. Socha. 2009. Role of

- different colours of aposematic insects in learning, memory and generalization of naïve bird predators. *Animal Behaviour* 77:327–336.
- Theron, E., K. Hawkins, E. Bermingham, R. E. Ricklefs, and N. I. Mundy. 2001. The molecular basis of an avian plumage polymorphism in the wild: A melanocortin-1-receptor point mutation is perfectly associated with the melanic plumage morph of the bananaquit, *Coereba flaveola*. *Current Biology* 11:550–557.
- Thompson, J. N. 1989. Concepts of coevolution. *Trends in Ecology and Evolution* 4:179–183.
- Tobias, J. A., R. Montgomerie, and B. E. Lyon. 2012. The evolution of female ornaments and weaponry: Social selection, sexual selection and ecological competition. *Philosophical Transactions of the Royal Society B: Biological Sciences* 367:2274–2293.
- Touchon, J. C., and K. M. Warkentin. 2008. Fish and dragonfly nymph predators induce opposite shifts in color and morphology of tadpoles. *Oikos* 117:634–640.
- Troscianko, J., and M. Stevens. 2015. Image calibration and analysis toolbox - a free software suite for objectively measuring reflectance, colour and pattern. *Methods in Ecology and Evolution* 6:1320–1331.
- Tuttle, E. M., A. O. Bergland, M. L. Korody, M. S. Brewer, D. J. Newhouse, P. Minx, M. Stager, et al. 2016. Divergence and functional degradation of a sex chromosome-like supergene. *Current Biology* 26:344–350.
- Uetz, G. W., D. L. Clark, J. A. Roberts, and M. Rector. 2011. Effect of visual background complexity and light level on the detection of visual signals of male *Schizocosa ocreata* wolf spiders by female conspecifics. *Behavioral Ecology and Sociobiology* 65:753–761.
- Uy, J. A. C., E. A. Cooper, S. Cutie, M. R. Concannon, J. W. Poelstra, R. G. Moyle, and C. E. Filardi. 2016. Mutations in different pigmentation genes are associated with parallel melanism in island flycatchers. *Proceedings of the Royal Society B: Biological Sciences* 283:20160731.
- Uy, J. A. C., R. G. Moyle, and C. E. Filardi. 2009. Plumage and song differences mediate species recognition between incipient Flycatcher species of the Solomon Islands. *Evolution* 63:153–164.
- van den Berg, C. P., J. Troscianko, J. A. Endler, N. J. Marshall, and K. L. Cheney. 2020. Quantitative Colour Pattern Analysis (QCPA): A comprehensive framework for the analysis of colour patterns in nature. *Methods in Ecology and Evolution* 11:316–332.
- Verhoeven, C., Z.-X. Ren, and K. Lunau. 2018. False-colour photography: a novel digital approach to visualize the bee view of flowers. *Journal of Pollination Ecology* 23:102–118.
- Wallace, A. R. 1877. The Colors of Animals and Plants. *The American Naturalist* 11:641–662.
- West-Eberhard, M. J. 1986. Alternative adaptations, speciation, and phylogeny. (A review). *Proceedings of the National Academy of Sciences of the United States of America* 83:1388–1392.
- Willink, B., A. García-Rodríguez, F. Bolaños, and H. Pröhl. 2014. The interplay between multiple predators and prey colour divergence. *Biological Journal of the Linnean Society* 113:580–589.
- Witzel, C., and K. R. Gegenfurtner. 2018. Color Perception: Objects, Constancy, and Categories. *Annual Review of Vision Science* 4:475–499.
- Wüster, W., C. S. E. Allum, I. B. Bjargardóttir, K. L. Bailey, K. J. Dawson, J. Guenioui, J. Lewis, et al. 2004. Do aposematism and Batesian mimicry require bright colours? A test, using European viper markings. *Proceedings. Biological sciences* 271:2495–9.



# **CHAPTER ONE**

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## **Determinants of intraspecific variations of coloration**



# Manuscript A

**A lot of convergence, a bit of divergence:  
environment and interspecific interactions  
shape color patterns in *Lissotriton* newts**

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## Abstract

Coexistence with related species poses evolutionary challenges to which populations may react in diverse ways: being exposed to similar environments, sympatric populations of two species may adopt similar phenotypes. However, selection may also favor trait divergence as a way to reduce competition for resources or mates. Visible body characteristics such as coloration and external morphology are involved to varying degrees in intraspecific signaling as well as in the adaptation to the environment, and as a consequence may be diversely affected by interspecific coexistence. Here, we study how colorimetric and morphological traits, involved or not in sexual attraction, behave in a pair of two related newt species when they come into sympatry. Importantly, we did not only estimate the raw trait responses to sympatry, but also the responses after accounting for environmental variations. Hence, we focused on phenotypic variation in sympatry beyond what would be expected purely from environmental gradients. We found evidence for convergence in dorsal and caudal coloration, and for divergence in male and female ventral coloration. The belly is exposed mostly during sexual interactions, and its colors may have diverged as a way to reduce opportunities for interspecific interactions and the associated loss of energy, suggesting a case of reproductive character displacement for both sexes (RCD). However, other sexual traits did not diverge in sympatry, suggesting that RCD does not uniformly affect all sexual traits. Our study emphasizes the contrasting response patterns of traits involved in different functions and calls for the need to consider whole body coloration.

**Keywords:** Reproductive Character Displacement, natural selection, sexual selection, crypsis, *Lissotriton*





## **Introduction**

Patterns of convergence and divergence among species have generated a large amount of interest in many areas of evolutionary ecology, from community assembly (Grime 2006) to biogeography (Bergmann 1847) and evolution (Brown and Wilson 1956; Grant 1972). For evolutionary biologists, convergence is a powerful way to reveal the footprint of adaptation on the phenotype of current organisms (Elmer and Meyer 2011; Losos 2011) while divergence due to ecological character displacement is a testimony of the role of resources competition on phenotypic evolution (Schluter 2000; Pfennig and Pfennig 2009). Divergence can also arise with a reproductive character displacement (RCD), where interspecific interactions affect mate choice and result in increased dissimilarity between species' phenotypes in sympatry (we agree here with Goldberg and Lande 2006 that reproductive character displacement is distinct from the pattern of increased prezygotic isolation in sympatry that may result from this process). Patterns of phenotypic convergence and divergence thus depend on the interplay between various selective forces, and different body parts may show different responses depending on the sets of selective forces they are exposed to (Shultz and Burns 2017). At the individual scale, patterns of convergence and divergence may thus help deciphering the role of local adaptation, ecological competition and interspecific reproductive interactions in shaping the phenotype. Yet, relatively few studies have compared patterns of convergence/divergence of several body parts within a species (but see Stuart–Fox and Ord 2004; Jang et al. 2009).

Body coloration is a prime example of traits subjected to various selective pressures, as it is involved in sexual signaling, avoiding (crypsis) or deterring (aposematism) predators, or regulating body temperature (Hill and McGraw 2006; Rudh and Qvarnström 2013). Depending on the position of the color patch on the body, each of these functions may predominate. A range of situations exists from patches that are usually concealed and exposed only during displays, such as the dewlaps of *Anolis*, used in social interactions and mate attraction (Nicholson et al. 2007; Cook et al. 2013), to parts usually visible and exposed, assumed to be mostly affected by environment- or predator-driven natural selection (Stuart–Fox and Ord 2004). For this reason, it is interesting to investigate convergence and divergence patterns in a set of color traits including both sexual signals and nonsexual traits within the same species pairs.

However, a difficulty arises when comparing patterns of divergence or convergence across traits, some of which are suspected to be under natural selection. Trait selective

optima may vary across a species' range according to large-scale or small-scale environmental gradients (Delhey et al. 2019; Price et al. 2019), so that allopatric and sympatric populations may experience different environmental conditions. Trait convergence or divergence in the sympatric zone is usually established by comparing interspecific differences in phenotypic means in sympatric versus allopatric populations of both species (Kawano 2002). However, these differences may not reflect a specific evolutionary response to interspecific contacts but a phenotypic response of each species to the environmental gradients experienced within their own ranges. Thus, accounting for the small and large-scale variation of environmental factors acting on the phenotype is required to properly investigate divergence or convergence in nature (Goldberg and Lande 2006).

We investigated patterns of divergence and convergence among multiple traits in a pair of related European newt species by analyzing spatial variation in size, ornaments and coloration of *Lissotriton helveticus* and *L. vulgaris* within and outside their zone of sympatry. We accounted for the influence of the environment on these traits by modelling phenotypic variation as a function of environmental gradients (mainly climatic) within each species' range. Some of these traits are involved in sexual interactions, while other are not and are assumed to be mainly under natural selection. We thus aim at improving our understanding of the contributions of natural selection and species recognition in the evolution of whole body coloration in animals.

## **Materials and Methods**

### **Biological models**

The Smooth Newt *Lissotriton vulgaris* has a large distribution range across Europe (Figure S1, we follow Speybroeck et al. 2020 for the taxonomy of the lineages that make up the Smooth Newt complex). From central France to northern Germany, one of its lineages shares a wide area of sympatry with the Palmate Newt *L. helveticus*, which reaches as far as southern France and north-western Iberia. In European newts, interspecific reproductive interactions are common (Babik et al. 2003; Pabijan et al. 2017) and in the sympatric zone, *L. helveticus* and *L. vulgaris* are frequently syntopic, and have similar reproductive phenology and ecological requirements during the breeding period (Griffiths 1986; Chadwick et al. 2006).

In the two species, both sexes share a conspicuous ventral coloration made of a yellow-orange belly on pale background. This conspicuous coloration is involved in female choice (Secondi et al. 2012; Secondi and Théry 2014) and influences male mating choice in a related newt (Lüdtke and Foerster 2018). In addition to colors, males exhibit various ornaments (e.g. caudal filament, crest, hind-foot web) displayed during courtship (Halliday 1977) and affecting female choice in both species (Gabor and Halliday 1997; Cornuau et al. 2014). However, most non-sexual traits are very similar between the two species, which share the same largely brown dorsal coloration. We described their phenotypes based on measurements of all reported sexual secondary characters, and on spectral measurements of body parts suspected to be under different selection pressures, namely the dorsal background color and the vivid coloration of the central belly and of the latero-ventral section of the tail. Previous results suggested divergence for the shape of the tail in females in the contact zone, but failed to find a similar pattern in males (Johanet et al. 2009). However, this study was restricted to syntopic and allotopic sites within the contact zone, and did not consider coloration.

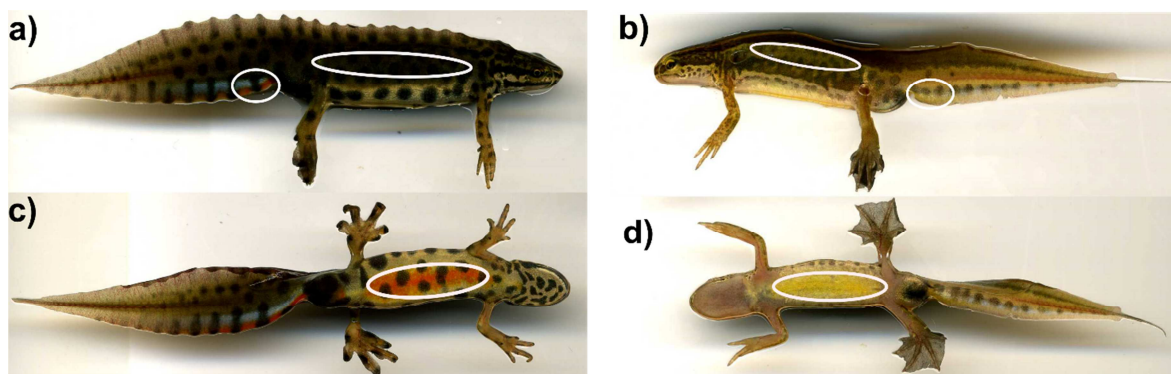
## Sampling

We sampled *L. helveticus* and *L. vulgaris* (all belonging to the northern lineage of *L. v. vulgaris*, see Pabijan et al. 2017) in the allopatric and sympatric part of their distribution ranges (Figure S1). Newts were caught in ponds during the breeding period from March 2012 to May 2014. In the allopatric zones we caught 59 males and 70 females *L. vulgaris*, and 118 males and 100 females *L. helveticus*. In the sympatric zone, we captured 137 males and 127 females *L. vulgaris*, and 312 males and 237 females *L. helveticus*. We excluded males without fully developed sexual ornaments and individuals with aberrant color measurements (i.e. value outside the typical range of variation while the photography did not reveal a particular coloration). The removal of aberrant spectrophotometric measures did not qualitatively change the results. Final sample size was 51 males and 70 females *L. vulgaris* and 95 males and 93 females *L. helveticus* in allopatry and 117 males and 117 females *L. vulgaris* and 294 males and 228 females *L. helveticus* in sympatry (69 sampling sites in total).

## Morphology, pattern and spectrometry measurements

All newts were anaesthetized prior to manipulation by immersion in Tricaine methane sulphonate (MS222, 0.2 g/l) for 5 minutes. Using a digital caliper, we measured snout-to-vent length and tail height in both sexes, plus the caudal filament length in male. Each measurement was taken twice and the mean was used in further analyses. For males, we also recorded the extent of the hind-foot web, the shape of the dorsal and caudal crest, the number of dark spots on the belly, and the number and size of the dark spots on the throat and the flank (scoring of the traits detailed Table 1, pictures of males of both species in Figure 1). The morphological variables were all correlated to the body size. For females, we used the residuals of the linear regression of tail height by snout-to-vent length to remove size effects. In males, we had 11 ornamental traits, and we used a Principal Component Analysis (PCA) to reduce the number of variables to a few independent traits, while accounting for size effects.

For coloration, we measured the reflectance spectra between 300 and 700nm of dorsal, ventral and caudal body parts in all individuals. Two measurements were taken for each body part. We averaged and smoothed the reflectance spectra for each individual, with the R package pavo (Maia et al. 2013). Flanks coloration was not measured, as their upper and lower coloration are respectively the same as dorsal and outer (lateral) ventral coloration. However, we quantified the number of dark spots on the flanks, which are sexually dimorphic and are probably a sexual signal (see above). For ventral coloration, we measured the vividly-colored central band of the belly, which are involved in reproductive interactions in newts (Secondi and Théry 2014; Lütke and Foerster 2018). The exact location of the measurements is described in Figure 1 (for the ventral coloration of the



**Figure 1.** Location of the dorsal, caudal and ventral measurements of coloration in *L. vulgaris* (a, c) and *L. helveticus* (b, d). Photos credit: J. Secondi.

Smooth Newt we made sure to measure the vivid coloration between the black spots).

We used a Jaz spectrometer (Ocean Optics), and a coaxial optic fiber (Avantes FCR-7UV200-2-45-ME). The probe was held with an angle of 45°. Reflectance spectra of each body part (Figure S2) are multidimensional traits, and from each of them we were able to extract several variables: the hue in the visible range (401-700nm), the saturation and brightness in the UV range (300-400 nm) and in the visible range separately. We did not describe the full 300-700nm range with only one variable because the spectra were bimodal (Figure S2), and because there is evidence that females use UV to assess males (Secondi and Théry 2014). We did not estimate hue for the back because we observed no peaks in the spectra, and overall a very low reflectance (<6%, see Figure S2). The most appropriate color variables for each part of the spectrum were selected among those described by Montgomerie (2006), and were given the same name. We measured brightness with B2, in UV (B2\_UV) and visible range (B2\_visible); saturation in the UV range using S1 (S1\_UV) and in the visible range using S6 (S6\_visible). For the belly, we chose another saturation index (S9), more appropriate to describe carotenoid-based coloration, which probably applies to newt ventral coloration (Kopecký and Šichtař 2014). We measured hue in the visible range with the H3 variable (H3\_visible). In summary, we extracted five (belly, tail) or four (dorsum) variables describing, in the visible and UV ranges, the background coloration of dorsum and the vivid coloration of central belly and proximal latero-ventral section of the tail.

## **Environmental factors**

We considered both large-scale and local environmental factors that can affect the expression of traits in newts. We extracted 19 bioclimatic variables from WorldClim v2 (Fick and Hijmans 2017), with 1km resolution, and used the first two axes of a PCA to summarize climatic variation among sites. The first axis accounted for 44% of the variance, and separated warm sites with high winter rainfall (negative side) from cold sites with high summer rainfall (positive side; Table S1). The second axis accounted for 24% of the variance, and described a gradient of temperature seasonality (high seasonality for negative values of PC2). We also included altitude (1-km resolution, Danielson and Gesch 2011) in our models. At a local scale, we quantified variation of water transparency by measuring the water transmission spectrum for each pond, and averaging transmission over the whole spectrum (method described in Secondi et al. 2017). Mean values of environmental variables in the sympatric and allopatric zones are presented in Table S2.

## Analysis 1: describing patterns of convergence/divergence

The aim of this first analysis was to detect patterns of divergence and convergence. We used Linear Mixed-effect Models, implemented in 'lme4' R package (Bates et al. 2015). We analyzed males and females separately, and ran one model for each trait. The model (1) included species identity, context (sympatry *versus* allopatry) and their interaction as fixed factors, and sampling site as a random factor.

$$(1) \text{ Trait} \sim \text{species} * \text{context} + \text{sampling site}$$

We investigated homoscedasticity and normality of the traits using diagnostic plots of models' residuals. After log-transformation of several traits to ensure normality (specified in Table S3), we did not find patterns indicating violation of the linear model hypotheses. We also standardized all the traits before analysis (mean-centered and divided by the standard deviation). To quantify convergence/divergence in sympatry, we computed an index for each standardized trait measuring the absolute difference between species mean trait in sympatry minus the absolute difference between species mean trait in allopatry. The index was positive if the trait diverged in sympatry and negative if it converged.

In order to limit type-I errors due to repeated testing of the same hypothesis on independent datasets, we used a cross validation procedure: we randomly divided the full dataset into two non-overlapping sub-datasets (half of the data in each), and we repeated the analysis on each. We only retained the effects that were significant in the global model and in both sub-datasets.

## Analysis 2: Testing the effect of sympatry and environmental factors on trait variation

We assessed the relative contribution of the environment on phenotypic variation in sympatry, and whether the divergence and convergence patterns detected in Analysis 1 held when the environmental variation was accounted for. For each sex and trait separately, we first created a full model, which included the fixed and random factors already present in model (1), plus all environmental variables, and their interaction with species identity (2). This model thus fitted species-specific responses to each environmental variable for each trait.

The full model thus reads:

$$(2) \text{ Trait} \sim \text{species} * (\text{context} + \text{Bioclimatic axis 1} + \text{Bioclimatic axis 2} + \text{altitude} + \text{water transmission}) + \text{sampling site}$$

Next, we applied a backward model selection procedure based on F-tests, a type-III sum of square analysis, with the function step of the 'lmerTest' package (Kuznetsova et al. 2017), in order to reduce the number of predictors/explanatory variables. Only the environmental variables (or interactions) that explained significant amounts of trait variance were kept in the models. Species identity, context, and their interaction were always present in the model.

We performed the same cross-validation procedure as in Analysis 1. Likewise, we checked the validity of each model using the diagnostic plots of the residuals, which did not show any patterns indicating violation of the linear model hypotheses. We also measured the Variance Inflation Factor (VIF) for each model (Table S4). The VIF was low for most variables but high in case of interactions. Because collinearity inflates the standard errors, but does not bias the coefficients, the corresponding tests are less often significant (Morrissey and Ruxton 2018). A high VIF may also indicate overdispersion, but diagnostic plots of residuals did not show any evidence of it. The results presented below are thus robust, but we may have missed some interactions due to a lack of power. Finally, we used Moran's test, using the spdep R package (Bivand et al. 2015), to evaluate the spatial autocorrelation of the residuals.

## **Results**

### **PCA on male morphological traits**

The first three PCs on male ornamental traits accounted for respectively 67%, 9% and 8% of the variance and were retained for further analyses. The first axis separated the two species based on the size of the caudal filament, the shape of the foot webs and the crest, the number and size of spots on the throat, the number of spots on the belly and the size of spots on the flank (Table 1). The second axis separated individuals with few vs many dark spots on the flanks and the third axis separated large individuals with many dark spots on the flanks from small individuals with few spots; these two axes had no obvious biological meaning.



## Phenotypic Evolution in Newts

**Table 1.** Description of the males morphological traits included in Principal Component Analysis, and their correlations with the first three components. In bold the strongest correlation for each axis.

Traits	Description and trait coding	Axis 1	Axis2	Axis 3
Lma	Snout to vent length (mm)	-0.67	-0.34	0.51
Tail	Maximum tail height (mm)	-0.72	-0.36	0.44
Fil	Caudal filament length (mm)	0.87	-0.23	0.15
Webshape	Shape of the web: (0) no web, (1) non-finger-binding web, (2) finger-binding web	0.82	-0.27	0.23
Crestshape	Shape of the dorsal crest: (0) no crest, (1) serrated crest	-0.98	0.09	-0.03
Caudalshape	Shape of the caudal crest: (0) no crest, (1) smooth crest, (2) serrated crest,	-0.95	0.10	-0.01
Throat_number	Density of dark spotting on throat: (0) no points, (1) few spots, (2) many spots	-0.92	0.13	-0.01
Throat_size	Size of dark spots on throat: (0) no points, (1) depigmented, (2) small spots, (3) medium spots, (4) small and large spots, (5) large spots, (6) mottling	-0.90	0.09	-0.03
Belly	Number of dark spots on belly	-0.79	0.22	0.09
Flank_number	Spotting on flanks: (0) no spot, (1) few spots, (2) many spots	0.40	0.70	0.54
Flank_size	Size of dark spots on flank: (0) no spots, (1) small spots, (2) medium spots, (3) large spots, (4) continuous line	-0.80	-0.25	-0.15
Percent of the variance accounted by the PCA		67%	9%	8%

## Patterns of divergence and convergence in sympatry

The results of model (1) indicate that the two *Lissotriton* species differ by many traits (10 male and 6 female traits), most of which presented significant species-by-sympatry interactions (7 for males and 6 for females, model results presented Table S3). Our index of divergence/convergence (Figure 2) indicated the existence of several patterns. Male ornamental traits (summarized as PC1-3) and female morphological traits (SVL, tail height) did not show any signs of convergence or divergence. Dorsal and caudal coloration traits tended to converge in sympatry, while a pattern of divergence was observed for the

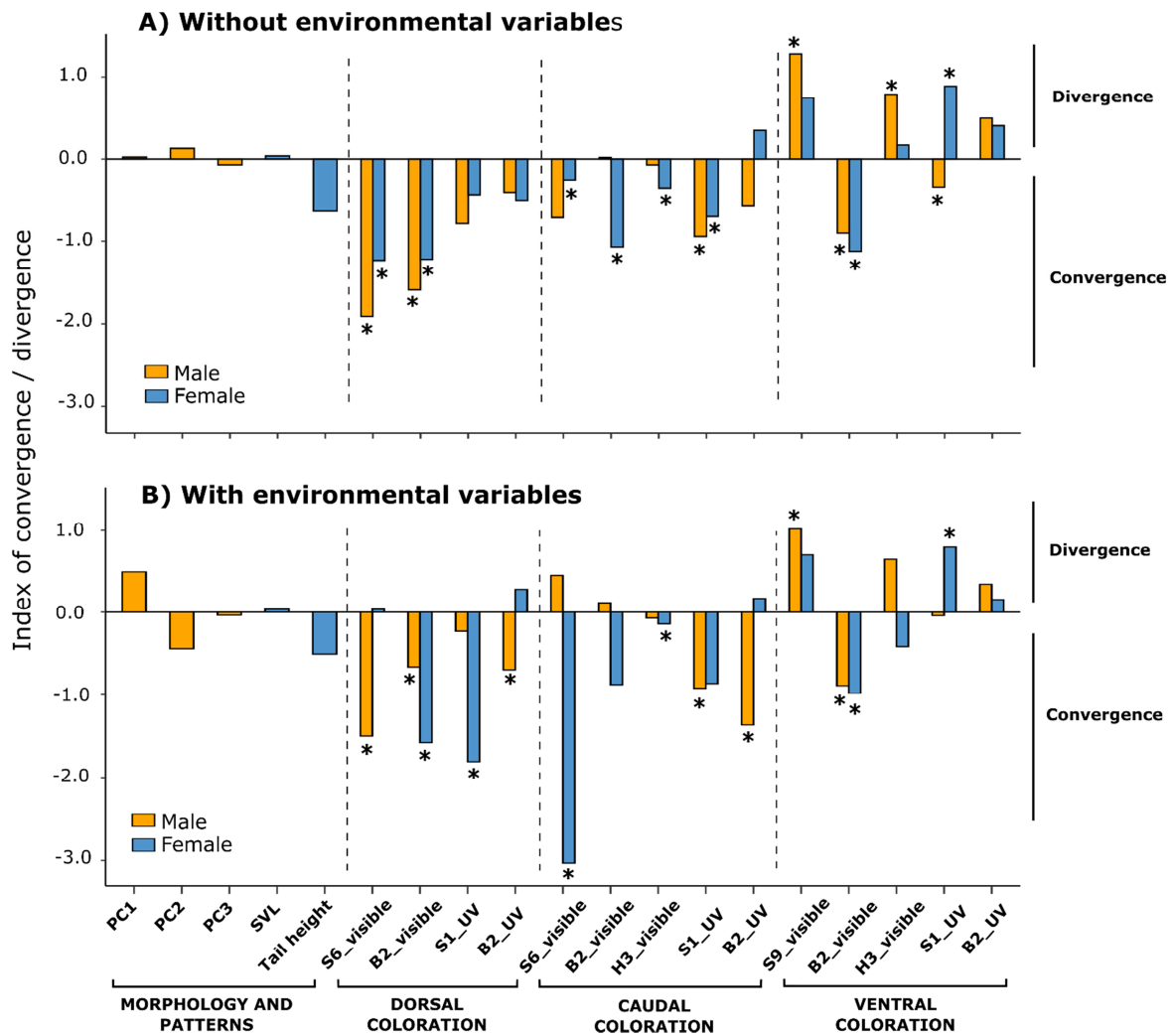
variables describing the coloration of the ventral band (except brightness in the visible range). Overall, males and females showed similar patterns of convergence and divergence.

### **Responses to environmental variables and their impact on divergence and convergence**

Environmental effects may result in convergence (i) when both species react similarly to an environmental variable that takes different values in sympatry and in allopatry or (ii) when species react differently to the same variable in such a way that their difference is decreased in sympatry. For these reasons, the patterns visible in Figure 2 may not reflect an actual response of species' phenotypes to sympatry, but rather to the environment.

Indeed, model (2) revealed many significant effects of environmental variables on male and female traits. Male morphology of both species, including PC1 (which differentiates the two species), was mainly influenced by the two bioclimatic axis (Table S5). The overall coloration of both sexes co-varied with bioclimatic variables and altitude, while water transmission had a generally lesser influence. Focusing on the effects that were retained after cross-validation, and common to both species, colorimetric traits were largely affected by the environment. First, dorsal brightness in the UV-spectrum decreased in cold and wet climates (positive values of climatic axis 1) in females, and in regions with less thermal contrasts between seasons (axis 2) in both sexes. It also increased with altitude in females, as did dorsal saturation and brightness in the visible spectrum. Second, in the ventral parts, saturation in the visible spectrum increased in both males and females as climates became colder/wetter (axis 1) and less seasonal (axis 2). In females, it also increased in sites with clearer waters. Finally, the ventral hue increased with altitude in females.

However, in general, the inclusion of environmental factors did not strongly modify the observations on convergence and divergence made in the first analysis (see Table S5 for the results of model (2) and Figure 2 for the pattern of convergence/divergence after controlling the effects of environmental variables). Results were only modified for four out of the twelve traits converging in the first analysis, as their convergence was entirely explained by common response to environment. For three other traits, significant patterns of convergence appeared only when the environment was taken into account (Table S5). Moran's tests for spatial correlation of the residuals were not significant for any model ( $p > 0.05$ ).



**Figure 2.** Score of the convergence / divergence index for morphological and color variables in males and females of *Lissotriton helveticus* and *L. vulgaris*, obtained from (A) the analysis 1, without inclusion of environmental variables, or (B) the analysis 2, with the environmental variables included. Index = |marginal mean LH sympatry - marginal mean LV sympatry| - |marginal mean LH allopatry - marginal mean LV allopatry| (LH = *L. helveticus*, LV= *L. vulgaris*). For the analysis without environmental variable, the marginal mean were equal the mean value of species trait. Positive values represent species divergence in sympatry, and negative values species convergence. To allow comparison, all traits were standardized. Stars indicate significant interactions between species and context (sympatry vs allopatry) i.e. significant pattern of convergence or divergence.

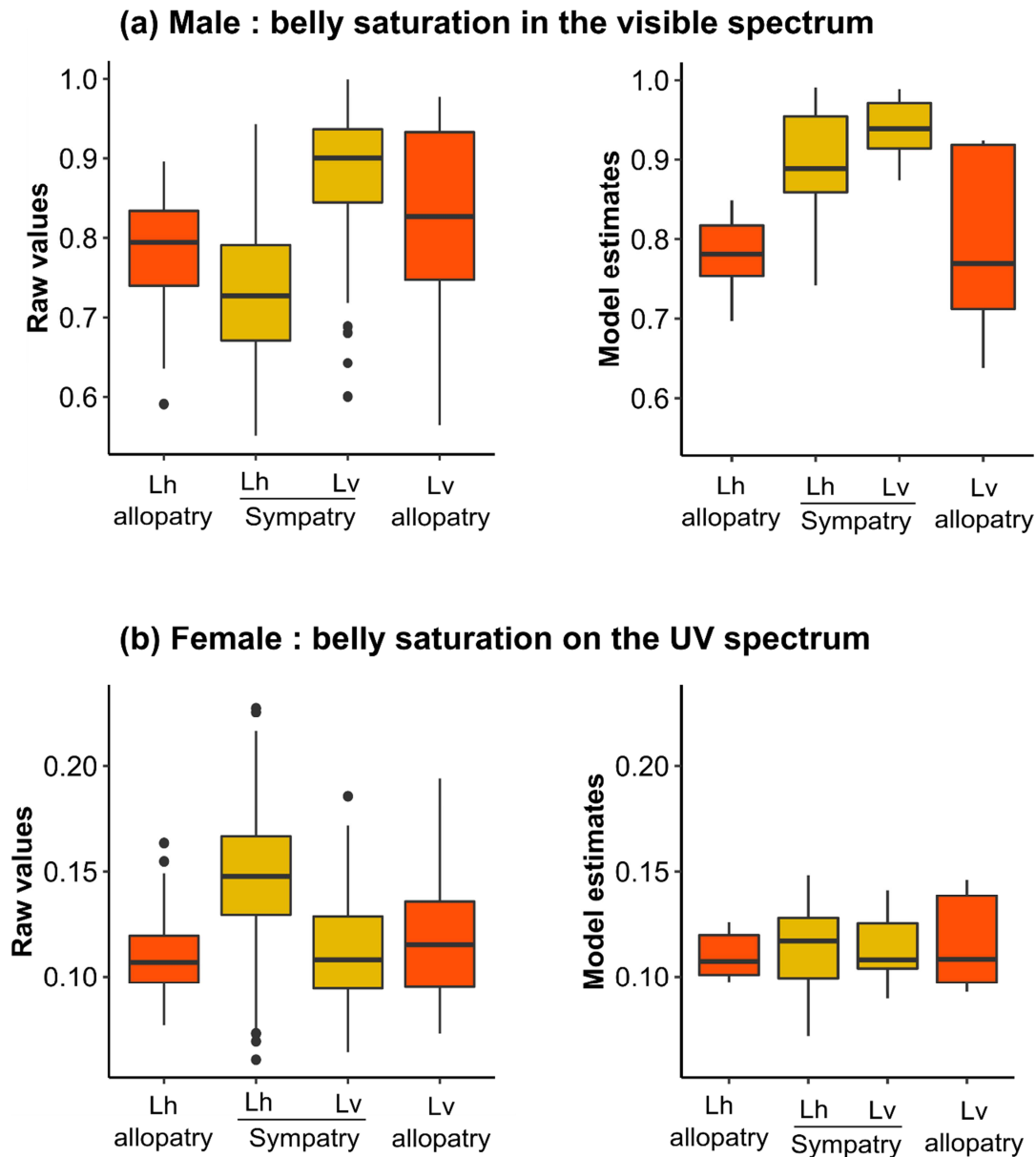
Patterns of divergence were found in the second analysis for some ventral colorimetric variables in both sexes (as in the first analysis not corrected by environmental variables). In Figure 3 we compared, for the two divergent traits, the raw data in sympatry and allopatry to what would be predicted by the environmental variations only. Interestingly, divergence was in both traits the result of a change in *L. helveticus* (Figure 3). For male ventral saturation, sympatric populations of *L. helveticus* populations displayed much less saturated colors than predicted by the environment, resulting in a larger difference with sympatric *L. vulgaris* compared to the difference between the two species in allopatry. In females, ventral UV saturation of *L. helveticus* in sympatry was higher than those of *L. vulgaris*, also resulting in a larger interspecific difference in sympatry, a difference that could not be explained by environmental variations as neither of the two species was affected by the environmental gradients.

## **Discussion**

Our analyses revealed a contrasted pattern of phenotypic variation, with the convergence of dorsal and caudal coloration and the divergence of ventral coloration in sympatric populations of *L. vulgaris* and *L. helveticus*. The divergence of ventral coloration in sympatry was not explained by environmental gradients and thus constitutes a case of divergent character displacement.

### **Environmental effects on newt phenotype**

Our analyses revealed that the phenotype of the two *Lissotriton* species was mainly influenced by large-scale gradients rather than local environmental conditions (water color), in contrast with growing evidence for strong effects of local conditions on phenotypes, in both newts (Secondi et al. 2007) and other taxa (de Abreu et al. 2018; Price et al. 2019). It is possible, however, that water coloration fluctuates too much in time (e.g. reduced transparency after rainfall) to trigger stable plastic and/or evolutionary responses (Sasaki and Ellner 1995).



**Figure 3.** Saturation of ventral body parts in males and females of *Lissotriton helveticus* (Lh) and *L. vulgaris* (Lv) in sympatry and allopatry. See methods for the computation of the variables. The left panel represents the raw color data and the right panel the values predicted from linear mixed models when accounting for the effects of environmental variables and species but without the interaction between the species and the context (sympatry or allopatry). The difference between the two graphs shows how interspecific interactions in sympatry change species coloration (on the left), compared to what is predicted from species-specific responses to the environmental conditions (on the right).

Environmental selection is known to influence the evolution of both sexual and non-sexual traits (Andersson 1982; Endler 1984), leading to phenotypic convergence between sister species (Wilkens 2001) or distantly related taxa (Mahler et al. 2013) exposed to the same conditions. In many vertebrates, dorsal coloration has a critical role for thermal physiology (Clusella-Trullas et al. 2007; Alho et al. 2010) and crypsis (Toledo and Haddad 2009) (Rosenblum 2006; Rudh and Qvarnström 2013), which could explain the influence of climate and altitude on newts' dorsal coloration. We did not however observe a decrease in dorsal brightness in cooler environments (Bogert's rule; Bogert 1949), which would be expected under the thermal adaptation hypothesis and. The crypsis hypothesis thus seems the most plausible to explain this pattern, but we could not directly characterize predation pressure (a daunting task at such continental scale) to test it. For the sexual traits, climatic variables could also correlate with conditions of transmission of sexual signals in the breeding habitat, and variable access to trophic resources leading to differential allocation of resources to sexual traits (Snell-Rood 2012; Macías-Ordóñez, 2013). More generally, we fully acknowledge that climate can co-vary with other biotic or abiotic factors, such as the geographic distribution of some predators. However, the lack of spatial autocorrelation indicates that the effects of climate are not entirely fortuitous and cannot be explained by the broad scale geographic patterns of climatic variation across Europe.

Despite their strong effects on body coloration, environmental variables did not fully account for all the observed convergence of dorsal and caudal colors. It is unlikely that the observed convergence arose from introgression because there is no interspecific gene flow between these two species (Arntzen et al. 1998). Thus, we probably missed some factors, biotic or environmental, affecting newt phenotype and uncorrelated with the environmental variables included in the analysis. Again, predators are good candidates and should be characterized in future studies of this system, as they have been shown to explain patterns of phenotypic convergence in other species (Dennis et al. 2011). In conclusion, while we are not confident that climate and altitude per se affect the phenotype of Smooth and Palmate newts, our clear patterns of common responses to bioclimatic variables and convergence in the zone of sympatry strongly support similar responses of the two species to shared environmental factors. Common garden experiments would be required to establish if these common responses reveal convergent local adaptation or shared plastic responses.

## Evidence for reproductive character displacement in both males and females of *Lissotriton*

We observed clear patterns of divergence in the ventral coloration in both sex. These patterns were maintained after accounting for environmental effects, and thus probably constitute a case of reproductive character displacement (Goldberg and Lande 2006; Adams and Collyer 2007). Generally, RCD is thought to arise either from reproductive interference between species or from selection against hybridization (Pfennig and Pfennig 2009).

In Smooth and Palmate newts, selection for enhanced interspecific recognition and avoidance of hybridization is probably strong in sympatry, as costs of hybridization are high and interspecific recognition is not perfect (Spurway and Callan 1960; Secondi et al. 2010). Hybrids between these two species are extremely rare in nature (Arntzen et al. 1998; unpublished data), but this might mainly be due to strong post-mating barriers, as no back-crosses have been detected (Spurway and Callan 1960; Arntzen et al. 1998; unpublished data). Indeed, males of both species frequently court heterospecific females (Roberts 1990), and male *L. vulgaris* exhibit little avoidance of heterospecific mating in experimental tests (Secondi et al. 2010, 2014). Because interspecific mating results in sterile offspring, females pay a very high cost for mating with a heterospecific male. Similarly, mating attempts are costly in males because of energy and time invested in complex displays, but also because spermatophores used for transferring sperm to the female are limiting (Verrell 1986). Both sexes should thus pay a high cost for interspecific mating, which is expected to select for species discrimination and preference for conspecific mates in sympatry (*i.e.* reinforcement, Dobzhansky 1940) by both females and males. As ventral colors have been found to influence mate choice (Secondi and Théry 2014; Lüdtkke and Foerster 2018), the most likely hypothesis is that divergence of ventral coloration in sympatry indeed reflects selection for enhanced interspecific recognition, even though we did not demonstrate enhanced species recognition and reduced interspecific courtship or mating in sympatry.

Interestingly, RCD was present in both females and males, while it is usually observed in male only (see Dyer et al. 2014). Such pattern could stem from the mutual mate choice observed in newts (Lüdtkke and Foerster 2018). Females frequently reject potential mates, while males do not engage automatically in courtship and often interrupt interactions with females (Roberts 1990). Heterospecific courting is likely to be costly for both sexes as the long and elaborated courtship increases predation risk. In addition sperm is limiting in *Lissotriton* (Halliday 1976), so that wasting gametes has a cost for both females and males. For all these reasons, it is not surprising that males appear to discriminate and

preferentially court homospecific females rather than heterospecific ones, as has been observed in *L. helveticus* (Secondi et al. 2010). In such a context, the evolution of divergent signals in females, facilitating this discrimination, is as likely as in males.

The divergence observed in ventral colors in sympatry, whether in males or in females, was driven by changes in *L. helveticus* only. Asymmetries in prezygotic isolation and RCD have been described multiple taxa (Cooley 2007; Yukilevich 2012; Dyer et al. 2014), and can be caused by either unidirectional hybridization or asymmetrical cost of hybridization (Smadja and Ganem 2005; Lemmon 2009). None of these hypotheses can be excluded for now. The hypothesis of asymmetric hybridization cost arising from different abundances of the two species in the sympatric zone is unlikely, as the more abundant population should pay the lower cost, but in our case, we sampled a higher number of *L. helveticus* in most sympatric sites (24 out of the 33). Further investigations are required to investigate the origin of the asymmetry.

While RCD is expected to affect only traits that participate in sexual signaling, not every trait is expected to exhibit RCD patterns in a given species pair. In Smooth and Palmate newts, not all sexual signals followed the same pattern: caudal coloration converged in sympatry and male morphological ornaments did not show any clear response. Heterogeneous responses of different sexual traits in sympatry has no obvious explanation (and does not need to), however a plausible hypothesis is the fact that some sexual traits are already quite differentiated between the two species in allopatry, thus limiting the potential benefits of further divergence (e.g. no caudal filament and almost no webbing in *L. vulgaris* compared to *L. helveticus*). Even among colorimetric variables describing ventral coloration, some did not diverge (or even converged) and the same hypothesis may apply: the change in only one color component (e.g. the hue) may suffice to dramatically alter the perceived color of the belly. Amphibians are known to be able to discriminate subtle variations of coloration, even in non-optimal environmental conditions (e.g. at night, Gomez et al. 2010), and it is doubtless that the difference in saturation and hue caused by divergence between sympatry and allopatry can be perceived by newts.

## Conclusion

Our study illustrates how the evolution of coloration depends on the balance between opposite selective forces on different parts of the body. In the sympatric zone, similar environments may select for phenotypic convergence while interspecific competition



for resources and mate choice may favor trait divergence. In agreement with the few previous comparable studies (Stuart–Fox and Ord 2004; Gomez and Théry 2007), we found that the balance between these two forces changes with the function of the trait: non-sexual characters involved in thermal regulation or crypsis (*i.e.* dorsal colors) tended to converge in sympatry, while less exposed body parts (*i.e.* ventral colors) involved in mate choice exhibited divergent reproductive character displacement, presumably due to selection for species recognition. Yet, even among traits known to function as sexual signals, only a few underwent RCD, which is not unexpected as mate recognition is often multifactorial and RCD in some signal modalities can be enough to facilitate the recognition of conspecific mates. In addition, some sexual traits are probably less constrained than others, for example the ventral colors that diverged in our study were located in concealed body parts, mostly visible at short range during displays, and thus probably weakly constrained by natural selection. However, many sexual signals are visible under most circumstances (e.g. bright dorsal plumage in northern cardinals Wolfenbarger 1999). Quantifying the relative contribution of natural selection (e.g. thermal constraints, predation risk), sexual selection and selection for interspecific recognition to the evolution of concealed or exposed sexual color signals across groups would thus significantly improve our understanding of the evolution of whole body coloration in animals.

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## References

- Adams, D. C., and M. L. Collyer. 2007. Analysis of character divergence along environmental gradients and other covariates. *Evolution* (N. Y). 61:510–515.
- Alho, J. S., G. Herczeg, F. Söderman, A. Laurila, K. Jönsson, and J. Merilä. 2010. Increasing melanism along a latitudinal gradient in a widespread amphibian: local adaptation, ontogenic or environmental plasticity? *BMC Evol. Biol.* 10:317.
- Andersson, M. 1982. Sexual selection, natural selection and quality advertisement. *Biol. J. Linn. Soc.* 17:375–393.
- Arntzen, J. W., P. Wijer, R. Jehle, E. Smit, and J. Smit. 1998. Rare hybridization and introgression in smooth and palmate newts (Salamandridae: *Triturus vulgaris* and *T. helveticus*). *J. Zool. Syst. Evol. Res.* 36:111–122.
- Babik, W., J. M. Szymura, and J. Rafinski. 2003. Nuclear markers, mitochondrial DNA and male secondary sexual traits variation in a newt hybrid zone (*Triturus vulgaris* x *T. montandoni*). *Mol. Ecol.* 12:1913–1930.
- Bates, D., M. Mächler, B. Bolker, and S. Walker. 2015. Fitting Linear Mixed-Effects Models using lme4. *J. Stat. Softw.* 67:1–48.
- Bergmann, C. 1847. Über die verhältnisse der warmeo-konomie der thiere zu ihrer grosse. *Gottinger Stud.* 3:595–708.
- Bivand, R., M. Altman, L. Anselin, R. Assunção, O. Berke, A. Bernat, and G. Blanchet. 2015. Package ‘spdep.’
- Bogert, C. M. 1949. Thermoregulation in reptiles, a factor in evolution. *Evolution* (N. Y). 3:195–211.
- Chadwick, E. A., F. M. Slater, and S. J. Ormerod. 2006. Inter- and intraspecific differences in climatically mediated phenological change in coexisting *Triturus* species. *Glob. Chang. Biol.* 12:1069–1078.
- Clusella-Trullas, S., J. H. van Wyk, and J. R. Spotila. 2007. Thermal melanism in ectotherms. *J. Therm. Biol.* 32:235–245.
- Cook, E. G., T. G. Murphy, and M. A. Johnson. 2013. Colorful displays signal male quality in a tropical anole lizard. *Naturwissenschaften* 100:993–996.
- Cooley, J. R. 2007. Decoding asymmetries in reproductive character displacement. *Proc. Acad. Nat. Sci. Philadelphia* 156:89–96.
- Cornuau, J. H., D. S. Schmeller, R. Pigeault, A. Sibeaux, A. Tourat, and A. Loyau. 2014. Information-content of morphological and behavioural sexual traits in the Palmate newt (*Lissotriton helveticus*). *Behav. Processes* 108:36–42.
- Danielson, J. J., and D. B. Gesch. 2011. Global multi-resolution terrain elevation data 2010 (GMTED2010).
- de Abreu, F. H. T., J. Schiatti, and M. Anciães. 2018. Spatial and environmental correlates of intraspecific morphological variation in three species of passerine birds from the Purus–Madeira interfluvium, Central Amazonia. *Evol. Ecol.* 32:191–214.
- Delhey, K., J. Dale, M. Valcu, and B. Kempenaers. 2019. Reconciling ecogeographical rules: rainfall and temperature predict global colour variation in the largest bird radiation. *Ecol. Lett.* 22:726–736.
- Dennis, S. R., M. J. Carter, W. T. Hentley, and A. P. Beckerman. 2011. Phenotypic convergence along a gradient of predation risk. *Proc. R. Soc. B Biol. Sci.* 278:1687–1696.

- Dobzhansky, T. 1940. *Genetics and the origin of species*. Columbia University Press.
- Dyer, K. A., B. E. White, J. L. Sztepanacz, E. R. Bewick, and H. D. Rundle. 2014. Reproductive character displacement of epicuticular compounds and their contribution to mate choice in *Drosophila subquinaria* and *Drosophila recens*. *Evolution* (N. Y). 68:1163–1175.
- Elmer, K. R., and A. Meyer. 2011. Adaptation in the age of ecological genomics: Insights from parallelism and convergence.
- Endler, J. A. 1984. Natural and sexual selection on color patterns in poeciliid fishes. Pp. 95–111 in.
- Fick, S. E., and R. J. Hijmans. 2017. WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. *Int. J. Climatol.* 37:4302–4315.
- Gabor, C. R., and T. R. Halliday. 1997. Sequential mate choice by multiply mating smooth newts: females become more choosy. *Behav. Ecol.* 8:162–166.
- Goldberg, E., and R. Lande. 2006. Ecological and reproductive character displacement of an environmental gradient. *Evolution* (N. Y). 60:1344–1357.
- Gomez, D., C. Richardson, T. Lengagne, M. Derex, S. Plenet, P. Joly, J. P. Léna, and M. Théry. 2010. Support for a role of colour vision in mate choice in the nocturnal European treefrog (*Hyla arborea*). *Behaviour* 147:1753–1768.
- Gomez, D., and M. Théry. 2007. Simultaneous crypsis and conspicuousness in color patterns: comparative analysis of a neotropical rainforest bird community. *Am. Nat.* 169:S42–S61.
- Griffiths, R. A. 1986. Feeding niche overlap and food selection in smooth and palmate newts, *Triturus vulgaris* and *T. helveticus*, at a pond in Mid-Wales. *J. Anim. Ecol.* 55:201.
- Grime, J. P. 2006. Trait convergence and trait divergence in herbaceous plant communities: Mechanisms and consequences. *J. Veg. Sci.* 17:255–260.
- Halliday, T. R. 1977. The courtship of european newts: an evolutionary perspective. Pp. 185–232 in *The Reproductive Biology of Amphibians*.
- Halliday, T. R. 1976. The libidinous newt. An analysis of variations in the sexual behaviour of the male smooth newt, *Triturus vulgaris*. *Anim. Behav.* 24:398–414.
- Hill, G. E., and K. J. McGraw. 2006. *Bird coloration Volume 2: function and evolution*. Harvard University Press.
- Jang, Y., Y.-J. Won, and J. Choe. 2009. Convergent and divergent patterns of morphological differentiation provide more evidence for reproductive character displacement in a wood cricket *Gryllus fultoni* (Orthoptera: Gryllidae). *BMC Evol. Biol.* 9:27.
- Johanet, A., J. Secondi, O. Pays, A. Pagano, T. Lodé, and C. Lemaire. 2009. A case of reproductive character displacement in female palmate newts (*Lissotriton helveticus*). *C. R. Biol.* 332:548–557.
- Kawano, K. 2002. Character displacement in giant rhinoceros beetles. *Am. Nat.* 159:255–271.
- Kopecký, O., and J. Šichtař. 2014. Sex differences in ventral side colouration of Alpine newts. *Isr. J. Ecol. Evol.* 60:29–34.
- Kuznetsova, A., P. B. Brockhoff, and R. H. B. Christensen. 2017. lmerTest Package: Tests in Linear Mixed Effects Models. *J. Stat. Softw.* 82.
- Lemmon, E. M. 2009. Diversification of conspecific signals in sympatry: geographic overlap drives multidimensional reproductive character displacement in frogs. *Evolution* (N. Y). 63:1155–1170.
- Losos, J. B. 2011. Convergence, adaptation, and constraint. *Evolution* (N. Y). 65:1827–1840.

- Lüdtke, D. U., and K. Foerster. 2018. Choosy males court both large, colourful females and less colourful but responsive females for longer. *Anim. Behav.* 146:1–11.
- Macías-Ordóñez, R. 2013. Macroecology of Sexual Selection: Large-Scale Influence of Climate on Sexually Selected Traits. *Sex. Sel.* 1–32.
- Mahler, D. L., T. Ingram, L. J. Revell, and J. B. Losos. 2013. Exceptional convergence on the macroevolutionary landscape in island lizard radiations. *Science* 341:292–295.
- Maia, R., C. M. Eliason, P.-P. Bitton, S. M. Doucet, and M. D. Shawkey. 2013. pavo : an R package for the analysis, visualization and organization of spectral data. *Methods Ecol. Evol.* 4:906–913.
- Montgomerie, R. 2006. Analyzing colors. Pp. 90–147 in *Bird Coloration Vol. I: Mechanisms and Measurements*.
- Morrissey, M. B., and G. D. Ruxton. 2018. Multiple regression is not multiple regressions: the meaning of multiple regression and the non-problem of collinearity. *Philos. Theory, Pract. Biol.* 10.
- Nicholson, K. E., L. J. Harmon, and J. B. Losos. 2007. Evolution of Anolis Lizard Dewlap Diversity. *PLoS One* 2:e274.
- Pabijan, M., P. Zieliński, K. Dudek, M. Stuglik, and W. Babik. 2017. Isolation and gene flow in a speciation continuum in newts. *Mol. Phylogenet. Evol.* 116:1–12.
- Pfennig, K. S., and D. W. Pfennig. 2009. Character displacement: ecological and reproductive responses to a common evolutionary problem. *Q. Rev. Biol.* 84:253–276.
- Price, N., S. Green, J. Troscianko, T. Tregenza, and M. Stevens. 2019. Background matching and disruptive coloration as habitat-specific strategies for camouflage. *Sci. Rep.* 9:1–10.
- Roberts, J. 1990. Reproductive isolation in Smooth and Palmate newts (*Triturus vulgaris vulgaris* and *T. helveticus helveticus*). Unpublished PhD thesis, Open University.
- Rosenblum, E. B. 2006. Convergent evolution and divergent selection: lizards at the White Sands ecotone. *Am. Nat.* 167:1–15.
- Rudh, A., and A. Qvarnström. 2013. Adaptive colouration in amphibians. *Semin. Cell Dev. Biol.* 24:553–561.
- Sasaki, A., and S. Ellner. 1995. The evolutionarily stable phenotype distribution in a random environment. *Evolution (N. Y.)* 49:337–350.
- Schluter, D. 2000. Ecological Character Displacement in Adaptive Radiation. *Am. Nat.* 156:S4–S16.
- Secondi, J., A. Aumjaud, O. Pays, S. Boyer, D. Montembault, and D. Violleau. 2007. Water turbidity affects the development of sexual morphology in the palmate newt. *Ethology* 113:711–720.
- Secondi, J., V. Dupont, A. Davranche, N. Mondy, T. Lengagne, and M. Théry. 2017. Variability of surface and underwater nocturnal spectral irradiance with the presence of clouds in urban and peri-urban wetlands. *PLoS One* 12:e0186808.
- Secondi, J., A. Johanet, O. Pays, F. Cazimajou, Z. Djalout, and C. Lemaire. 2010. Olfactory and visual species recognition in newts and their role in hybridization. *Behaviour* 147:1693–1712.
- Secondi, J., V. Lepetz, and M. Théry. 2012. Male attractiveness is influenced by uv wavelengths in a newt species but not in its close relative. *PLoS One* 7:e30391.
- Secondi, J., M. Okassa, S. Sourice, and M. Théry. 2014. Habitat-dependent species recognition in

- hybridizing newts. *Evol. Biol.* 41:71–80.
- Secondi, J., and M. Théry. 2014. An ultraviolet signal generates a conflict between sexual selection and species recognition in a newt. *Behav. Ecol. Sociobiol.* 68:1049–1058.
- Shultz, A. J., and K. J. Burns. 2017. The role of sexual and natural selection in shaping patterns of sexual dichromatism in the largest family of songbirds (Aves: Thraupidae). *Evolution* (N. Y). 71:1061–1074.
- Smadja, C., and G. Ganem. 2005. Asymmetrical reproductive character displacement in the house mouse. *J. Evol. Biol.* 18:1485–1493.
- Snell-Rood, E. C. 2012. The effect of climate on acoustic signals: Does atmospheric sound absorption matter for bird song and bat echolocation? *J. Acoust. Soc. Am.* 131:1650–1658.
- Speybroeck, J., W. Beukema, C. Dufresnes, U. Fritz, D. Jablonski, P. Lymberakis, I. Martinez-Solano, E. Razzetti, M. Vamberger, M. Vences, J. Voros, and P. A. Crochet. 2020. Species list of the European herpetofauna-2020 update by the Taxonomic Committee of the Societas Europaea Herpetologica.
- Spurway, H., and H. Callan. 1960. The vigour and male sterility of hybrids between the species *Triturus vulgaris* and *T. helveticus*. *J. Genet.* 84–116.
- Stuart-Fox, D. M., and T. J. Ord. 2004. Sexual selection, natural selection and the evolution of dimorphic coloration and ornamentation in agamid lizards. *Proc. R. Soc. London. Ser. B Biol. Sci.* 271:2249–2255.
- Toledo, L. F., and C. F. B. Haddad. 2009. Colors and some morphological traits as defensive mechanisms in anurans. *Int. J. Zool.* 2009:1–12.
- Wilkens, H. 2001. Convergent adaptations to cave life in the *Rhamdia Laticauda* catfish group (Pimelodidae, Teleostei). *Environ. Biol. Fishes* 62:251–261.
- Wolfenbarger, L. L. R. 1999. Red coloration of male northern cardinals correlates with mate quality and territory quality. *Behav. Ecol.* 10:80–90.
- Yukilevich, R. 2012. Symmetrical patterns of speciation uniquely support reinforcement in *Drosophila*. *Evolution* (N. Y). 66:1430–1446.

## Annexes: Supplementary Tables and Figures

**Table S1.** Description of the WorldClim bioclimatic variables included in Principal Component Analysis, and their correlations with the first four components. In bold the strongest correlation for each axis.

Bioclimatic variables	Axis 1	Axis 2	Axis 3	Axis4
Annual Mean Temperature	-0.92	-0.11	-0.32	0.00
Mean Temperature of Warmest Quarter	-0.74	-0.54	-0.26	0.12
Mean Temperature of Coldest Quarter	-0.90	0.23	-0.34	-0.06
Annual Precipitation	-0.41	0.54	0.67	0.20
Precipitation of Wettest Month	-0.60	0.10	0.78	0.07
Precipitation of Driest Month	0.67	0.53	-0.21	0.41
Precipitation Seasonality (Coefficient of Variation)	-0.63	-0.45	0.53	-0.20
Precipitation of Wettest Quarter	-0.57	0.21	0.79	0.04
Precipitation of Driest Quarter	0.58	0.62	-0.15	0.41
Precipitation of Warmest Quarter	0.85	0.11	0.39	0.07
Precipitation of Coldest Quarter	-0.76	0.42	0.43	0.14
Mean Diurnal Range (Mean of monthly (max temp - min temp))	-0.28	-0.61	0.05	0.64
Isothermality	-0.70	0.40	-0.09	0.22
Temperature Seasonality (standard deviation *100)	0.47	-0.81	0.20	0.19
Max Temperature of Warmest Month	-0.71	-0.60	-0.20	0.19
Min Temperature of Coldest Month	-0.83	0.36	-0.34	-0.19
Temperature Annual Range	0.31	-0.86	0.19	0.36
Mean Temperature of Wettest Quarter	0.26	-0.49	0.24	-0.59
Mean Temperature of Driest Quarter	-0.89	-0.09	-0.30	0.16
Percent of the variance accounted by the PCA	44%	24%	16%	8%

**Table S2.** Quantitative summary (Mean  $\pm$  Standard Deviation) of environmental variables used in the analysis 2.

Environmental variables	Allopatry	Sympatry	Allopatry
	L. helveticus		L.vulgaris
Altitude	384.42 $\pm$ 362.04	146.08 $\pm$ 93.40	352.60 $\pm$ 120.97
PC1	-2.03 $\pm$ 1.75	1.59 $\pm$ 1.33	3.68 $\pm$ 0.24
PC2	-0.44 $\pm$ 2.55	1.756 $\pm$ 0.89	-2.15 $\pm$ 0.61
Water transmission	89.44 $\pm$ 8.61	85.33 $\pm$ 9.76	77.90 $\pm$ 9.98

**Tables S3:** raw summary of regression coefficients from linear mixed effect models of the analysis 1 (without environmental covariates), made on the full dataset, and the associated values of the sympatric divergence index.

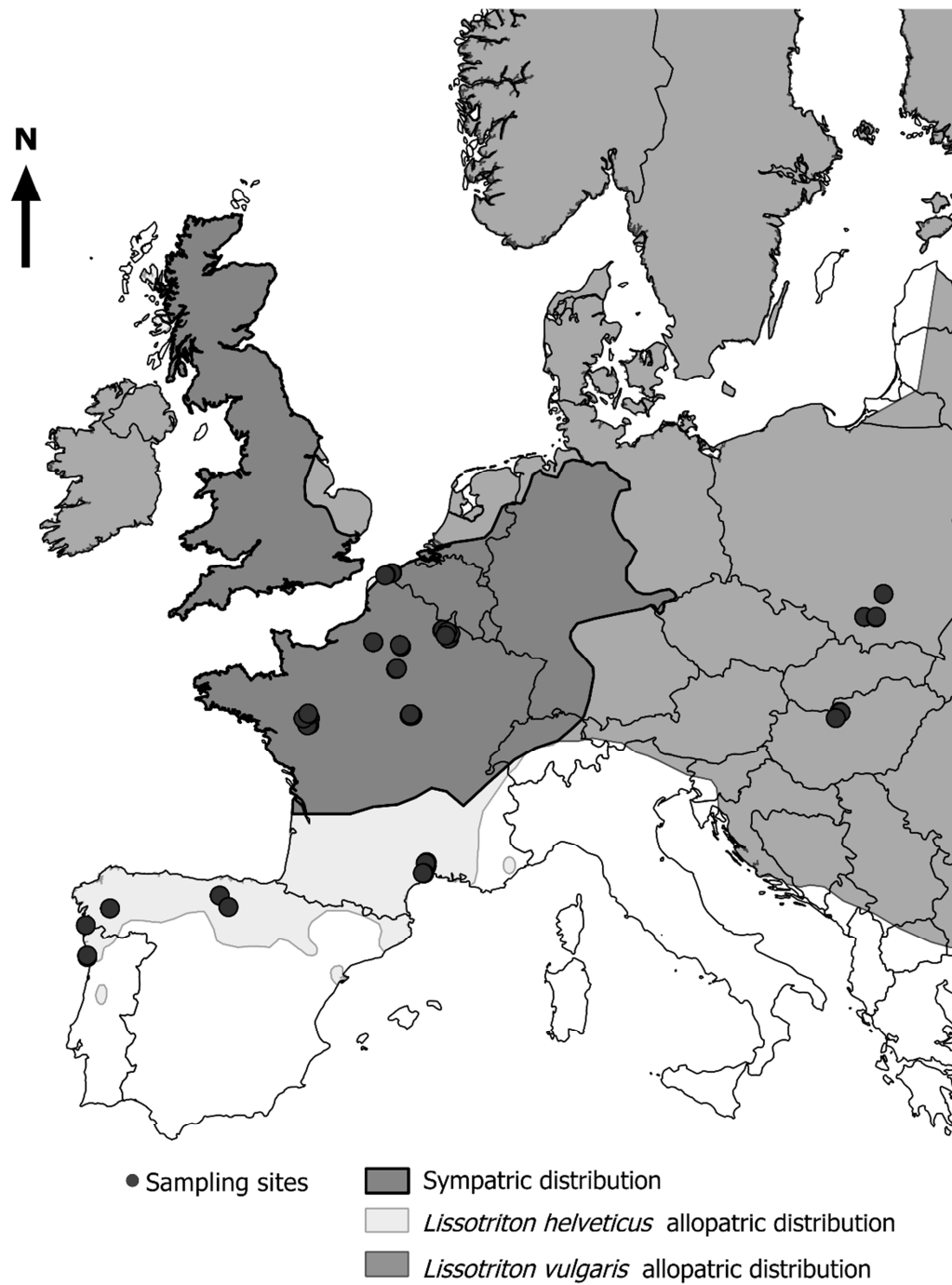
This table is not available in the printed version of the thesis due to its size, but can be found in the file MS1 Intraspecific variations in newts at:  
<https://1drv.ms/f/s!AhWgyBtt5X3FamXDhTlzauD5uXo>

**Tables S4:** Variance Inflation Factor for the linear mixed effect models of the analysis 2 made on the full dataset.

This table is not available in the printed version of the thesis due to its size, but can be found in the file MS1 Intraspecific variations in newts at:  
<https://1drv.ms/f/s!AhWgyBtt5X3FamXDhTlzauD5uXo>

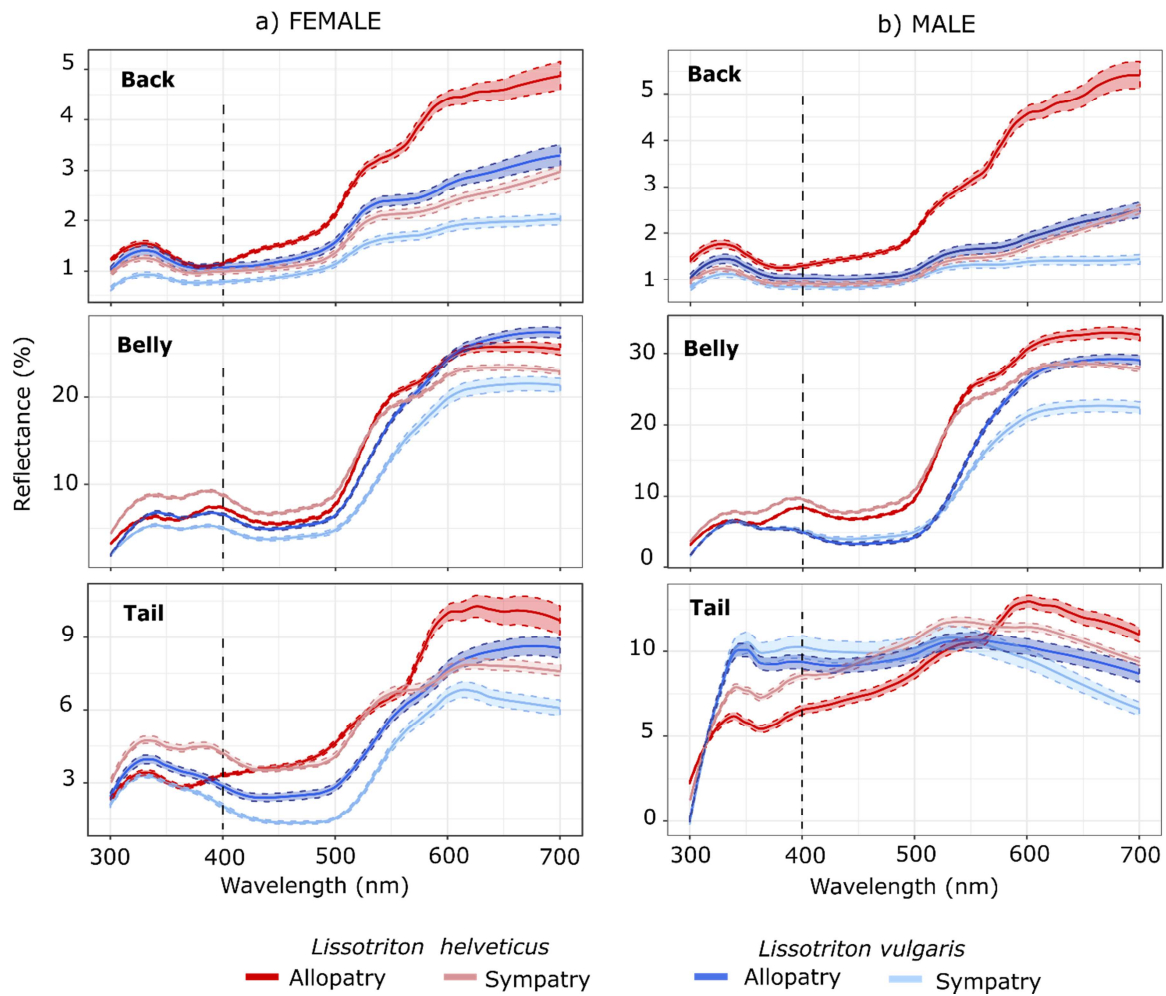
**Tables S5:** raw summary of regression coefficients from linear mixed effect models of the analysis 2 (with environmental covariates), made on the full dataset.

This table is not available in the printed version of the thesis due to its size, but can be found in the file MS1 Intraspecific variations in newts at:  
<https://1drv.ms/f/s!AhWgyBtt5X3FamXDhTlzauD5uXo>



**Figure S1.** Map of the distribution ranges of *Lissotriton helveticus* and *L. vulgaris* with the location of the sites sampled in the study. Species' distributions were obtained from IUCN Red List maps (IUCN 2018).





**Figure S2.** Mean spectral reflectance of female (a) and male (b) dorsal, ventral and caudal patches for *Lissotriton vulgaris* and *Lissotriton helveticus* originating from the sympatric and the allopatric zone. Dashed lines and shaded area represent the standard error around each spectrum. The black vertical dashed lines separate the UV range (left) from the visible range (right).

# **CHAPTER TWO**

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## **Evolution of mimicry**



# Manuscript B

## Looking for mimicry in a snake assemblage using deep learning

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### **Abstract**

Batesian mimicry is a canonical example of evolution by natural selection, popularized by highly colorful species resembling unrelated models with astonishing precision. However, Batesian mimicry could also occur in inconspicuous species and rely on subtle resemblance. Although potentially widespread, such instances have been rarely investigated, such that the real frequency of Batesian mimicry has remained largely unknown. To fill this gap, we developed a new approach using deep learning to quantify visual resemblance between putative mimics and models from photographs. We applied this method to Western Palearctic snakes. Potential nonvenomous mimics were revealed by an excess of resemblance to sympatric venomous snakes compared to random expectations. We found that 8% of the non-venomous species were potential mimics, although they resembled their models imperfectly. This study is the first to quantify the frequency of Batesian mimicry in a whole community of vertebrates, and shows that even concealed species can act as potential models. Our approach should prove useful to detect mimicry in other communities, and more generally it highlights the benefits of deep learning for quantitative studies of phenotypic resemblance.

**Keywords:** Imperfect mimicry, Camouflage, Deep Neural Network, Batesian mimicry.



## **Introduction**

Batesian mimicry occurs when palatable or harmless species evolve resemble to an unpalatable or dangerous model species and consequently gains protection from predators (Bates 1862). Mimicry is a canonical example of evolution through natural selection; it was already discussed by Darwin with Wallace (Darwin 1887) and still constitutes a proliferous field of research (Sherratt 2002; Nishikawa et al. 2015; Joshi et al. 2017; Bosque et al. 2018). Nevertheless, some fundamental aspects of this defensive strategy remain poorly known, notably its frequency in plants and animals, mainly because detecting mimicry is no simple matter.

Research on Batesian visual mimicry has long been confined to a few spectacular cases, like hoverflies (Penney et al. 2012), butterflies (Kitamura and Imafuku 2015) or kingsnakes (Brodie and Janzen 1995), which possess conspicuous colors and show a resemblance to their models so striking that it cannot be fortuitous. This perfection is a double-edged sword: it served as an evidence for Darwinian evolution but it also provided a distorted picture of what mimicry can be. Indeed, neither conspicuousness nor extreme resemblance is a necessary condition for Batesian visual mimicry. Even inconspicuous or camouflaged venomous species may be spotted by predators, which can recognize them using cues that have not necessarily evolved as signals. Harmless and palatable species can mimic these cues to gain additional protection from mimicry while being themselves cryptic (Barlow and Wiens 1977; Mound et al. 2005; Gianoli and Carrasco-Urra 2014; Corcobado et al. 2016). Last, experimental evidence suggests that imperfect resemblance between mimics and models, known as imperfect mimicry, can provide protection against predators (Dittrich et al. 1993). Focusing on canonical examples of striking mimicry using conspicuous signals is thus likely to bias our perception of mimicry and underestimate its frequency.

The detection of visual mimicry within a community requires a quantification of the resemblance between species. In mimicry studies, two main categories of methods have been traditionally used to estimate resemblance. The first methods analyze predefined features, for example morphometric measures, the geometry of color patterns or the distribution of orientations and spatial frequencies in light changes in images (Penney et al. 2012; Taylor et al. 2016; Troscianko et al. 2017; Endler et al. 2018). These methods usually attempt to model physiological perceptual processes and can thus provide information on the role of these processes in the evolution of mimicry. However, they may be unable to capture the high dimensionality of the feature space relevant for assessing visual



resemblance (Zhang et al. 2018). The second category of methods relies on psychological tests with human subjects (e.g., Cuthill and Bennett 1993). These methods can provide more accurate assessments of overall perceptual resemblance; however, extensive tests and very large sample sizes are necessary to buffer the individual variations between evaluators (Ishihara et al. 2001; Kitayama et al. 2003).

Artificial Intelligences, and especially deep convolutional neural networks (hereafter DCNNs), offer new opportunities to model perceptual resemblance (Jozwik et al. 2017; Cichy and Kaiser 2019). DCNNs are a class of artificial neural networks composed by large number (>50) of learnable filters (hence the “deep”) and which use a convolution (i.e. a mathematical operation on two functions that produces a third function expressing how the shape of one is modified by the other) to re-encode the data with which they are fed (Goodfellow et al. 2016). A psychophysical experiment conducted with thousands of humans showed that measures of resemblance between complex stimuli (e.g., pictures of a crowd) by DCNNs predict the average human-perceived resemblance better than any previous models (Zhang et al. 2018). Moreover, DCNNs can estimate resemblance despite noise arising from differences in position, orientation, occlusion and illumination among images (Jozwik et al. 2017). Thus, DCNNs are highly suited to exploit the nearly inexhaustible resource of non-standardized photographs posted on the World Wide Web. DCNNs have revolutionized many scientific fields in recent years, including biology (e.g. in genomics: Zhou and Troyanskaya 2015, medicine: Shen et al. 2017) but the potential of DCNN as a model of human and animal perception, which can handle very large databases of non-standardized data, has been overlooked in visual ecology thus far.

Several cases of Batesian visual mimicry between venomous and non-venomous snakes of the Western Palearctic (Europe, North Africa and the Middle-East) have been reported in literature, sometimes based on experimental or behavioral data but mainly using a human subjective assessment of the visual resemblance between potential model and mimic (Gans 1961; Werner and Frankenberg 1982; Werner 1983; Valkonen et al. 2011a; Aubret and Mangin 2014; Valkonen and Mappes 2014). None of the venomous species in the study area possesses highly conspicuous colors, and most of them are cryptic in their environment (Turk 1958; Heatwole and Davison 1976; Werner 1983). However the zigzag dorsal patterns of vipers has been shown to elicit avoidance in bird predators (Valkonen et al. 2011b) in addition to providing camouflage (Santos et al. 2014). In spite of these case studies, no attempt has been made to evaluate the extent of mimicry in the Western Palearctic snake assemblage. Western Palearctic snakes thus appear as a good model to measure the frequency of mimicry in situations more complicated than most classic

examples described in the literature. Here we incorporated DCNN into a workflow to identify potential mimics among all 122 snakes species known in the Western Palearctic at the time of the study.

To identify candidate mimics, we developed a DCNN method to quantify visual resemblance and combined this information with geographic distributions. We classified as potential mimic any non-venomous species that met two criteria: (i) its resemblance with (at least) one of the venomous species of the West Palearctic was particularly high and (ii) the degree of sympatry with the most-resembling venomous species was higher than expected from a random draw. The literature mentions several species in our assemblage that may be mimetic; we did not incorporate this as prior information, but rather posteriorly compared our results to the literature.

## **Materials and Methods**

We analyzed all 122 snake species of the Western Palearctic (based on Geniez 2015, with the additional species described since), 35 venomous species and 87 non-venomous species (see Table S1 for information on venomousness, the number of images analyzed and the source of distribution data).

### **Image datasets**

We created two image datasets, one for the non-venomous snakes (838 images; mean = 9.6 images per species) and one for the venomous snakes (1802 images; mean = 50.0 per species; details in Table S1). All venomous species exhibit some level of intraspecific variation, which can be geographically structured (and often formally recognized as different subspecies) or be present in most populations as individual variation. Moreover, some species exhibit distinct morphotypes that can be easily identified by humans (see Figure S1 for examples of different morphotypes in *Vipera aspis*). We did not treat these conspecific morphotypes as distinct classes. As DCNNs can solve nonlinear problems, we expected the networks to learn that these different morphotypes belong to a single species. However, in general, classes with high variation are more difficult to recognize for DCNNs (e.g. Fuentes et al. 2018); we thus used more images for venomous species with several morphotypes (up to

84 images per species, see Table S1). All images represent different individuals except for a few species for which only a few images were available.

In addition to the 35 species of Western Palearctic venomous snakes (each representing a distinct class of pictures), we added one class of “foreign” venomous snakes to the venomous dataset. This class was made of 80 photographs of 80 different species living outside the Western Palearctic but in climates similar to those of the study area, i.e. temperate and desert (list of the species used in Table S1)

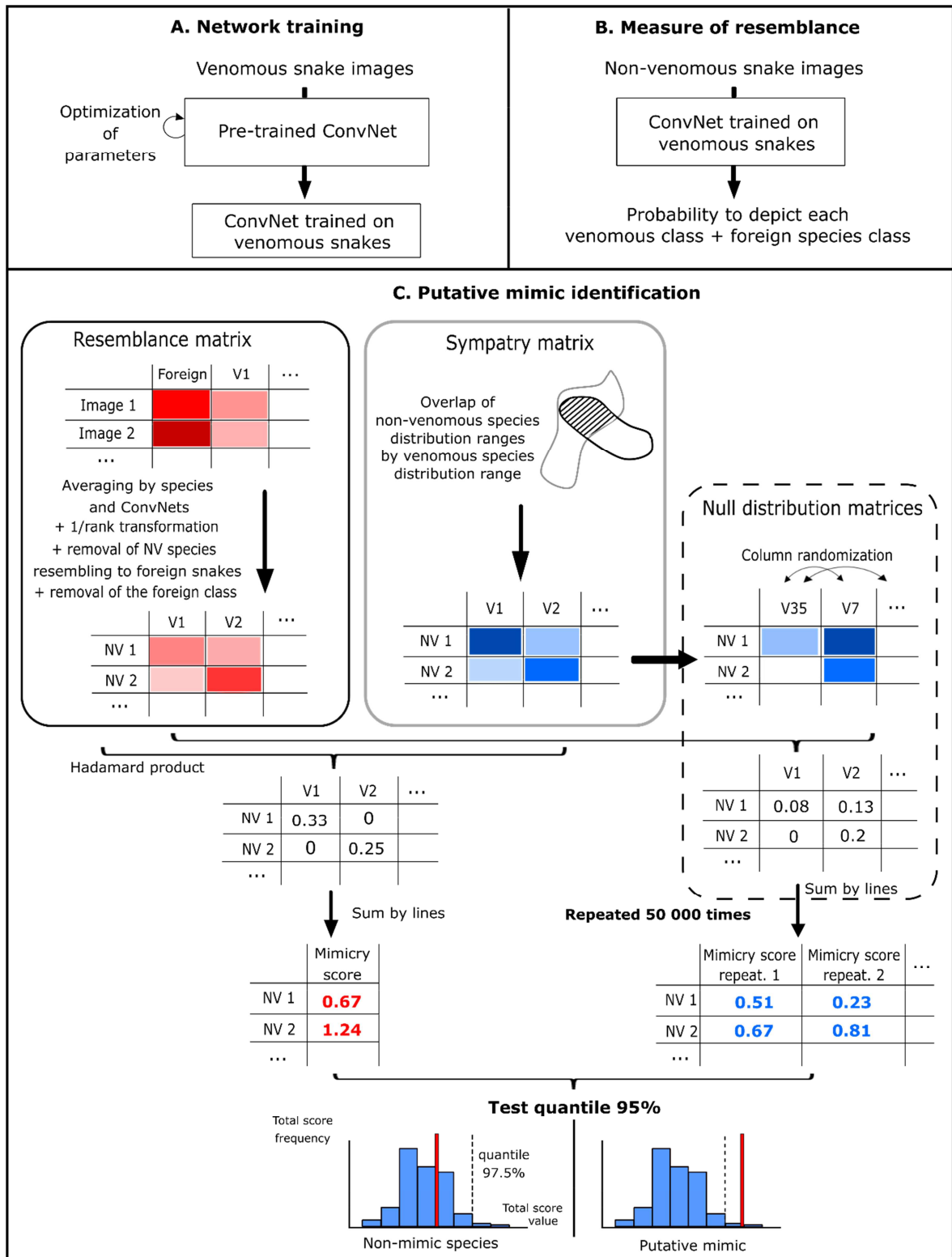
We selected only images showing a clear dorsal view of the whole specimen (see Figure S1 for examples). Eighty percent of the images were collected on the Internet, the remaining being taken in the field by the authors or colleagues. An expert herpetologist (PG) validated all identifications individually. Because images are rare for several of the studied species, our datasets may appear small compared to those of other studies using DCNNs. However, recent investigations revealed that, with an appropriate training (see *transfer learning* below), DCNNs can reach very high performances even when trained on limited datasets (Körschens et al. 2018). This is confirmed by our results (see below).

### Geographic distribution dataset

We constructed distribution ranges from three types of data: (1) when available, we used distribution maps downloaded from the IUCN Red List website ([www.iucnredlist.org](http://www.iucnredlist.org), accessed on February 2018); (2) otherwise, we reconstructed by hand the spatialized distribution maps from pictures of distribution ranges found on the <http://apps.who.int/bloodproducts/snakeantivenoms/database/> website and from other specialized literature; (3) when no distribution map was available, we built maps from occurrence data found in the specialized literature (references listed in Table S1). We used QGIS 2.18 (Quantum GIS Development Team 2013) to create the distribution maps. Each distribution map was depicted by a spatialized polygon projected in WGS 84.

### Methodological workflow

DCNNs were first trained to recognize the different venomous species of the Western Palearctic plus the “foreign” class (Figure 1A). Trained DCNNs were then asked to classify pictures of non-venomous snakes into one of the venomous species or into the “foreign” category (Figure 1B).



**Figure 1.** Workflow of the three-step method used to detect candidate mimic species among non-venomous snakes and candidate model species among venomous ones. (A) Training of the artificial neural networks to recognize 35 venomous snake species plus the “foreign” absolute measure of resemblance but instead a measure of the relative resemblance with each species of venomous snakes plus the “foreign” class. (B) Measuring the resemblance of non-venomous snakes (one measure per image) with each species of venomous snakes plus the “foreign” class. (C) Building the resemblance matrix and the sympatry matrix, and testing an excess of resemblance between sympatric species using a null distribution to identify candidate mimics.

limit the risk of picking up a “most resembling venomous snake” even if the image does not particularly resemble any of the venomous snakes (Dalyac et al. 2014). The “foreign” class, which groups together a high diversity of patterns, is expected to act as an attractor for such nonvenomous species. Thus, we are confident that most non-venomous species consistently identified by the DCNN as one of the “non-foreign” venomous species do show a true resemblance.

The mean attribution probability (averaged across all available pictures) of each non-venomous species to each venomous species was then used to build a matrix of resemblance between non-venomous and venomous species. These probabilities were transformed into resemblance scores, i.e. in inversed ranks of probability ( $1/\text{rank}$ , in ascending order; Figure 1C). Thus, the classes of venomous snakes to which one particular nonvenomous snake was attributed most often, i.e. ranking first or second, had the highest resemblance score. This step allows to reduce the prediction variability arising from the use of different training algorithms (Sameen and Pradhan 2017; Kim et al. 2019) and prevents overconfident predictions (Ju et al. 2018). It also increases the match between measure of resemblance by DCNNs and the perceptual space of animals (see Zhang et al. 2018 for work on human). Next, we deleted from this matrix the foreign venomous species class, as well as all non-venomous species for which the “foreign” class received the highest resemblance score, thus discarding species that were clearly not mimics (60 non-venomous species discarded). In parallel, we built a sympatry matrix of the same size as the resemblance matrix, which gives the proportion of the geographic distribution of each non-venomous species that overlaps with the distribution of each venomous species. Following a previous study (Miller et al. 2019), we considered that the proportion of sympatry reflected the potential extent of selection for mimicry. Even if a few allopatric species pairs have distribution ranges close enough to allow predators to associate both snake species, most allopatric pairs are distant by hundreds or thousands of kilometers, which limits this possibility (Pfennig and Mullen 2010). The last step of our workflow was to calculate “total scores” by summing, for each non-venomous species, the resemblance scores weighted by sympatry scores (Hadamard matrix product).

Candidate mimics and models were identified statistically by comparing total scores to a null distribution of “randomized total scores”. This null distribution was generated by randomly shuffling the venomous species in the sympatry matrix before weighting by the resemblance score. The weighted resemblance scores were then summed for each non-venomous species to obtain the randomized total score. We replicated this operation 50,000 times (Figure 1C). Species for which the total scores fell in the right tail (e.g., the 5%

highest scores) of the null distribution were more similar to sympatric venomous species than expected by chance and were thus considered candidate mimics.

Since we tested the same hypothesis of an excess of resemblance multiple times, the chance of false discovery (i.e. significant result arising by chance) also increased. We used the Fisher's method, also named Fisher's combined probability test (Fisher 1925), to test if the  $p$ -values of the individual mimicry tests (estimated as the proportion of randomized scores higher or equal to the real score) were distributed according to a uniform distribution, or presented an excess of low values, indicating a significant excess of resemblance with the sympatric venomous snakes. We combined the  $p$ -values of the 27 non-venomous species (out of 87) that were not eliminated as being classified into the "foreign" class into a  $\chi^2$  statistic, and compared it to a  $\chi^2$  distribution with  $2 \times 27$  degrees of freedom. This effectively provides a global test of the hypothesis that mimicry affected the evolution of phenotype in the Western Palearctic snake assemblage. All statistical analyses were performed with R (Development Core Team 2008).

Our method did not include phylogenetic correction for the measure of the resemblance because all the venomous and non-venomous snakes belong to two distant and monophyletic clades. The resemblance between these clades thus cannot be attributed to phylogenetic relationship (see discussion).

## **DCNN training**

Training a DCNN to recognize snake species from scratch would have required a very large dataset of images to achieve good performances. Following other studies (e.g., Norouzzadeh et al. 2018), we used "transfer learning" in which a DCNN pre-trained on a very large dataset is fine-tuned with a new, smaller dataset. All details about the structure and the training of DCNNs are available in the Supplementary Methods. All the codes are available at [https://github.com/t-desolan/Mimicry\\_snakes](https://github.com/t-desolan/Mimicry_snakes). The images used for training and testing can be provided upon request.

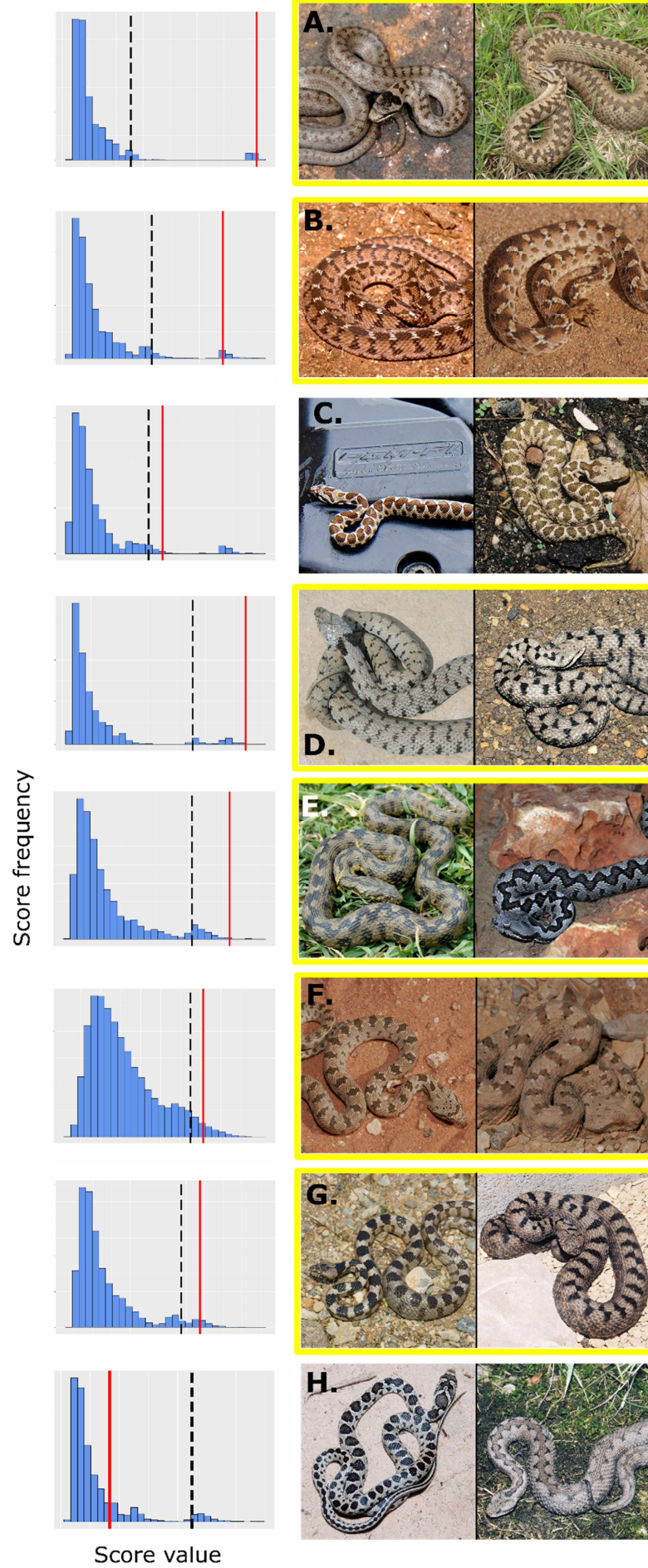
## **Test of background influence**

To confirm that network predictions were based on the snakes and not on the background, snakes were cut out in 60 photos and pasted onto photos of ground only. Ground photos

were similar to backgrounds found in snake photos, and depicted ground with rock, dead leaves, plants or sand. Of the 60 snake images, 20 belonged to venomous snakes, 20 to non-venomous snakes previously identified as mimetic and 20 to non-venomous snakes previously identified as non-mimetic. Then, we used non-parametric Multivariate Analysis Of Variance, with adonis function of vegan package (Dixon 2003) in R, to test the effect of background replacement on resemblance scores. We controlled variation between species by entering species names as an explanatory variable. We checked for multivariate homoscedasticity using the betadisper function of vegan package (non-significant, effect of background:  $F = 0.15$ ,  $p = 0.69$ ).

**Figure 2.** The seven mimic and model species identified in our analysis (A-G) and one example pair of sympatric but non-resembling species (H), with mimicry scores (red), quantiles 95% (black dashed line) and the null distribution (blue) of each species pair. Species framed in yellow have been previously reported as mimetic in the literature (Gans 1961; Werner and Frankenberg 1982; Werner 1983; Valkonen et al. 2011a; Aubret and Mangin 2014). (A) *Coronella austriaca* – *Vipera berus* ( $p$ -value=0.004). (B) *Dasypeltis sahelensis* – *Echis pyramidum* ( $p$ -value=0.02). (C) *Hemorrhois nummifer* – *Montivipera xanthine* ( $p$ -value=0.04). (D) *Natrix helvetica* – *V. aspis* ( $p$ -value=0.003). (E) *N. maura* – *V. latastei* ( $p$ -value=0.004). (F) *Spalerosophis diadema* – *Pseudocerastes fieldi* ( $p$ -value=0.02). (G) *Telescopus fallax* – *V. ammodytes* ( $p$ -value=0.02). (H) *H. hippocrepis* – *V. latastei* ( $p$ -value=0.18). Photo credits: Matthieu Berroneau, Olivier Buisson, Luis García Cardenete, Alexandre Cluchier, Philippe Geniez, André Langenbach, Jean Muratet, Gilles Pottier and Jean-François Trape.







## **Results**

After training, DCNNs were able to recognize the species of a Western Palearctic venomous snake with an accuracy of  $77\% \pm 0.004$  SE (percent of pictures correctly classified, evaluated on the validation dataset; random guess: 2.78% accuracy) and a top-3 accuracy of  $92\% \pm 0.015$  SE (probability that one of the three classes with the highest prediction matches the correct class), indicating that DCNNs learned the most distinctive traits of each species (precision per class detailed in Figure S2).

Comparing weighted resemblance scores to the null distribution, with a 5% threshold, our method identified seven non-venomous species out of the 87 (8%) as candidate mimics of venomous snakes: these species have a higher resemblance with their sympatric venomous species than with a random assemblage of venomous snakes. The list of potential mimics and models are given in Figure 2 and with more details in Figure S3. Fisher's method confirmed the overall significance of excessive resemblance with sympatric venomous snakes across the tested non-venomous species set ( $\chi^2 = 109.3$ ,  $p < 0.001$ ). In addition, resemblance scores were not driven by similarity between image backgrounds, as they were not affected by shuffling backgrounds among images (non-parametric MANOVA performed with 60 images, background effect:  $F = 0.74$ ,  $p = 0.67$ ).

## **Discussion**

As far as we know, we provide here the first attempt to quantify the extent of mimicry in an assemblage of vertebrates at a continental scale (and we know of only one similar study in invertebrates: Wilson et al. 2015). Although the hypothesis of mimicry would have to be validated experimentally for each particular species pair, our analysis indicates the existence in Western Palearctic snakes of a pattern typical of Batesian mimicry (i.e. unusually high resemblance between sympatric pairs of nontoxic and toxic preys) that influenced the phenotypic evolution of this assemblage. The visual comparison of models and mimics suggests that their phenotypic resemblance is based on coloration and pattern (Figure 2 and S2).

Venomous and non-venomous species of snakes in our community belong to different families so relatedness cannot explain this pattern and mimicry itself probably evolved at least six times, as all but two of the candidate mimetic species belong to different

genera (the exceptions are the two *Natrix* species; see Figure S4 for the phylogenetic trees of the venomous and non-venomous species).

### **Are our candidate mimic species really mimicking venomous snakes?**

Several arguments from the natural history of snakes support the validity of our method to identify mimics and models. Firstly, six of the seven species identified as potential mimics by our method have been mentioned as such in the literature (Gans 1961; Werner and Frankenberg 1982; Werner 1983; Valkonen et al. 2011a; Aubret and Mangin 2014; see Figure 2). Secondly, the mimicry hypothesis is supported for all the detected species by additional evidence, based on behavioral or/and acoustic traits. The putative mimics *Natrix helvetica*, *N. maura*, *Coronella austriaca*, *Spalerosophis diadema* and *Telescopus fallax* displace their maxillary bones to produce a triangular head shape when disturbed, increasing visual resemblance with their viper model (Werner and Frankenberg 1982; Werner 1983; Valkonen et al. 2011a; Aubret and Mangin 2014). In addition, *Natrix maura* also produces a hissing sound similar to the sympatric *Vipera aspis* (Aubret and Mangin 2014) while the non-venomous *Dasypeltis sahelensis* and its sympatric venomous *Echis pyramidum* possess the same modified scales on the flanks that are rubbed to produce a similar warning signal when disturbed (Gans 1961). Finally, even if it has not been reported yet as a potential mimic in the literature, *Hemorrhoids nummifer* shows the same behavioral and acoustic warning signal as the sympatric vipers *Montivipera xanthina* and *Macrovipera lebetina* (authors' personal observation).

Although additional cues support the mimicry hypothesis for all detected species, our method can only suggest candidate mimics and a proper demonstration of mimicry would require experimental data. Indeed, most studies of interspecific resemblance are fundamentally limited in their ability to distinguish between mimicry and other factors of resemblance. Resemblance could arise by ecological convergence because of similar abiotic environmental constraints or similar selective pressures for camouflage (Rajpurohit et al. 2008; Stevens et al. 2013). These factors could lead to overestimating the frequency of mimicry in our results. This issue potentially affects all mimicry studies that do not test predator behavior in a natural environment. However, because most studies focus on conspicuously colored and presumably aposematic species (Brodie and Janzen 1995; Cheney and Marshall 2009; Penney et al. 2012) researchers usually assume that resemblance can only be attributed to mimicry. This assumption is seldom tested even if conspicuous colors

may have camouflage function (Mochida et al. 2015; Barnett et al. 2018). In Western Palearctic snakes, body colors are not conspicuous. It would thus be interesting to perform predation tests in natural conditions to investigate whether the features copied by potential mimics are those effectively used by predators to avoid models (e.g., as in Kristiansen et al. 2018). Nevertheless, as discussed previously, all identified mimics use behavioral or acoustic imitation in addition to color pattern and, as resemblance in several ontogenetically independent traits can hardly be attributed to ecological convergence, to our view mimicry remains the most likely explanation.

### **DCNNs can identify imperfect mimicry of camouflaged models**

Remarkably, we found that even well camouflaged species (e.g., vipers of the genus *Pseudocerastes*; Figure 2) could be a model for non-venomous species. The lack of any pattern or color signaling venomousness in this species confirms that mimicry can be effective without conspicuous traits, and advocate for a better separation between the concepts of Batesian mimicry, communication signaling and conspicuousness. The link between these three concepts seems to represent a historical legacy of the easier detection of mimicry when it involves conspicuous aposematic models. The method presented here, which uses DCNN-based resemblance coupled with information on geographic co-occurrence, avoids a biased selection favoring conspicuous organisms when studying Batesian mimicry.

An additional advantage of our DCNN-based approach is its ability to suggest mimicry even when resemblance is imperfect. Most of the species identified by our method are imperfect mimics, at least from our human point of view, as their colors and patterns match only partially those of their model (Figure 2). Although any of the eleven non-exclusive hypotheses proposed in the literature to explain imperfect mimicry (see Kikuchi and Pfennig 2013; Dalziell and Welbergen 2016 for a detailed list) could apply to Western Palearctic snakes, only the “back-up signal” hypothesis is currently supported by ecological evidence. This hypothesis suggests that imperfect mimicry can be reinforced by complementary signals (Johnstone 1996). It is convincing especially for mimics that show similarities in behavioral, morphological and acoustic warning signals with their sympatric venomous species (Gans 1961; Werner 1983; Valkonen et al. 2011a; Aubret and Mangin 2014). In addition, variability of models could help to maintain imperfect mimicry it may prevent predators from associating a specific phenotype to venomousness (Kikuchi and

Pfennig 2010) and thus make them more careful and less likely to attack snakes with imperfectly matching phenotypes. This explanation is plausible at least for the three candidate models *Vipera ammodytes*, *V. berus* and *V. aspis*, which exhibit significant intraspecific and often intra-population variation in coloration (Figure S1). Finally, mimicry is a matter of receiver perception. A poor mimicry for our vision system may be better for another vision system. The different snakes' predators are likely to display different visual systems (e.g. bird and mammals), each shaping the evolution of mimicry in different ways.

### **Performance of the method to identify putative mimics**

The ability to detect imperfect and non-conspicuous mimicry makes our method less conservative compared to methods entirely based on human detection, for evaluating the frequency of mimicry. Even so, we may have failed to identify potential mimic-model pairs. Firstly, the 5<sup>th</sup> percentile threshold in the null distribution used to interpret resemblance scores is entirely conventional and may overlook some mimicry systems. We found two species below but close to the 5<sup>th</sup> percentile threshold. The first species, *Dolichophis jugularis*, displays the same black coloration as its sympatric species *Walterinnesia morgani*, but it has a different timing of activity (one is diurnal while the other is nocturnal) and is thus probably not a mimic. For the second species, *Rhagerhis moilensis*, coloration mimicry of the sympatric *Cerastes cerastes* cannot be discarded and should be subjected to further examination. Therefore, the 5<sup>th</sup> percentile threshold should be interpreted cautiously and we recommend not drawing firm conclusions without examining species close to a decision threshold. Secondly, we may have failed to identify candidate mimics and models because, for the non-venomous polymorphic species (e.g. Leviton 1970; Kark et al. 1997; Mebert 2011), we could not test mimicry for each morphotype separately. Instead, we had to analyze all morphotypes together, and test mimicry at the species level. This complicates the detection of mimicry as only some morphotypes of these polymorphic species may mimic venomous snakes (e.g., in *Hemorrhhis ravergeri*, Werner and Frankenberg 1982, see below). Testing mimicry for each morphotype would require mapping the geographical distribution of each morphotype, known but this information was not available for most polymorphic species. Thirdly, DCNNs cannot detect resemblance from information not present in the data: mimicry based on UV coloration (see next paragraph), behavior, acoustic or olfactive signals will not be identified. These three limits may lead to an under-estimation of the mimicry frequency in natural communities.

Among the species suggested as mimetic in literature, three were not identified in our study (Gans 1961; Werner and Frankenberg 1982; Werner 1983). One of them, *Hemorrhois ravergeri* is considered a mimic of the sympatric *Vipera ammodytes transcaucasiana* (Werner and Frankenberg 1982). Our method failed to identify it as such probably because it exhibits a strong polymorphism, with only some of its variants thought to mimic vipers (Werner and Frankenberg 1982). A second species, *Dasypeltis bazi*, could have been missed by our approach because of the limited number of pictures in our dataset (six in total, this rare and poorly-known species was recently described by Saleh and Sarhan 2016) and the marked variation in coloration between individuals. However, the third unrecognized mimic, *Telescopus dhara* (Werner 1983), is not polymorphic, and we believe that claims for mimicry in this species are too optimistic, as it bears no particular resemblance with the proposed model *Echis coloratus* from visual inspection.

### Use of DCNNs to assess phenotypic resemblance

Several lines of evidence indicate that our method provides accurate estimation of resemblance. Firstly, the performance of DCNNs in image classification were remarkably high. It is similar or superior to that of other studies (e.g., Carranza-Rojas et al. 2017; Van Horn et al. 2018), despite a limited size of the training dataset and the extreme resemblance among several vipers that makes their identification challenging when the geographic origin is unknown, even for experts (Geniez 2015). In comparison, a previous DCNN-based analysis of species recognition based on 579,184 unstandardized training images representing 5098 species reached 68,5% and 88,2% top-1 and top-5 accuracy, respectively (Van Horn et al. 2018). Thus, our analysis confirms previous researches in demonstrating that DNNs can reach high level of accuracy even when trained on small datasets (Hansen et al. 2018; Körschens et al. 2018; Mathis et al. 2018). Secondly, we avoided “by-default” classification, as more than two-third of the non-venomous species were identified as a foreign species class and removed in further analysis. These results support the idea that DCNNs can be effective tools to measure phenotypic resemblance.

Our study highlights the benefits of DCNNs for studying resemblance between phenotypes in ecology and evolution. DCNNs have been previously used in ecology to analyze big data, e.g., to identify species from camera trap images (Norouzzadeh et al. 2018). However, their capacity to model biological perception has been thus far overlooked (Cichy and Kaiser 2019). A growing body of literature in humans has shown that DCNNs

predict the perceived resemblance between complex stimuli better than other models, even those specifically tuned with neurophysiological data (Yamins et al. 2014; Kriegeskorte 2015; Kubiľius et al. 2016). Of particular relevance for the study of camouflage and mimicry, one study showed that whichever perceptual task a DCNN is trained for (e.g., recognizing objects from images, segmenting foreground in video frames), DCNN accurately predicts perceptual similarity even near the discrimination threshold (Zhang et al. 2018).

In mimicry systems (and more generally in most communication systems) the relevant observer is not human. Are the performances of DCNNs at reproducing human perception also relevant to other animals? These performances are based on three characteristics (Kriegeskorte 2015). The first one, the capacity of DCNNs to solve high-level tasks (Zhang et al. 2018), is shared by all animals. The second is their general architecture (a stack of convolution, activation and pooling functions) which reproduces operations performed sequentially and iteratively by neurons in the different human visual areas (Güçlü and van Gerven 2015). Similar operations are also found, beyond humans, in at least all vertebrates (Renoult et al. 2019). The third is the structure of input data. In this study, we used RGB images that match human trichromatic vision (i.e. based on three cone types). Other animals have different cone types (e.g., two in carnivores, four in birds), tuned to different wavelengths. How these differences affect DCNN models of perceptual space is a nearly unexplored avenue of research (but see Bergeron and Fuller 2018). Yet, indirect evidence suggests that the impact would be relatively minor. Indeed, studies on bird plumages that used both RGB images and bird-specific cone stimulations showed no qualitative differences (Dale et al. 2015; Miller et al. 2019). Accordingly, several authors have argued that RGB images provide good insight into ecology, evolution, and behavior in nonhuman species (Seddon et al. 2010; Bergeron and Fuller 2018). In addition, in our particular case, the use of ranks to express relative resemblance makes our mimicry-detection method insensitive to how precisely the similarity measure produced by DCNNs scales to similarity perceived by animals.

A potentially important limitation of RGB images, however, is to eliminate wavebands to which humans –but not other vertebrates– are blind. The ultraviolets (UV), in particular, may be problematic. Regarding our study, none of the main predators of snakes in the Western Palearctic use UV cues for foraging (Jacobs 2009; Lind et al. 2013, 2014). Mammalian predators are not able to see in UV because they do not have UV cones (Jacobs 2009). Raptors have UV cones, but previous suggestion that they use UV cues to detect preys (Viitala et al. 1995) is contentious as their ocular media only transmits UV poorly (Lind et al 2013). Thus, UV-based mimicry seems unlikely in Western Palearctic snakes.

Last, we analyzed images with minimum standardization (images were cropped to center the snake, but not standardized for luminance, position or background). Centering was necessary because of the small size of our training dataset, but it could be omitted if a sufficient amount of images were available. Although standardization removes irrelevant information from the data, we think that non-standardized images are ecologically more relevant. Indeed, predators experience substantial variation in perceptual conditions, in relation to snake position, background, shading, light intensity and color. This variation could affect the evolution of mimic phenotypes; for example, it has been suggested to explain imperfect mimicry (Kikuchi and Pfennig 2013). While RGB pictures from the Internet may not precisely match variation experienced by predators, drastically limiting all variation (by standardization) would make the dataset un-natural. Standardized images would be nevertheless useful in other contexts, e.g. to determine which visual features contribute the most to deceive predators.

## Conclusion

Our method rationalizes the search for mimicry evolution, and as such is designed to select candidate mimics and models on the basis of a strong coincidence between resemblance and sympatry. It is complemented by biological observations that strengthen the case for mimicry (versus other forms of convergent evolution). It is an improvement over pure naturalistic intuition because it is quantitative and has a clear rationale – a way to avoid the pitfalls of story-telling. By allowing a more comprehensive description of the diversity of mimicry, our methods could help to understand the selective forces acting on this defensive strategy, but also generates new models to test hypotheses on mimicry developed in literature. We propose that Artificial Intelligence has a great potential for the study of mimicry. Although there is still room for methodological improvement, DCNNs allow to measure resemblance while accounting for the high dimensionality of visual phenotypes, simultaneously accounting for colors, texture, patterns and proportions, without requiring highly standardized data that may be difficult to collect for many species. Beyond mimicry, the ability to handle complex visual patterns makes DCNNs highly promising tools for modeling resemblance in general, with broad perspectives of applications in evolution and ecology.

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## References

- Aubret, F., and A. Mangin. 2014. The snake hiss: potential acoustic mimicry in a viper-colubrid complex. *Biological Journal of the Linnean Society* 113:1107–1114.
- Barlow, A., K. Baker, C. R. Hendry, L. Peppin, T. Phelps, K. A. Tolley, C. E. Wüster, et al. 2013. Phylogeography of the widespread African puff adder (*Bitis arietans*) reveals multiple Pleistocene refugia in southern Africa. *Molecular Ecology* 22:1134–1157.
- Barlow, B. A., and D. Wiens. 1977. Host-parasite resemblance in australian mistletoes: the case for cryptic mimicry. *Evolution* 31:69–84.
- Barnett, J. B., C. Michalis, N. E. Scott-Samuel, and I. C. Cuthill. 2018. Distance-dependent defensive coloration in the poison frog *Dendrobates tinctorius*, Dendrobatidae. *Proceedings of the National Academy of Sciences of the United States of America* 115:6416–6421.
- Bates, H. W. 1862. Contributions to an Insect Fauna of the Amazon Valley. Lepidoptera: Heliconidae. *Transactions of the Linnean Society of London* 23:495–566.
- Bergeron, Z. T., and R. C. Fuller. 2018. Using Human Vision to Detect Variation in Avian Coloration: How Bad Is It? *The American naturalist* 191:269–276.
- Bosque, R. J., J. P. Lawrence, R. Buchholz, G. R. Colli, J. Heppard, and B. Noonan. 2018. Diversity of warning signal and social interaction influences the evolution of imperfect mimicry. *Ecology and Evolution* 8:7490–7499.
- Brodie, E. D., and F. J. Janzen. 1995. Experimental Studies of Coral Snake Mimicry: Generalized Avoidance of Ringed Snake Patterns by Free-Ranging Avian Predators. *Functional Ecology* 9:186–190.
- Carranza-Rojas, J., H. Goeau, P. Bonnet, E. Mata-Montero, and A. Joly. 2017. Going deeper in the automated identification of Herbarium specimens. *BMC Evolutionary Biology* 17:181.
- Cheney, K. L., and N. J. Marshall. 2009. Mimicry in coral reef fish: how accurate is this deception in terms of color and luminance? *Behavioral Ecology* 20:459–468.
- Chollet, F. 2015. Keras: Deep learning library for theano and tensorflow.
2017. Xception: Deep learning with depthwise separable convolutions. Pages 1800–1807 in *Conference on Computer Vision and Pattern Recognition*. IEEE.
- Cichy, R. M., and D. Kaiser. 2019. Deep Neural Networks as Scientific Models. *Trends in Cognitive Sciences* 23:305–317.
- Corcobado, G., M. E. Herberstein, and S. Pekár. 2016. The role of ultraviolet colour in the assessment of mimetic accuracy between Batesian mimics and their models: A case study using ant-mimicking spiders. *Science of Nature* 103:90–101.
- Crochet, P.-A., J. B. Rasmussen, T. Wilms, P. Geniez, J.-F. Trape, and W. Böhme. 2008. Systematic status and correct nomen of the western North African cat snake: *Telescopus tripolitanus* (Werner, 1909) (Serpentes: Colubridae), with comments on the other taxa in the *dharabobtus* group. *Zootaxa* 1703:25–46.
- Cuthill, I. C., and A. T. D. Bennett. 1993. Mimicry and the eye of the beholder. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 253:203–204.
- Dale, J., C. J. Dey, K. Delhey, B. Kempnaers, and M. Valcu. 2015. The effects of life history and sexual selection on male and female plumage colouration. *Nature* 527:367–370.
- Dalyac, A., M. Shanahan, and J. K. College. 2014. Tackling class imbalance with deep convolutional neural networks. Imperial College.

- Dalziell, A. H., and J. A. Welbergen. 2016. Mimicry for all modalities. *Ecology Letters* 19:609–619.
- Darwin, C. 1887. *Life and Letters of Charles Darwin*. John Murray, London.
- Deng, J., W. Dong, R. Socher, L.-J. Li, Kai Li, and Li Fei-Fei. 2009. ImageNet: A large-scale hierarchical image database. Pages 248–255 in *Conference on Computer Vision and Pattern Recognition*. IEEE.
- Development Core Team. 2008. *R: A Language and Environment for Statistical Computing*. Austria.
- Dittrich, W., F. Gilbert, P. Green, P. McGregor, and D. Grewcock. 1993. Imperfect Mimicry: A Pigeon's Perspective. *Proceedings of the Royal Society B: Biological Sciences* 251:195–200.
- Dixon, P. 2003. VEGAN, a package of R functions for community ecology. *Journal of Vegetation Science* 14:927–930.
- Endler, J. A., G. L. Cole, and A. M. Kranz. 2018. Boundary strength analysis: Combining colour pattern geometry and coloured patch visual properties for use in predicting behaviour and fitness. (S. Blomberg, ed.) *Methods in Ecology and Evolution* 9:2334–2348.
- Ferchaud, A.-L., S. Ursenbacher, M. Cheylan, L. Luiselli, D. Jelić, B. Halpern, Á. Major, et al. 2012. Phylogeography of the *Vipera ursinii* complex (Viperidae): mitochondrial markers reveal an east-west disjunction in the Palaearctic region. *Journal of Biogeography* 39:1836–1847.
- Fisher, R. A. 1925. *Statistical Methods for Research Workers*. Oliver and Boyd, Edinburgh.
- Fuentes, A. F., S. Yoon, J. Lee, and D. S. Park. 2018. High-Performance Deep Neural Network-Based Tomato Plant Diseases and Pests Diagnosis System With Refinement Filter Bank. *Frontiers in Plant Science* 9.
- Gans, C. 1961. Mimicry in procryptically colored snakes of the genus *Dasypeltis*. *Evolution* 15:72–91.
- Geniez, P. 2015. *Serpents d'Europe, d'Afrique du Nord et du Moyen-Orient*. Delachaux et Niestlé SA, Paris.
2018. *Snakes of Europe, North Africa and the Middle East : a photographic guide*. Princeton University Press.
- Gianoli, E., and F. Carrasco-Urra. 2014. Leaf Mimicry in a Climbing Plant Protects against Herbivory. *Current Biology* 24:984–987.
- Gonçalves, D. V., F. Martínez-Freiría, P.-A. Crochet, P. Geniez, S. Carranza, and J. C. Brito. 2018. The role of climatic cycles and trans-Saharan migration corridors in species diversification: Biogeography of *Psammophis schokari* group in North Africa. *Molecular Phylogenetics and Evolution* 118:64–74.
- Goodfellow, I., Y. Bengio, and A. Courville. 2016. *Deep learning*. The MIT Press.
- Güçlü, U., and M. A. J. van Gerven. 2015. Deep Neural Networks Reveal a Gradient in the Complexity of Neural Representations across the Ventral Stream. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 35:10005–14.
- Hansen, M. F., M. L. Smith, L. N. Smith, M. G. Salter, E. M. Baxter, M. Farish, and B. Grieve. 2018. Towards on-farm pig face recognition using convolutional neural networks. *Computers in Industry* 98:145–152.
- Heatwole, H., and E. Davison. 1976. A review of caudal luring in snakes with notes on its occurrence in the Saharan sand viper, *Cerastes vipera*. *Herpetologica* 32:332–336.
- Ishihara, K., S. Ishihara, M. Nagamachi, S. Hiramatsu, and H. Osaki. 2001. Age-related decline in color perception and difficulties with daily activities—measurement, questionnaire, optical

- and computer-graphics simulation studies. *International Journal of Industrial Ergonomics* 28:153–163.
- IUCN. 2020. The IUCN Red List of Threatened Species. Version 2020-2.
- Jacobs, G. H. 2009. Evolution of colour vision in mammals. *Philosophical Transactions of the Royal Society B: Biological Sciences* 364:2957–2967.
- Johnstone, R. A. 1996. Multiple Displays in Animal Communication: ‘Backup Signals’ and ‘Multiple Messages’. *Philosophical Transactions of the Royal Society B: Biological Sciences* 351:329–338.
- Joshi, J., A. Prakash, and K. Kunte. 2017. Evolutionary Assembly of Communities in Butterfly Mimicry Rings. *The American Naturalist* 189:E58–E76.
- Jozwik, K. M., N. Kriegeskorte, K. R. Storrs, and M. Mur. 2017. Deep Convolutional Neural Networks Outperform Feature-Based But Not Categorical Models in Explaining Object Similarity Judgments. *Frontiers in Psychology* 8:1726–1754.
- Ju, C., A. Bibaut, and M. J. van der Laan. 2018. The Relative Performance of Ensemble Methods with Deep Convolutional Neural Networks for Image Classification. *Journal of Applied Statistics* 45:2800–2818.
- Kark, S., I. Warburg, and Y. L. Werner. 1997. Polymorphism in the snake *Psammophis schokarion* both sides of the desert edge in Israel and Sinai. *Journal of Arid Environments* 37:513–527.
- Kelly, C. M. R., N. P. Barker, M. H. Villet, D. G. Broadley, and W. R. Branch. 2008. The snake family Psammophiidae (Reptilia: Serpentes): Phylogenetics and species delimitation in the African sand snakes (*Psammophis* Boie, 1825) and allied genera. *Molecular Phylogenetics and Evolution* 47:1045–1060.
- Kikuchi, D. W., and D. W. Pfennig. 2010. High-model abundance may permit the gradual evolution of Batesian mimicry: an experimental test. *Proceedings. Biological sciences* 277:1041–8.
- Kikuchi, D. W., and D. W. Pfennig. 2013. Imperfect Mimicry and the Limits of Natural Selection. *The Quarterly Review of Biology* 88:297–315.
- Kim, J., A.-D. Nguyen, and S. Lee. 2019. Deep CNN-Based Blind Image Quality Predictor. *IEEE Transactions on Neural Networks and Learning Systems* 30:11–24.
- Kindler, C., M. Chèvre, S. Ursenbacher, W. Böhme, A. Hille, D. Jablonski, M. Vamberger, et al. 2017. Hybridization patterns in two contact zones of grass snakes reveal a new Central European snake species. *Scientific Reports* 7:7378.
- Kindler, C., P. de Pous, S. Carranza, M. Beddek, P. Geniez, and U. Fritz. 2018. Phylogeography of the Ibero-Maghrebian red-eyed grass snake (*Natrix astreptophora*). *Organisms Diversity & Evolution* 18:143–150.
- Kitamura, T., and M. Imafuku. 2015. Behavioural mimicry in flight path of Batesian intraspecific polymorphic butterfly *Papilio polytes*. *Proceedings of the Royal Society B: Biological Sciences* 282:20150483.
- Kitayama, S., S. Duffy, T. Kawamura, and J. T. Larsen. 2003. Perceiving an Object and Its Context in Different Cultures. *Psychological Science* 14:201–206.
- Körschens, M., B. Barz, and J. Denzler. 2018. Towards Automatic Identification of Elephants in the Wild. *arXiv* 1812.04418.
- Kriegeskorte, N. 2015. Deep Neural Networks: A New Framework for Modeling Biological Vision and Brain Information Processing. *Annual Review of Vision Science* 1:417–446.
- Kristiansen, E. B., S. D. Finkbeiner, R. I. Hill, L. Prusa, and S. P. Mullen. 2018. Testing the adaptive

- hypothesis of Batesian mimicry among hybridizing North American admiral butterflies. *Evolution* 72:1436–1448.
- Kubilius, J., S. Bracci, and H. P. Op de Beeck. 2016. Deep Neural Networks as a Computational Model for Human Shape Sensitivity. *PLOS Computational Biology* 12:e1004896.
- Leviton, A. 1970. Review of the snakes of the genus *Lytorhynchus*. *Proceedings of the California Academy of sciences* 37:249–274.
- Lind, O., M. Mitkus, P. Olsson, and A. Kelber. 2013. Ultraviolet sensitivity and colour vision in raptor foraging. *The Journal of experimental biology* 216:1819–26.
- Lind, O., M. Mitkus, P. Olsson, and A. Kelber. 2014. Ultraviolet vision in birds: the importance of transparent eye media. *Proceedings of the Royal Society B: Biological Sciences* 281:20132209–20132209.
- Mangiacotti, M., L. Limongi, M. Sannolo, R. Sacchi, M. A. L. Zuffi, and S. Scali. 2014. Head shape variation in eastern and western Montpellier snakes. *Acta Herpetologica* 9:167–177.
- Mathis, A., P. Mamidanna, K. M. Cury, T. Abe, V. N. Murthy, M. W. Mathis, and M. Bethge. 2018. DeepLabCut: markerless pose estimation of user-defined body parts with deep learning. *Nature Neuroscience* 21:1281–1289.
- Mebert, K. 2011. Geographic Variation of Morphological Characters in the Dice Snake (*Natrix tessellata*). *Mertensiella* 18:11–19.
- Miller, E. T., G. M. Leighton, B. G. Freeman, A. C. Lees, and R. A. Ligon. 2019. Ecological and geographical overlap drive plumage evolution and mimicry in woodpeckers. *Nature Communications* 10:1602.
- Mizsei, E., D. Jablonski, S. A. Roussos, M. Dimaki, Y. Ioannidis, G. Nilson, and Z. T. Nagy. 2017. Nuclear markers support the mitochondrial phylogeny of *Vipera ursinii*–*renardi* complex (Squamata: Viperidae) and species status for the Greek meadow viper. *Zootaxa* 4227:75.
- Mochida, K., W. Y. Zhang, and M. Toda. 2015. The function of body coloration of the hai coral snake *Sinomicrurus japonicus boettgeri*. *Zoological Studies* 54:33–39.
- Mound, L. A., P. Reynaud, and P. Reynaud. 2005. Franklinothrips; a pantropical Thysanoptera genus of ant-mimicking obligate predators (Aeolothripidae). *Zootaxa* 864:1.
- Nishikawa, H., T. Iijima, R. Kajitani, J. Yamaguchi, T. Ando, Y. Suzuki, S. Sugano, et al. 2015. A genetic mechanism for female-limited Batesian mimicry in *Papilio* butterfly. *Nature Genetics* 47:405–409.
- Norouzzadeh, M. S., A. Nguyen, M. Kosmala, A. Swanson, M. S. Palmer, C. Packer, and J. Clune. 2018. Automatically identifying, counting, and describing wild animals in camera-trap images with deep learning. *Proceedings of the National Academy of Sciences of the United States of America* 115:E5716–E5725.
- Penney, H. D., C. Hassall, J. H. Skevington, K. R. Abbott, and T. N. Sherratt. 2012. A comparative analysis of the evolution of imperfect mimicry. *Nature* 483:461–464.
- Pfennig, D. W., and S. P. Mullen. 2010. Mimics without models: causes and consequences of allopatry in Batesian mimicry complexes. *Proceedings. Biological sciences* 277:2577–85.
- Rajpurohit, S., R. Parkash, and S. Ramniwas. 2008. Body melanization and its adaptive role in thermoregulation and tolerance against desiccating conditions in drosophilids. *Entomological Research* 38:49–60.
- Renoult, J. P., B. Guyl, T. C. Mendelson, A. Percher, J. Dorignac, F. Geniet, and F. Molino. 2019. Modelling the Perception of Colour Patterns in Vertebrates with HMAX. *bioRxiv* 552307.
- Safaei-Mahroo, B., H. Ghaffari, H. Fahimi, S. Broomand, M. Yazdanian, E. Najafi Majd, S. S.

- Hosseinian Yousefkhani, et al. 2015. The Herpetofauna of Iran: Checklist of Taxonomy, Distribution and Conservation Status. *Asian Herpetological Research* 6:257–290.
- Saleh, M., and M. Sarhan. 2016. The egg-eating snake (Colubridae: *Dasypeltis*) of Faiyum, Egypt, with the description of a new species. *Bulletin de la Société Herpétologique de France* 160:25–48.
- Sameen, M., and B. Pradhan. 2017. Severity Prediction of Traffic Accidents with Recurrent Neural Networks. *Applied Sciences* 7:476.
- Santos, X., M. Vidal-García, J. C. Brito, S. Fahd, G. A. Llorente, F. Martínez-Freiría, X. Parellada, et al. 2014. Phylogeographic and environmental correlates support the cryptic function of the zigzag pattern in a European viper. *Evolutionary Ecology* 28:611–626.
- Schätti, B., and A. Schmitz. 2006. Re-assessing *Platyceps ventromaculatus* (Gray, 1834) (Reptilia: Squamata: Colubrinae). *Revue suisse de zoologie*. 113:747–768.
- Schätti, B., F. Tillack, and C. Kucharzewski. 2014. *Platyceps rhodorachis* (JAN, 1863) - A study of the racer genus *Platyceps* BLYTH, 1860 east of the tigris (Reptilia: Squamata: Colubridae). *Vertebrate Zoology* 64:297–405.
- Seddon, N., J. A. Tobias, M. Eaton, A. Ödeen, and B. E. Byers. 2010. Human Vision Can Provide a Valid Proxy for Avian Perception of Sexual Dichromatism. *The Auk* 127:283–292.
- Shen, D., G. Wu, and H.-I. Suk. 2017. Deep Learning in Medical Image Analysis. *Annual Review of Biomedical Engineering* 19:221–248.
- Sherratt, T. N. 2002. The evolution of imperfect mimicry. *Behavioral Ecology* 13:821–826.
- Srivastava, N., G. Hinton, A. Krizhevsky, I. Sutskever, and R. Salakhutdinov. 2014. Dropout: A Simple Way to Prevent Neural Networks from Overfitting. *Journal of Machine Learning Research* 15:1929–1958.
- Stevens, M., C. P. Rong, and P. A. Todd. 2013. Colour change and camouflage in the horned ghost crab *Ocypode ceratophthalmus*. *Biological Journal of the Linnean Society* 109:257–270.
- Taigman, Y., M. Yang, M. Ranzato, and L. Wolf. 2014. DeepFace: Closing the Gap to Human-Level Performance in Face Verification. Pages 1701–1708 in *Conference on Computer Vision and Pattern Recognition*. IEEE.
- Tamar, K., J. Šmíd, B. Göçmen, S. Meiri, and S. Carranza. 2016. An integrative systematic revision and biogeography of *Rhynchocalamus* snakes (Reptilia, Colubridae) with a description of a new species from Israel. *PeerJ* 4:e2769.
- Taylor, C. H., T. Reader, and F. Gilbert. 2016. Hoverflies are imperfect mimics of wasp colouration. *Evolutionary Ecology* 30:567–581.
- Trape, J.-F., and Y. Mané. 2006. *Guide des serpents d’Afrique occidentale; savane et désert*. Institut de Recherche pour le Développement (IRD).
- Trape, J.-F., and O. Mediannikov. 2016. Cinq serpents nouveaux du genre *Boaedon* Duméril, Bibron & Duméril, 1854 (Serpentes : Lamprophiidae) en Afrique centrale. *Bulletin de la Société Herpétologique de France* 159:61–111.
- Troscianko, J., J. Skelhorn, and M. Stevens. 2017. Quantifying camouflage: how to predict detectability from appearance. *BMC Evolutionary Biology* 17:7.
- Turk, J. 1958. Observations on Snakes and Snake Bite in the Eastern Mediterranean Area. *Journal of the Royal Army Medical* 104:63–71.
- Valkonen, J. K., and J. Mappes. 2014. Resembling a Viper: Implications of Mimicry for Conservation of the Endangered Smooth Snake. *Conservation Biology* 28:1568–1574.

- Valkonen, J. K., O. Nokelainen, and J. Mappes. 2011a. Antipredatory Function of Head Shape for Vipers and Their Mimics. *PLoS ONE* 6:e22272.
- Valkonen, J., M. Niskanen, M. Björklund, and J. Mappes. 2011b. Disruption or aposematism? Significance of dorsal zigzag pattern of European vipers. *Evolutionary Ecology* 25:1047–1063.
- Van Horn, G., O. Mac Aodha, Y. Song, Y. Cui, C. Sun, A. Shepard, H. Adam, et al. 2018. The INaturalist Species Classification and Detection Dataset. Pages 8769–8778 in *Conference on Computer Vision and Pattern Recognition*. IEEE .
- Viitala, J., E. Korplmäki, P. Palokangas, and M. Koivula. 1995. Attraction of kestrels to vole scent marks visible in ultraviolet light. *Nature* 373:425–427.
- Wade, E. 2001. Review of the false smooth snake genus *Macroprotodon* (Serpentes, Colubridae) in Algeria with a description of a new species. *Bulletin of The Natural History Museum Zoology Series* 67:85–107.
- Werner, Y. L. 1983. Behavioural triangulation of the head in three boigine snakes: possible cases of mimicry. *Israel Journal of Zoology* 32:205–228.
- Werner, Y. L., and E. Frankenberg. 1982. Head triangulation in two colubrine snakes: probable behavioural reinforcement of batesian mimicry. *Israel Journal of Zoology* 31:137–150.
- Wilson, J. S., J. P. Jahner, M. L. Forister, E. S. Sheehan, K. A. Williams, and J. P. Pitts. 2015. North American velvet ants form one of the world’s largest known Müllerian mimicry complexes. *Current Biology* 25:R704–R706.
- World Health Organization. 2010. Database of venomous snakes.
- Yamins, D. L. K., H. Hong, C. F. Cadieu, E. A. Solomon, D. Seibert, and J. J. DiCarlo. 2014. Performance-optimized hierarchical models predict neural responses in higher visual cortex. *Proceedings of the National Academy of Sciences of the United States of America* 111:8619–24.
- Zhang, R., P. Isola, A. A. Efros, E. Shechtman, and O. Wang. 2018. The Unreasonable Effectiveness of Deep Features as a Perceptual Metric. Pages 586–595 in *Conference on Computer Vision and Pattern Recognition*. IEEE.
- Zheng, J. 2009. Predicting software reliability with neural network ensembles. *Expert Systems with Applications* 36:2116–2122.
- Zhou, J., and O. G. Troyanskaya. 2015. Predicting effects of noncoding variants with deep learning-based sequence model. *Nature methods* 12:931–4.



## Annexes: Supplementary Methods

### *(a) Image pre-processing*

Snake images were squared by cropping around subjects and downsized to 299 x 299 pixels. The venomous snake dataset was split into two: 90% of each class images was used for training and 10% for validation (i.e., to monitor performances while tuning training hyperparameters). We used an image augmentation procedure to improve invariance to noise and to limit overfitting: before every training epoch, images were rotated with an angle sampled between 0° and 180°, randomly flipped horizontally and vertically, up to 20% zoomed in or out, and RGB color channel randomly shifted by 0.2. Images of non-venomous snakes were squared and downsized only.

### *(b) DCNNs training and predictions*

Training a DCNN to recognize snake species from scratch would have required a very large dataset of images to achieve good performances. Following other studies (e.g., Norouzzadeh et al. 2018), we used “transfer learning” in which a DCNN pre-trained on a very large dataset is fine-tuned with a new, smaller dataset. In this study, we transferred the Xception network (Chollet 2017) pre-trained on ImageNet (a dataset of 14 million images belonging to more than 1 000 classes; Deng et al. 2009). We tested other networks also trained on ImageNet (Alexnet, VGG16 and ResNet50), which all yielded qualitatively similar results but reached lower performance in species recognition. These lower performances might be explained by the difficulty of the task: strong similarity between species, and high variation in shape, position, background. To limit overfitting, we added several dropout layers (Srivastava et al. 2014). We replaced the original final classification layer by a first fully-connected convolutional layer (2048 units), a dropout layer (dropout probability: 0.7), a second fully-connected convolutional layer (256 units), a second dropout layer (dropout probability: 0.7) and a 36-ways softmax classifier. The highest performances were reached by fine-tuning all layers (with a very small learning rate) rather than the last added layers only. We trained three networks separately using three different optimizers: Nadam (learning rate 0.00003, decay 0), Single Gradient Descent (learning rate 0.001, decay 0.00001, Nesterov momentum 0.9) and Adagrad (learning rate: 0.0005, decay: 0) (Zheng 2009; Ju et al. 2018). The training was stopped

when we visually estimated that the curves of validation loss did not decrease further for 20 consecutive epochs, i.e. after 50 epochs for training with Stochastic Gradient Descent, 120 epochs with Adagrad and 45 epochs with Nadam (Figure S5). We did not use a test set to measure the final accuracy, due to the limited number of images available for training, and because training and validation/test images were totally independent (different individuals and shooting conditions; see Taigman et al. 2014 for similar procedure).

We estimated the classification probabilities of each non-venomous snake image with each of the three DCNNs trained with different optimizers, and used the mean classification probability (over DCNNs) for further analyses. The whole training and prediction procedure was repeated 15 times, to produce 15 matrices of resemblance scores that were eventually averaged (arithmetic mean) into a single resemblance matrix. This step allows to mitigate idiosyncrasies caused by the dropout layers.

All DCNN analyses were performed with the Keras library (Chollet 2015) in Python language. All the codes are available at [https://github.com/t-desolan/Mimicry\\_snakes](https://github.com/t-desolan/Mimicry_snakes). The images used for training and testing can be provided upon request.



## Annexes: Supplementary Tables and Figures

**Table S1.** Number of photographs, rationale for species exclusion and source of distribution maps for each of the 35 venomous species and 87 non-venomous species of the Western Palearctic snakes, plus the 80 foreign species of the foreign class.

This table is not available in the printed version of the thesis due to its size, but can be found in the file MS2 Snake mimicry at:

<https://1drv.ms/f/s!AhWgyBtt5X3FamXDhTlzauD5uXo>



**Figure S1.** Sample of the intraspecific variability observed in the same population of *Vipera aspis* present in the Briançonnais (France, province of Hautes-Alpes). Photo credit: Grégory Deso



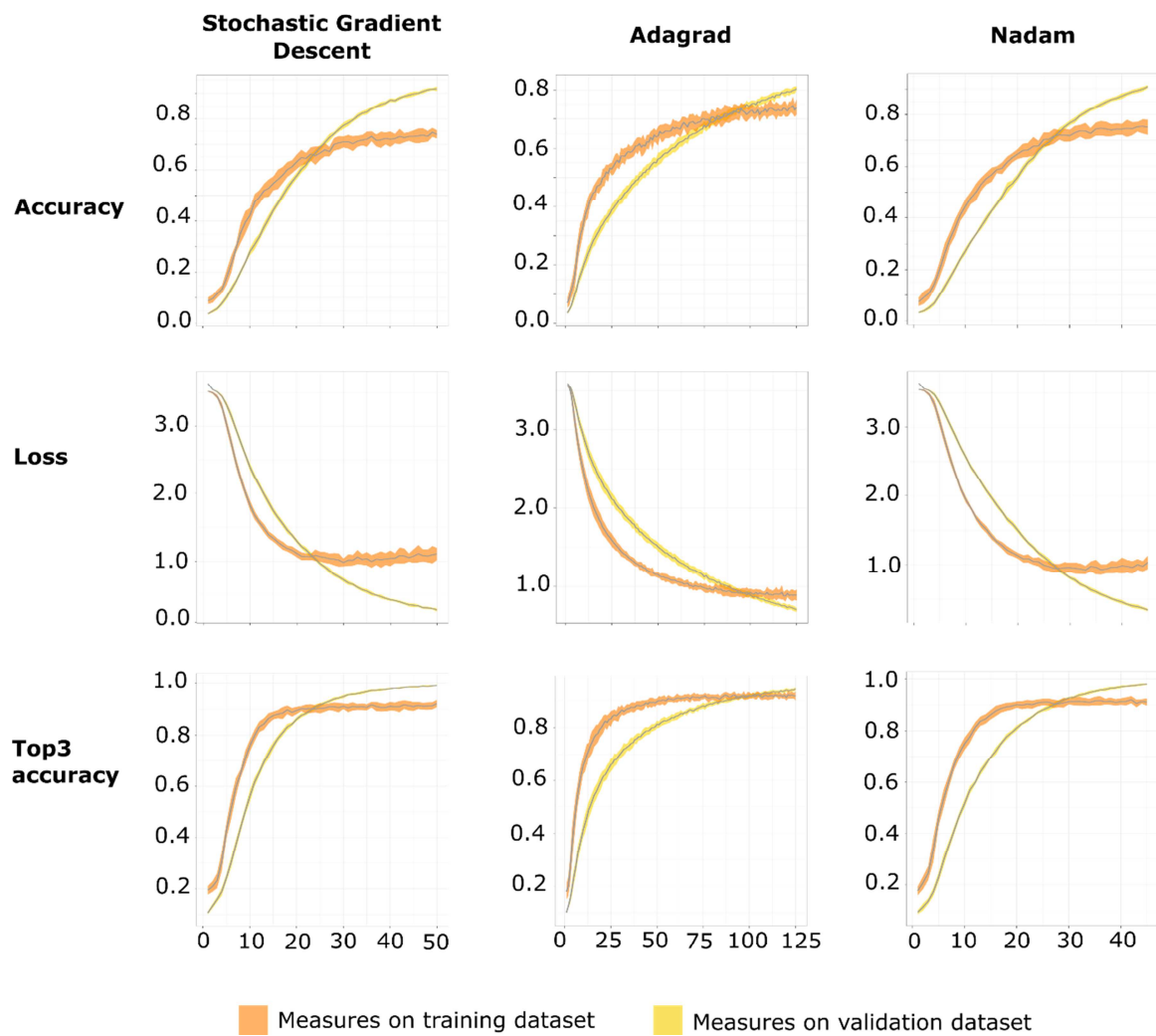




**Figure S3.** The seven mimic and model species identified in our analysis (A-G) and seven examples pair of non-mimetic species (H-N), with mimicry scores (red), quantiles 95% (black dashed line) and the null distribution (blue) of each species pair. Species framed in yellow have been previously reported as mimetic in the literature (Gans 1961; Werner and Frankenberg 1982; Werner 1983; Valkonen et al. 2011a; Aubret and Mangin 2014). (A) *Coronella austriaca* – *Vipera berus*. (B) *Dasypeltis sahelensis* – *Echis pyramidum*. (C) *Hemorrhois nummifer* – *Montivipera xanthina*. (D) *Natrix helvetica* – *V. aspis*. (E) *N. maura* – *V. latastei*. (F) *Spalerosophis diadema* – *Pseudocerastes fieldi*. (G) *Telescopus fallax* – *V. ammodytes*. (H) *H. hippocrepis* – *V. latastei*. (I) *Psammophis sibilans* – *V. seoanei*. (J) *Eryx miliaris* – *Montivipera bulgardaghica*. (K) *Platyceps chesneii* – *V. aspis*. (L) *Eryx jaculus* – *M. bulgardaghica*. (M) *Elaphe quatuorlineata* – *V. seoanei*. (N) *Eirenis lineomaculatus* – *V. ursini*. Photo credits: Matthieu Berroneau, José C. Brito, Olivier Buisson, Luis García Cardenete, Alexandre Cluchier, Pierre-André Crochet, Michel Geniez, Philippe Geniez, André Langenbach, Jean Muratet, Gilles Pottier and Jean-François Trape.



**Figure S4.** Phylogeny of the venomous (right) and the non-venomous species included in the analysis, the mimetic and model species identified are respectively in red and blue. The only possible case of phylogenetically inherited mimicry (i.e. when mimicry evolved in the common ancestor and is transmit to the present species) concerns the two *Natrix* species. The phylogenetic tree was reconstructed from different bibliographic sources (Kalyabina-Hauf et al. 2004; Velo-Anton et al. 2012; Kyriazi et al. 2013; Saleh and Sarhan 2016; Stümpel et al. 2016; Kindler et al. 2017; Mizsei et al. 2017, Šmid et al. 2019).



**Figure S5.** Progression of accuracy, loss and top3 accuracy through the training period for the three ConvNet of network ensemble: Stochastic Gradient Descent network, Adagrad network and Nadam network.

# **Manuscript C**

## **The evolutionary importance of cues in protective mimicry**

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Predators often avoid toxic, unpalatable or otherwise unprofitable prey because of innate biases or past unpleasant experiences. In both cases, the association between prey appearance and unprofitability has favored an anti-predator strategy called "aposematism" (Poulton, 1890). In short, aposematic prey benefit from reduced predation because predators perceive the prey phenotype as a warning (Wallace, 1882). Aposematic traits take the form of spectacular conspicuous colorations in many taxa, in particular in snakes (Smith, 1977), amphibians (Rudh and Qvarnström, 2013), insects (Wilson et al., 2015; Motyka et al., 2018) and spiny plants (Lev-Yadun, 2016). As such, they have enthralled many evolutionary biologists and ecologists, whose research greatly improved our understanding of local adaptation (Mallet and Barton, 1989), speciation (Merrill et al., 2012), community dynamics (Chazot et al., 2014), and predator foraging decisions (Skelhorn et al., 2016). In the literature, aposematism is therefore often equated with colorations that are consistently conspicuous (but see recent studies on 'switchable' aposematic signals; e.g., Kang et al., 2016a, 2016b). We argue here that this focus on conspicuous colorations has led to researchers overlooking other components of prey phenotype. Aposematism relies not only on 'signaling traits' (like conspicuous coloration), which evolve through natural selection imposed by predators, but also on 'cues' (e.g., body shape, behavior or non-conspicuous coloration), the evolution of which is determined mainly by environment, sexual selection or developmental constraints (hampering evolutionary changes) (Maynard-Smith and Harper, 2003; Scott-Phillips, 2008). In this opinion piece, we aim at highlighting the underappreciated role of cues in the evolution of warning signals in the context of protective mimicry.

### **Defining signals and cues in an evolutionary context**

Communication and signaling in animals have long been recognized to rely on the detection of both signals and cues (Scott-Phillips, 2008; Bro-Jørgensen, 2010; Higham and Hebets, 2013). Yet, the terms 'signal' and 'cue' have been fraught with semantic disagreement (Maynard-Smith and Harper, 2003; Scott-Phillips, 2008). In communication theory, cues and signals have been defined from an informational point of view. Cues are defined as incidental sources of information detected by 'unintended' receivers, while signal are information addressed to 'intended' receivers (Hasson, 1994; Greenfield, 2006). Yet, this distinction seems of little practical use in an evolutionary context. The terms 'unintended' and 'intended' do not make much sense in evolutionary biology and can be misleading (Font, 2018).



To better understand the implication of cues and signals for trait evolution, we need to consider definitions that are relevant in an evolutionary context. Several authors adopted adaptationist definitions of cues and signals, generally in addition to the informational definitions (Maynard-Smith and Harper, 2003; Scott-Phillips, 2008; Bro-Jørgensen, 2010). If we focus on predator-prey interactions: in prey, signals are defined as traits that have evolved predominantly through differential predator avoidance, while cues are traits that have evolved predominantly through other evolutionary forces. Of course, selection imposed by predators is likely to influence the evolution of all traits that are perceptible. The difference between cues and signals in a given species relies on the importance of selection that results from predator perception, compared to the other evolutionary forces acting on the trait. Therefore, a phenotypic trait that is a cue in one species can be a signal in another.

Distinguishing cues from signals in the field is challenging. The exact nature of selection acting on conspicuous signals has only recently been measured in the field (in butterflies; Chouteau et al., 2016). Therefore, the intensity of selection imposed by predators on non-conspicuous traits can be difficult to assess (e.g., on flight behavior in butterflies; Beccaloni, 1997).

Hereafter, we adopt the adaptationist definition of cues and signals to discuss the evolutionary importance of cues in protective mimicry. In examples, we classify phenotypic traits as signals or cues; but remember that those assertions require caution.

### **Distinguishing cue mimicry from signal mimicry**

Protective mimicry, when a species benefit from reduced predation by mimicking another unprofitable species, is one of the most celebrated examples of evolution by natural selection (Bates, 1862; Cott, 1940; Quicke, 2017). Species engaged in protective mimicry are traditionally defined as mimetic or model species, with the former being the species benefiting the most from mimicry (Cott, 1940; Ruxton et al., 2018). Communication between prey and predators determines the evolution of protective mimicry but, surprisingly, little attention has been given to the evolutionary implication of cues in protective mimicry. As signaling traits strongly stimulate the predator sensory system, most mimetic systems rely on ‘signal mimicry’, whereby the mimetic signaling traits are similar to the model’s signaling traits (Cott, 1940; Quicke, 2017). Nonetheless, predators also perceive cues, favoring the evolution of ‘cue mimicry’, whereby the mimetic signaling traits are similar to some of the

model's cues. In the case of cue mimicry, the same phenotypic trait is a signaling trait in the mimetic species, and a cue in the model species (Jamie, 2017).

In a recent review paper, Jamie (2017) proposed a conceptual framework considering the distinction between cues and signals to contrast and order all mimetic resemblances (protective mimicry, aggressive mimicry, rewarding mimicry). This framework highlights the evolutionary importance of cues in mimicry and distinguishes 'cue mimicry' from 'signal mimicry'. Nonetheless, Jamie (2017) provided no convincing examples of cues involved in protective mimicry but instead discussed cases of masquerade where inanimate objects are mimicked. To fill this gap, we present below convincing examples of 'cue mimicry' in the context of protective mimicry. We then discuss the evolutionary consequences of this defensive strategy for the evolution of mimetic signals.

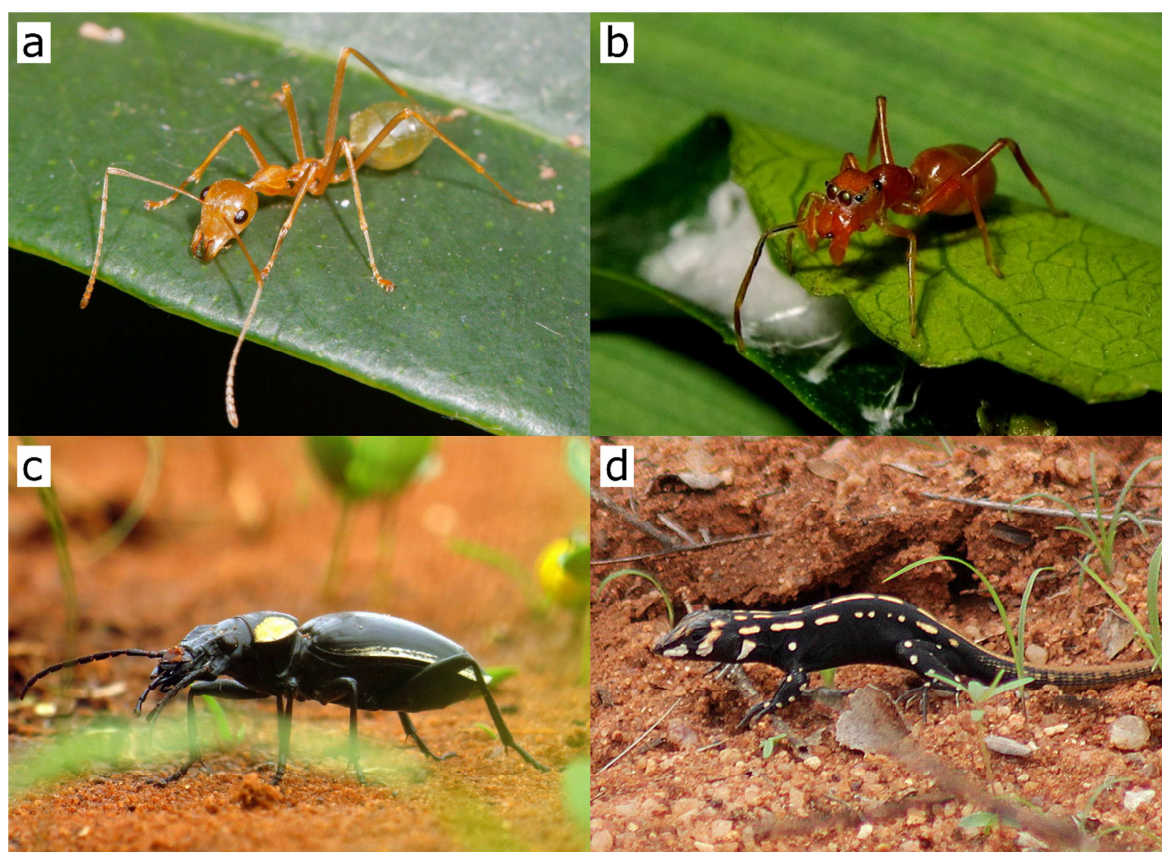
### **Cue mimicry in nature**

In nature, protective mimicry is often characterized by some forms of cue mimicry. One of its most striking examples is eyespots mimicry found in a wide variety of insects (Stevens, 2005). Eyespots usually take the form of a large dark central spot surrounded by a white border, and look like the eyes of large size vertebrates that are predators of the small birds attacking insects. The evolution of eyespots is determined by differential predation rate in insect mimetic species (De Bona et al., 2015), whereas the evolution of vertebrate eyes is determined by other selective pressures. As such, eyespots mimicry can be defined as cue mimicry. Another common example of cue mimicry is seen in the mimicry of ants. Ants are involved in many mimetic systems, especially with spiders as mimics, and yet rarely display conspicuous colorations (Huang et al., 2011). Most ant species can defend themselves (mandible, formic acid), making them highly unprofitable to most predators. Mimetic species with the same body shape, gait and colorations than the ants benefit from reduced predation (Figure 1a-b; Mclver and Stonedahl, 1993; Nelson and Card, 2016). In the ant models, the evolution of these traits is mainly determined by environment and developmental constraints (except aposematic conspicuous coloration in some ant species), while it is determined by predator selective pressures in their mimics. Finally, cue mimicry is also found in plants. Australian mistletoe species benefit from reduced herbivory by mimicking the foliage of their host-tree (Barlow and Wiens, 1977; Burns, 2010). Similarly, a vine, *Boquila trifoliolata*, has the same leaf shape as its supporting plant (Gianoli and Carrasco-Urra, 2014). In both examples, the supporting species is considered as the model

because they are little palatable. Here again, the foliage shape of models is probably determined by environment and developmental constraints, rather than herbivory. All these examples demonstrate that cue mimicry is not anecdotal and may be taxonomically widespread.

### Cue mimicry in association with signal mimicry

Cue mimicry can be associated with signal mimicry in the same mimetic system. This is especially the case when model and mimic belong to different guilds and have very different ecology. For instance, in southern Africa, juveniles of the *Heliobolus lugubris* lizard species mimic a sympatric and noxious beetle species (Figure 1c-d; Huey and Pianka, 1977). The two species have a similar conspicuous coloration, a black coloration with white spots, but the lizard also has the same gait as the beetle, thereby increasing mimetic resemblance.



**Figure 1.** Examples of cue mimicry in protective mimicry. The body shape of the ant *Oecophylla smaragdina* (a) is mimicked by the spider *Myrmaplata plataleoides* (b). In addition to signal mimicry, the gait of the beetle *Anthia thoracica* (c) is mimicked by the juvenile lizard *Heliobolus lugubris* (d). Photo credits: (a) Lek Khau; (b) Jason Alexander; (c) Tjeerd de Wit; (d) Christine Sydes.

Similar examples of signal mimicry paired with behavioral cue mimicry can be found in other mimetic systems involving vertebrate and invertebrate species (Vitt, 1992; Londoño et al., 2015). Likewise, some hoverfly species wave their darkened front legs, thereby mimicking the presence and movement of long antennae that are cues in conspicuously colored wasp models (Waldbauer, 1970).

Cue mimicry can also be associated with signal mimicry when model and mimic belong to the same guild. For instance, some aposematic species of *Dilophotes* beetles have different conspicuous mimetic signals (shared with different model species) depending on their sex (Motyka et al., 2018). In these species, males are smaller than females, and this sexual size dimorphism may have favored the evolution of sexual mimetic dimorphism. In model species, size is probably a cue, the evolution of which is primarily determined by developmental constraints. In the mimetic *Dilophotes* species, however, sexual size dimorphism strongly affects predator avoidance and has determined the evolution of different conspicuous mimetic signals in females and males. This example reveals how model's cues can potentially influence the signaling traits of the mimic, and thus demonstrates the importance of accounting for cues in theories on mimicry evolution.

### **Implications of cue mimicry for mimicry evolution**

Accounting for cue mimicry may shed light on puzzling patterns observed in mimetic systems. Imperfect mimicry is found in many taxa (Vereecken and Schiestl, 2008; Penney et al., 2012), while a perfect resemblance should theoretically evolve by natural selection. Several hypotheses may explain this paradox (Kikuchi and Pfennig, 2013). Among those hypotheses, the backup signal hypothesis suggests that a weak resemblance between mimic and model can be compensated by additional backup signals, thereby maintaining imperfect mimicry on other signaling traits (Johnstone, 1996). Following this backup signal hypothesis, cue mimicry may evolve as a backup to imperfect signal mimicry. In particular, if mimetic and model species belong to different guilds and show strong phenotypic differences, perfect mimicry may be difficult to evolve and cue mimicry is likely to evolve as a backup to imperfect signal mimicry. Such situation is nicely illustrated with the example of the juvenile lizards mimicking the beetle's gait (Figure 1c-d) (Huey and Pianka, 1977), and may occur in many vertebrate-invertebrate mimetic systems (Vitt, 1992; Londoño et al., 2015). Overall, even in mimetic system involving species from the same guild, cue mimicry can play an important role for the maintenance of imperfect mimicry (Silva et al., 2016).

Due to the differences of selective pressure acting on cues and signals, cues can maintain the diversity of mimetic signals among defended species (so-called ‘Müllerian mimicry’). Several distinct and stable Müllerian mimetic forms co-occurring in the same place have been observed, while a single shared mimetic form should provide increased protection against predators (Mallet and Joron, 1999). Several hypotheses may explain this phenomenon, like local adaptation (Mallet and Gilbert, 1995) or heterogeneity in micro-habitat use among species (Willmott et al., 2017), but to our knowledge, the implication of cues for the maintenance of Müllerian mimetic diversity has not been investigated. Predators use both signaling traits and cues to recognize their prey, so that mimicry based on signaling traits alone may not necessarily deceive predators. Contrary to signaling traits, selective pressure imposed by predation does not favor the evolution of shared cues in the model species. Differences in the cues of models could prevent the convergence of Müllerian mimetic forms and may explain the maintenance of diversity in some communities. Such a situation is illustrated by the case of *Dilophotes* beetles, the evolution of which is compelled by the size of their models (Motyka et al., 2018). This hypothesis remains to be investigated theoretically, and has received little empirical support so far, probably because of the oversight of cues in the literature on protective mimicry.

## Conclusion

The literature on aposematism and mimicry has mainly focused on conspicuous signaling traits. While such focus has allowed for rapid advancement of the field, we may have minimized the implication of cues for the evolution of mimicry. As illustrated above, protective mimicry can occur without any form of conspicuous signal and, by focusing on conspicuous coloration, we may underestimate the predominance of protective mimicry in natural communities. A rigorous framework should be employed to detect such mimicry based on non-conspicuous traits (see de Jager and Anderson, 2019).

The evolution of mimetic signals involves a wide variety of selective pressures, but also a large variety of traits (Rojas et al., 2018; Briolat et al., 2019). We highlighted here that the same phenotypic trait can be shaped by different selective forces in the different species involved in mimicry. Such distinction could help to disentangle the selective forces shaping the complex evolution of mimicry and may improve our comprehension of this defensive strategy.

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## References

- Barlow, B. A., and Wiens, D. (1977). Host-parasite resemblance in Australian mistletoes: The case for cryptic mimicry. *Evolution* (N. Y.) 31, 69–84. doi:10.1111/j.1558-5646.1977.tb00983.x.
- Bates, H. W. (1862). Contributions to an insect fauna of the Amazon valley. Lepidoptera: Heliconidae. *Trans. Linn. Soc. London* 23, 495–566. doi:10.1111/j.1096-3642.1860.tb00146.x.
- Beccaloni, G. W. (1997). Vertical stratification of ithomiine butterfly (Nymphalidae: Ithomiinae) mimicry complexes: The relationship between adult flight height and larval host-plant height. *Biol. J. Linn. Soc.* 62, 313–341. doi:10.1111/j.1095-8312.1997.tb01629.x.
- Briolat, E. S., Burdfield-Steel, E. R., Paul, S. C., Rönkä, K. H., Seymoure, B. M., Stankowich, T., et al. (2019). Diversity in warning coloration: selective paradox or the norm? *Biol. Rev.* 94, 388–414. doi:10.1111/brv.12460.
- Bro-Jørgensen, J. (2010). Dynamics of multiple signalling systems: Animal communication in a world in flux. *Trends Ecol. Evol.* 25, 292–300. doi:10.1016/J.TREE.2009.11.003.
- Burns, K. C. (2010). Is crypsis a common defensive strategy in plants? Speculation on signal deception in the New Zealand flora. *Plant Signal. Behav.* 5, 9–13. doi:10.4161/psb.5.1.10236.
- Chazot, N., Willmott, K. R., Santacruz Endara, P. G., Toporov, A., Hill, R. I., Jiggins, C. D., et al. (2014). Mutualistic mimicry and filtering by altitude shape the structure of Andean butterfly communities. *Am. Nat.* 183, 26–39. doi:10.1086/674100.
- Chouteau, M., Arias, M., and Joron, M. (2016). Warning signals are under positive frequency-dependent selection in nature. *Proc. Natl. Acad. Sci.* 113, 2164–2169. doi:10.1073/PNAS.1519216113.
- Cott, H. B. (1940). Adaptive coloration in animals. London: Methuen & Co.
- De Bona, S., Valkonen, J. K., López-Sepulcre, A., and Mappes, J. (2015). Predator mimicry, not conspicuousness, explains the efficacy of butterfly eyespots. *Proceedings. Biol. Sci.* 282, 20150202. doi:10.1098/rspb.2015.0202.
- de Jager, M. L., and Anderson, B. (2019). When is resemblance mimicry? *Funct. Ecol.*, 1365–2435.13346. doi:10.1111/1365-2435.13346.
- Font, E. (2018). Mimicry, camouflage and perceptual exploitation: The evolution of deception in nature. *Biosemiotics*, 1–18. doi:10.1007/s12304-018-9339-6.
- Gianoli, E., and Carrasco-Urra, F. (2014). Leaf mimicry in a climbing plant protects against herbivory. *Curr. Biol.* 24, 984–987. doi:10.1016/J.CUB.2014.03.010.
- Greenfield, M. D. (2006). “Honesty and deception in animal signals,” in *Essays in Animal Behaviour: Celebrating 50 Years of Animal Behaviour*, 279–298.
- Hasson, O. (1994). Cheating signals. *J. Theor. Biol.* 167, 223–238. doi:10.1006/JTBI.1994.1065.
- Higham, J. P., and Hebets, E. A. (2013). An introduction to multimodal communication. *Behav. Ecol. Sociobiol.* 67, 1381–1388. doi:10.1007/s00265-013-1590-x.
- Huang, J.-N., Cheng, R.-C., Li, D., and Tso, I.-M. (2011). Salticid predation as one potential driving force of ant mimicry in jumping spiders. *Proceedings. Biol. Sci.* 278, 1356–1364. doi:10.1098/rspb.2010.1896.
- Huey, R. B., and Pianka, E. R. (1977). Natural selection for juvenile lizards mimicking noxious beetles. *Science* 195, 201–203.

- Jamie, G. A. (2017). Signals, cues and the nature of mimicry. *Proceedings. Biol. Sci.* 284, 20162080. doi:10.1098/rspb.2016.2080.
- Johnstone, R. A. (1996). Multiple displays in animal communication: 'Backup signals' and 'Multiple messages'. *Philos. Trans. R. Soc. B Biol. Sci.* 351, 329–338. doi:10.1098/rstb.1996.0026.
- Kang, C., Cho, H.-J., Lee, S.-I., and Jablonski, P. G. (2016a). Post-attack aposematic display in prey facilitates predator avoidance learning. *Front. Ecol. Evol.* 4, 35. doi:10.3389/fevo.2016.00035.
- Kang, C., Moon, H., Sherratt, T. N., Lee, S.-I., and Jablonski, P. G. (2016b). Multiple lines of anti-predator defence in the spotted lanternfly, *Lycorma delicatula* (Hemiptera: Fulgoridae). *Biol. J. Linn. Soc.* 120, 115–124. doi:10.1111/bij.12847.
- Kikuchi, D. W., and Pfennig, D. W. (2013). Imperfect mimicry and the limits of natural selection. *Q. Rev. Biol.* 88, 297–315. doi:10.1086/673758.
- Lev-Yadun, S. (2016). "Defensive (anti-herbivory) coloration in land plants," in (Cham: Springer International Publishing). doi:10.1007/978-3-319-42096-7.
- Londoño, G. A., García, D. A., and Sánchez Martínez, M. A. (2015). Morphological and behavioral evidence of Batesian mimicry in nestlings of a lowland Amazonian bird. *Am. Nat.* 185, 135–141. doi:10.1086/679106.
- Mallet, J., and Barton, N. H. (1989). Strong natural selection in a warning-color hybrid zone. *Evolution (N. Y.)* 43, 421–431. doi:10.1111/j.1558-5646.1989.tb04237.x.
- Mallet, J., and Gilbert, L. E. (1995). Why are there so many mimicry rings? Correlations between habitat, behaviour and mimicry in *Heliconius* butterflies. *Biol. J. Linn. Soc.* 55, 159–180. doi:10.1111/j.1095-8312.1995.tb01057.x.
- Mallet, J., and Joron, M. (1999). Evolution of diversity in warning color and mimicry: Polymorphisms, shifting balance, and speciation. *Annu. Rev. Ecol. Syst.* 30, 201–233. doi:10.1146/annurev.ecolsys.30.1.201.
- Maynard-Smith, J., and Harper, D. (2003). *Animal signals*. Oxford University Press.
- McIver, J. D., and Stonedahl, G. (1993). Myrmecomorphy: Morphological and behavioral mimicry of ants. *Annu. Rev. Entomol.* 38, 351–377. doi:10.1146/annurev.en.38.010193.002031.
- Merrill, R. M., Wallbank, R. W. R., Bull, V., Salazar, P. C. A., Mallet, J., Stevens, M., et al. (2012). Disruptive ecological selection on a mating cue. *Proc. R. Soc. B Biol. Sci.* 279, 4907–4913. doi:10.1098/rspb.2012.1968.
- Motyka, M., Kampova, L., and Bocak, L. (2018). Phylogeny and evolution of Müllerian mimicry in aposematic Dilophotes : Evidence for advergence and size-constraints in evolution of mimetic sexual dimorphism. *Sci. Rep.* 8, 3744. doi:10.1038/s41598-018-22155-6.
- Nelson, X. J., and Card, A. (2016). Locomotory mimicry in ant-like spiders. *Behav. Ecol.* 27, 700–707. doi:10.1093/beheco/arv218.
- Penney, H. D., Hassall, C., Skevington, J. H., Abbott, K. R., and Sherratt, T. N. (2012). A comparative analysis of the evolution of imperfect mimicry. *Nature* 483, 461–464. doi:10.1038/nature10961.
- Poulton, E. (1890). *The colours of animals: Their meaning and use, especially considered in the case of insects.* , ed. D. Appleton and Co. New York.
- Quicke, D. L. J. (2017). *Mimicry, crypsis, masquerade and other adaptive resemblances*. Oxford: Wiley Blackwell.
- Rojas, B., Burdfield-Steel, E., De Pasqual, C., Gordon, S., Hernández, L., Mappes, J., et al. (2018).



- Multimodal aposematic signals and their emerging role in mate attraction. *Front. Ecol. Evol.* 6, 93. doi:10.3389/fevo.2018.00093.
- Rudh, A., and Qvarnström, A. (2013). Adaptive colouration in amphibians. *Semin. Cell Dev. Biol.* 24, 553–561. doi:10.1016/J.SEMCDB.2013.05.004.
- Ruxton, G. D., Allen, W. L., Sherratt, T. N., and Speed, M. P. (2018). *Avoiding attack : The evolutionary ecology of crypsis, aposematism, and mimicry.* 2de ed., Oxford University Press.
- Scott-Phillips, T. C. (2008). Defining biological communication. *J. Evol. Biol.* 21, 387–395. doi:10.1111/j.1420-9101.2007.01497.x.
- Silva, I., Crane, M., Artchawakom, T., Suwanwaree, P., and Strine, C. T. (2016). More than meets the eye: Change in pupil shape by a mock viper. *Front. Ecol. Environ.* 14, 453–454. doi:10.1002/fee.1420.
- Skelhorn, J., Halpin, C. G., and Rowe, C. (2016). Learning about aposematic prey. *Behav. Ecol.* 27, 955–964. doi:10.1093/beheco/arw009.
- Smith, S. M. (1977). Coral-snake pattern recognition and stimulus generalisation by naive great kiskadees (Aves: Tyrannidae). *Nature* 265, 535–536. doi:10.1038/265535a0.
- Stevens, M. (2005). The role of eyespots as anti-predator mechanisms, principally demonstrated in the Lepidoptera. *Biol. Rev.* 80, 573–588. doi:10.1017/S1464793105006810.
- Vereecken, N. J., and Schiestl, F. P. (2008). The evolution of imperfect floral mimicry. *Proc. Natl. Acad. Sci. U. S. A.* 105, 7484–7488. doi:10.1073/pnas.0800194105.
- Vitt, L. J. (1992). Mimicry of millipedes and centipedes by elongate terrestrial vertebrates. *Res. Explor.*, 76–95.
- Waldbauer, G. P. (1970). Mimicry of Hymenopteran antennae by Syrphidae. *Psyche A J. Entomol.* 77, 45–49. doi:10.1155/1970/28967.
- Wallace, A. R. (1882). Dr. Fritz Müller on some difficult cases of mimicry. *Nature* 26, 86–87. doi:10.1038/026086a0.
- Willmott, K. R., Robinson Willmott, J. C., Elias, M., and Jiggins, C. D. (2017). Maintaining mimicry diversity: Optimal warning colour patterns differ among microhabitats in Amazonian clearwing butterflies. *Proc. R. Soc. B Biol. Sci.* 284, 20170744. doi:10.1098/rspb.2017.0744.
- Wilson, J. S., Jahner, J. P., Forister, M. L., Sheehan, E. S., Williams, K. A., and Pitts, J. P. (2015). North American velvet ants form one of the world's largest known Müllerian mimicry complexes. *Curr. Biol.* 25, R704–R706. doi:10.1016/j.cub.2015.06.053.

# **CHAPTER THREE**

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**Effect of sexual and natural selection on coloration  
evolution**



# **Manuscript D**

## **Sexual selection does not always result in faster evolution or increased coloration diversity**

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Manuscript in preparation



### **Abstract**

Sexual selection has been hypothesized to generate more phenotypic diversity than natural selection over evolutionary time. This hypothesis has been supported by studies focusing on small ranges of taxa. Here, we investigated the evolution of coloration more broadly, in three groups of vertebrates (birds, lizards and newts). We compared the disparity and the rates of evolution of coloration among body parts under sexual and non-sexual selection, and between males and females. We expected higher disparity/evolutionary rates in males and in sexually selected body parts than in females and non-sexually selected parts. In birds, these expectations were fulfilled, while in newts and lacertids disparities and evolutionary rates were often similar between sexes and trait types. Thus, our results suggest that sexual selection is not always promoting fast phenotypic evolution, and that its impact on the evolutionary diversification of color patterns may be taxon-dependent.

**Keywords:** diversity, sexual coloration, camouflage, evolution, signals, communication



## **Introduction**

Understanding the origin of the astonishing diversity of colors and shapes visible in the animal kingdom is a fundamental question in evolution. Since Darwin, two main selective processes have been identified as shaping the evolution of phenotypes: natural selection, arising from differences in survival or fecundity, and sexual selection, the selection resulting from competition for mates and their gametes (the second being sometimes considered as a special case of the first; Darwin 1871; Mayr 1972; Starr et al. 2014). Over the past 50 years, an abundant literature demonstrated that sexual and natural selection can interact, and that phenotypic traits frequently evolve due to a combination of both processes (e.g. Shine and Shetty 2001; Kim and Choe 2003; Baeckens et al. 2018). However, we still have a largely incomplete understanding of the relative importance of natural and sexual selection, and identifying their contributions in generating the diversity of the phenotype observed in nature remains a major challenge for evolutionary biologists.

The variety of sexual traits observed in some iconic groups (e.g. birds of paradise), and the fact that sexual traits are often the first traits to diverge in recently separated taxa (e.g. Masta and Maddison 2002; Boul et al. 2007), has led biologists to hypothesize that sexual traits (i.e. traits that are under sexual selection) evolve more rapidly and generate more phenotypic diversity than non-sexual traits. Such a pattern could be explained by two factors. Firstly, natural selection could be more often stabilizing because of the limited number of potential phenotypic optima in a given environment (Rosenblum 2006; Tobler et al. 2011) while there are probably a larger number of potential optima for sexually-selected traits (Irwin 2008). For example, a coloration selected for crypsis should match as precisely as possible that of the habitat, while many different sorts of bright colors and costly ornaments can potentially be involved in sexual signaling. Secondly, several meta-analyses found that the strength of directional sexual selection is higher than the strength of directional natural selection in wild populations (Hoekstra et al. 2001; Kingsolver and Pfennig 2007; Kingsolver and Diamond 2011).

Although both evolutionary rate and phenotypic disparity are linked to the strength of directional selection, they do not necessarily present similar patterns when measured at large phylogenetic scales. Indeed, if traits evolve rapidly but are constrained to converge towards the same optima in distantly related species, phenotypic disparity can remain low while evolutionary rates are high. Accounting both components is thus important to understand the evolution of phenotypes.



Several studies recently investigated the influence of sexual selection on phenotype, and all found that traits subjected to sexual selection had either higher rates of evolution or higher disparity than other traits (list of the studies in Table S1), although evolutionary rates and diversity did not always give concordant signals. As far as we know, the empirical support for the hypothesis that sexual selection increases rates of evolution and phenotypic diversity is thus nearly univocal in the literature. However, all studies but two have focused on birds and fish, none of them included more than one clade of vertebrate, and only two studies investigated disparity. Thus, the generality of this pattern remains an open question.

Evolutionary rates measure the amount of change in a trait between ancestor and descendent lineages, standardized by time. However, this definition can be apprehended in two different ways. To allow comparison, evolutionary rates have to be in a dimensionless scale. Hence, they can be quantified as a change of a trait relative to the intraspecific variance of the trait. This is the approach of the “haldane” (Gingerich 1983), which is often used in microevolution studies. With this method, evolutionary rates will be all the greater that the change is high and the intraspecific variability is low. Furthermore, evolutionary rates can also be thought as a change of a trait relative to the average value of the trait. This method is comparable to the “darwin” (Haldane 1949), which is usually used in paleontological studies. In this case, rates will be higher if the changes is strong proportionally to the mean value of the trait. These two approaches can be interpreted in terms of standardization: the trait is divided either by its intraspecific variability or by its average value. The distinction between these two conceptions of evolutionary rates is especially important if the intraspecific variability of the trait is uncorrelated to its averaged values (e.g. in case of polymorphism).

The challenge of comparing the trait evolution is also methodological, in particular when trait have a very different nature (for example morphology and acoustic signal or behavioral sequences during display, but see Ligon et al. 2018). Fortunately, some traits, including coloration, have multiple functions and can be shaped by either sexual or natural selection. They are prime candidates to examine the relative influence of natural and sexual selections on phenotypes. For instance, colorations of body parts visible from a distance, like dorsal coloration, are usually used for camouflage, and are mainly under the influence of natural selection (Gomez and Théry 2007; Price et al. 2019). On the other hand, colorations of more concealed body parts, like throat or belly, are frequently involved in intrasexual competition and mating choice and are mainly sexually selected (Stuart-Fox and Ord 2004; Clark et al. 2011). Of course, this dichotomy is a simplification of the complex nature of

selective processes acting on body coloration but it allows using the coloration of various body parts to compare the effect of different selective pressures on phenotype.

Here, we used coloration data of 550 species coming from three major clades of vertebrates (Amphibia, Squamata and Aves), to provide for the first multi-clade analysis testing the influence of sexual selection on the disparity and the evolutionary rates of coloration. The intensity of sexual selection can be expected to differ between sexes, due to anisogamy and asymmetrical sex roles (Janicke et al. 2016), and between the body parts considered (see above). Here, we compared for each dataset the disparity and the rates of evolution between sexes and between body parts. We predicted that body parts involved in sexual selection should have a higher disparity and evolutionary rates than body part involved in survival selection, and that this effect should be stronger in males compared to females. We repeated the analysis for both types of standardization to account for the different ways in which rates of change can be standardized.

## **Material and methods**

### **Bird dataset**

We gathered a dataset of spectrometric measurement for 492 species of birds (list of species and sampling locations in Table S2). This dataset was divided in two subsets: (i) unpublished data on 285 species living in the Amazonian tropical forest, and (ii) previously published data on 104 species living on islands and 105 closely related mainland species or subspecies (Doutrelant et al. 2016). We measured, for all individuals of the two subsets, the coloration of six body parts: the back, the wing, the crown, the belly, the breast and the throat. Different species were measured by different persons but the same person always measured the two sexes and all colored patches of any given species ; there is thus no observer effect confounded with the sex of patch effects. We had on average  $2.8 \pm 1.4$  measurements per species, body part and sex over the three datasets. For Amazonian species, measurements were obtained from well preserved specimens available in the collection of the Museum National d'Histoire Naturelle (Paris). We measured the reflectance spectra between 300 and 700nm with a Jaz spectrometer (Ocean Optics), and a coaxial optic fiber (Avantes FCR-7UV200-2-45-ME). For insular and mainland species, the origin of specimens and the details of color measurements are presented in Doutrelant et al. 2016 (especially appendix S2).

When a species had a uniform coloration on several body parts, we only measured one body part and the measurement was duplicated for the others (e.g. only belly was measured when belly, breast and throat coloration were similar using human vision). This procedure was used in the two sub-datasets. When a species had a pattern of several colors on the same body part (e.g. speckled brown and white patterns) we used the mean value of colorimetric variables describing the colors present in the body part, independently of the proportion of each color in the body part for simplicity. We investigated the impact of these changes on the result by repeating the analysis when excluding the averaged and duplicated measurements, and the results remained unchanged.

We retrieved the phylogeny of birds from Birdtree (Jetz et al. 2012). We accounted for phylogenetic uncertainty by including in the analysis 100 random trees from the phylogeny built with all species and the Ericson backbone.

### Lizard dataset

We gathered spectrometric measurement for the dorsal, ventral and lateral body part of 45 species of Lacertidae (listed Table S2, names have been updated to follow current taxonomy). Lateral colorations were measured on conspicuous colored structures: either eyespots or outer ventral scales (OVS) depending on their presence in the species. For 41 species, the mean reflectance spectra were retrieved from a previous study (Pérez i de Lanuza et al. 2013). One of us (AB) also measured the reflectance spectra of seven species, four of which were not in the dataset of Pérez i de Lanuza et al. (2013). For these seven species, AB used a USB-2000 spectrophotometer, a HL2000 (Halogen-Deuterium) light source, and a 400- $\mu$ mR400-7-UV/VIS fiber-optic probe (all products of OceanOptics Inc., Dunedin, FL, USA). This allowed us to quantify intraspecific variability for seven species, with  $7.0 \pm 5.6$  spectra per species, sex and body part. For the three species already measured in Pérez i de Lanuza et al. (2013), we calculated the mean reflectance spectra across individuals, and then averaged this spectra with the mean spectra of Pérez i de Lanuza et al. (2013).

We used the phylogenetic tree built by Garcia-Porta et al. (2019) from genomic data of 262 species of Lacertidae. Only one species included in our analysis was absent from the tree; an undescribed species of *Podarcis* found in Morocco (Djebel Sirwa lineage in Kaliontzopoulou et al. 2011). This species was attached as sister species of *Podarcis vaucheri*, with the divergence date found in Kaliontzopoulou et al. (2011).

## Newt dataset

We sampled 1604 individuals belonging to 18 different lineages of European newts representing all lineages of *Lissotriton* plus three species of *Triturus* and one species of *Ommatotriton* and *Ichthyosaura* (list of lineages Table S2). All newts were anaesthetized prior to manipulation by immersion in Tricaine methane sulphonate (MS222, 0.2 g/l) for 5 minutes. JS measured coloration for the ventral, dorsal and caudal body part (see de Solan et al submitted for a more detailed description of this dataset). We measured the reflectance spectra between 300 and 700 nm using a Jaz spectrometer (Ocean Optics), and a coaxial optic fiber (Avantes FCR-7UV200-2-45-ME). We had on average  $44.3 \pm 63.3$  color measurements per species, sex and body part.

In addition, we characterized newts color patterns by scoring the number and size of the dark spots on the belly, the throat and the flank (six variables, detail of the scores in Table S3). These variables were scored on a subset of the full dataset, with  $17.3 \pm 8.9$  measurements on average per species and sexes. We then made a Principal Component Analysis (PCA) on the 6 scores, in order to reduce the number of variables. We kept in further analysis the first three axes, accounting for 87% of the total variance (see Table S3 for traits contributions to each axis).

**Table 1.** Classification of the measures of color and pattern in two categories: the non-sexual traits and the sexual traits, and the associated literature supporting the classification.

	Non-sexual traits	Sexual traits	References
Newt dataset	- Dorsal coloration	- Tail coloration - Belly coloration - Coloration patterns	Halliday 1977; Fernandez and Collins 1988; Secondi et al. 2007; Hantak and Kuchta 2018; Lütke and Foerster 2018
Bird dataset	- Dorsal coloration - Wing coloration	- Crown coloration - Belly coloration - Breast coloration - Throat coloration	Doucet et al. 2007; Gomez and Théry 2007; Friedman and Remeš 2015; Shultz and Burns 2017; Marcondes and Brumfield 2019
Lizard dataset	- Dorsal coloration	- Belly coloration - Eyespot/OVS coloration	Lopez and Martin 2001; De Lanuza et al. 2014; Reguera et al. 2014; Abalos et al. 2016; Díaz et al. 2017

We built the phylogeny of the European newts using genetic data and bibliographic sources (see details in Supplementary methods).

### Classification into sexual and non-sexual colorations

For each clade separately, body parts were classified a priori as sexual or non-sexual based on the literature (see Table 1, details in Supplementary Materials), though we are aware that this binary classification is a simplification as different body parts may have different functions depending on species.

### Spectrometric analyses

All spectra (from all body parts and datasets) were first smoothed with the `prospec` function of `pavo` package (Maia et al. 2013). Then, we used a vision model (`vismodel` function) to summarize spectra into variables: the luminance and the quantum catch of each cone typical of the clade (4 types for birds and lizards, 3 for newts). Depending on the dataset, we used different settings for the vision models. For birds, we used the cones sensibility of a UV-sensitive bird, *Parus caeruleus* (Hart et al. 2000), and Forest shade spectrum as illuminant (Gomez and Théry 2007). Luminance was estimated as the number of photons caught by double cones, the photoreceptors specialized in achromatic vision in birds (Osorio and Vorobyev 2005). We repeated the analyses with the color vision of *Puffinus pacificus* (Hart 2004), a violet-sensitive bird, and the results remained unchanged. For lizards, we used the color vision of *Podarcis muralis* (described in Martin et al. 2015), and the CIE D65 illuminant (Schanda 2007). We measured the luminance as the stimuli of long-wavelength sensitive cones (as in Pérez i de Lanuza et al. 2018). For the newt analysis, we used the color vision of *Lissotriton vulgaris* (Korenyak and Govardovskii 2013), and the CIE D65 illuminant. We estimated the luminance as the stimuli of long-wavelength sensitive cones. Finally, we measured the transmission spectra of the water in the 77 sampled ponds (see Secondi et al. 2017 for methodology), and we used the mean spectrum as environmental transmission spectrum.

Coloration was quantified by quantum catches instead of hue and chroma, used in many vision studies, because they collectively contain more information, and because the evolution of the hue is complicated to model, since it is a circular variable (like an angle). To allow comparison between variables, we applied two different standardization. For each color variable, sex and body part, we divided the measurements by either (i) the mean

intraspecific standard deviation, or (ii) the mean value of the trait. Finally, we averaged the measurements across individuals, reducing the data to one measurement for each color variable, body part, sex and species. We did not standardize by the mean the axes of the PCA for the newt dataset because PCA axes for males and females are already scaled.

### **Measure of disparity and rate of evolution**

For each data set, we measured multivariate disparity with the `dispRity` package (Guillerme 2018). For each body part and sex separately, disparity was measured as the average Euclidean distance between a given species and the centroids of all species of the clade, in the multivariate space of color variables. This method provides a single measure of disparity for all colors variables describing a given body part, for each species, body part and sex. We repeated the disparity analysis for both type of standardization.

We estimated the rate of evolution along each branch of the phylogeny using the `RRphylo` function of the `RRphylo` package (Castiglione et al. 2018). We made one model of evolution for each color variable, body part and sex (description and number of `RRphylo` model fitted Table S5). We repeated the analysis for both types of standardization. `RRphylo` uses a phylogenetic ridge regression to reconstruct the ancestral state at each node. Then, for each branch, it calculates the rate of evolution as the difference between states divided by the branch length. `RRphylo` does not make any assumption on the tempo and the mode of evolution and is thus more flexible to describe the rate of evolution than methods based on the Brownian model of evolution (Kratsch and McHardy 2014). Because ancestral state reconstruction is more accurate for the terminal nodes than for internal nodes, it is likely that the most accurate rates of evolution are found at the tips of the phylogeny. Thus, we only used the rates of the tip branches (results remained unchanged when including internal branches). We synthesized into a single measure the `RRphylo` rates of all the color variables characterizing a spectrum, by computing the squared root of the sum of the squared evolutionary rates (i.e. an estimate of the Euclidean distance travelled in multivariate phenotypic space per unit of evolutionary time). We thus obtained a synthetic rate of color evolution for each body part, sex and species. This step avoided pseudoreplication of the evolutionary rates with the different color variables.

In addition to the `RRphylo` rates, we also measured Brownian rates of evolution, with one rate for all species, using the `mvMOPRH` package (Clavel et al. 2015). We made this analysis to allow a comparison of our results with the rest of the literature, as Brownian motion models are used in many comparative studies (see Table S1). We could not use

variable-rates Brownian models, such as the one implemented in BayesTrait (Pagel and Meade 2007), as they usually failed to converge in preliminary analyses.

To account for the uncertainty of birds and newts phylogenies, we repeated the RRphylo and the Brownian models on all the phylogenetic trees (100 in each case). We used the mean rate, averaged across all trees, in statistical analysis. We did not account for the phylogenetic uncertainty in lizards because all the nodes of the tree provided by (Garcia-Porta et al. 2019) were highly supported.

### Statistical tests

To compare disparity of coloration between sexes and body parts, we used a Linear Mixed-effect Model with phylogenetic correlation, implemented in 'spaMM' (Rousset 2014). We made one model per dataset. The model included sex, body part and their interaction as fixed effects, and species identity and its interaction with sex as random effects. Phylogenetic correlation was accounted by adding a variance-covariance matrix at the species level, in which the correlation between two species was constrained to decrease in proportion to  $1 - (\text{distance between species}) / (\text{maximum possible distance between two species present the dataset})$ . Distances were estimated as the sums of branch lengths between two species in the phylogenetic tree. We tested the significance of the sex by body part interaction with a LRT test. When the interaction was significant, we made additional models to test separately the effect of body part within each sex, and the effect of sex for each body part, keeping the same random effects than in the full models.

Secondly, we tested for differences in the rate of evolution between sexes and body parts. We analyzed the RRphylo rates of evolution with the same models as for the disparity analysis. For the Brownian rates of evolution, we used a simple linear model with the same fixed factor than for the disparity analysis. We only modeled the Brownian rate of evolution of birds, because Brownian models generate one evolutionary rate per model, leaving too small sample sizes for the tests to have any meaning in lizards and newts dataset (respectively 5 and 4 rates per category of sex-body part).

The statistical tests were repeated for both type of standardization. The measurements of disparity and evolutionary rates (Brownian and RRphylo) were log-transformed before analysis to insure normality. For each model, we investigated homoscedasticity and normality using diagnostic plots of models' residuals. We did not find patterns indicating violation of the linear model hypotheses.

### **Specific adjustments for each clade**

A few adjustments had to be made to take into account some peculiarities of each dataset. First, nine lizard species showed sexual coloration on their back, a body part considered as non-sexual in lizards in general (Galán 2008; species specified in Table S1). We could not analyze these nine species separately from the rest of the dorsal rates, due to the phylogenetic correction and the random effects included in the model. We thus removed the corresponding evolutionary rates and disparity measurements before analyses.

Second, the data on patterns in newts (PCA scores) differ in nature from the color variables, which together make a coherent multivariate dataset obtained by homogeneous procedures. We therefore treated patterns separately (testing only the sex effect, though with otherwise the same analysis as for colors).

Third, in bird, we checked that pooling together the two sub-datasets did not globally change the results by performing analyses separately (see Supplementary methods).

## **Results**

The results presented hereafter were obtained from data standardized by the intraspecific variability but both types of standardization lead to overall similar conclusion (results for the data standardized by the mean trait values can be found in Supplementary Material). The type of standardization had a low effect for the birds and newts datasets, but it influenced the results found in lacertids. For them, the standardization by the mean leads to a general increase of the disparity and evolutionary rates in males, without changing the comparison between body parts (see Supplementary Material).

In birds, the results were generally in agreement with our predictions. For most body parts, including all sexual colorations, males had higher disparities and evolutionary rates than females – the only coloration without a strong male-female difference in disparity of evolutionary rate being wing coloration (Figure 1 and 2, see also table S6 and S7, model split by body parts). In addition, in both sexes, the four sexual coloration had higher disparities and evolutionary rates than the two nonsexual ones (Figure 1 and 2, table S6 and S7, model split by sex). The likelihood-ratio test revealed a significant interaction between body parts and sex for disparity, but not for evolutionary rates ( $\chi^2=35.9$ ,  $p<0.001$  and  $\chi^2=7.4$ ,  $p=0.18$

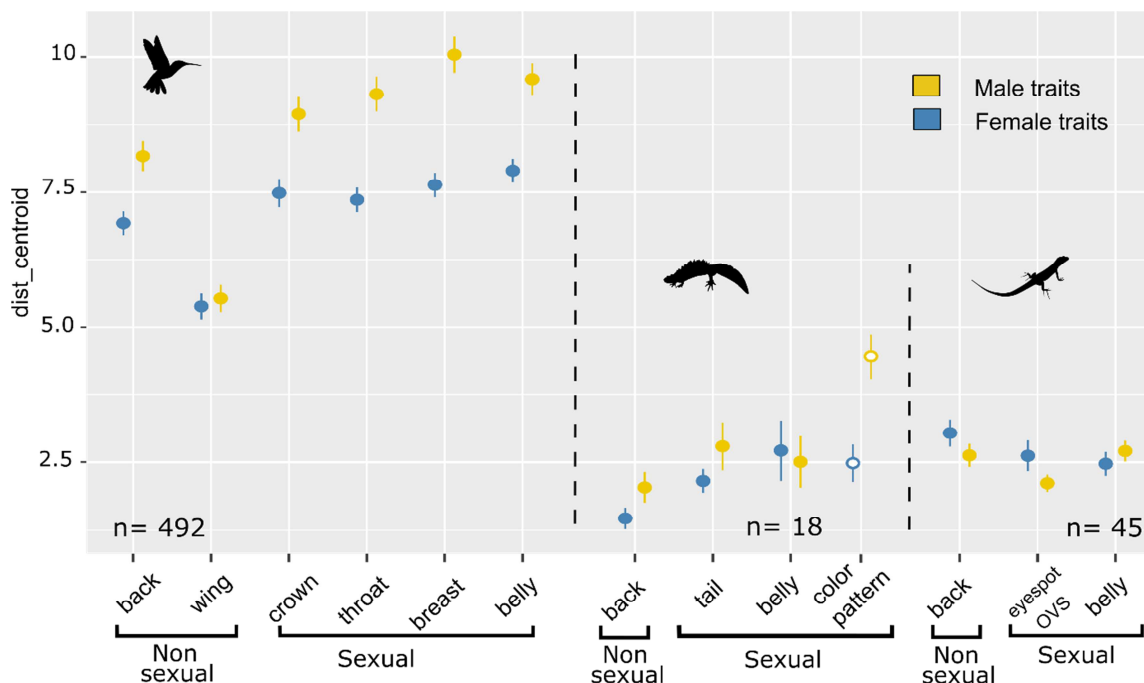


## Sexual selection and evolutionary rates

respectively), indicating that disparity differed more between sexual and non-sexual colorations for males than for females.

In newts, the results were only partially in agreement with our prediction. Coloration of body parts under sexual selection (tail and belly) had a higher disparity and evolutionary rates than dorsal coloration, a nonsexual trait (table S6). However, for all body parts, disparity and evolutionary rates of coloration variables were similar between sexes (Figure 1 and 2, table S6). Last, the PCA scores based on sexually selected patterns were significantly more disparate and had higher evolutionary rates in males than in females (disparity:  $t=10.27$ ,  $p<0.001$ ; evolutionary rates:  $t=3.0$ ,  $p=0.003$ ). The likelihood-ratio test indicated a lack of interaction between body parts and sex for disparity and evolutionary rates ( $\chi^2=1.8$ ,  $p=0.39$  and  $\chi^2=3.6$ ,  $p=0.17$  respectively).

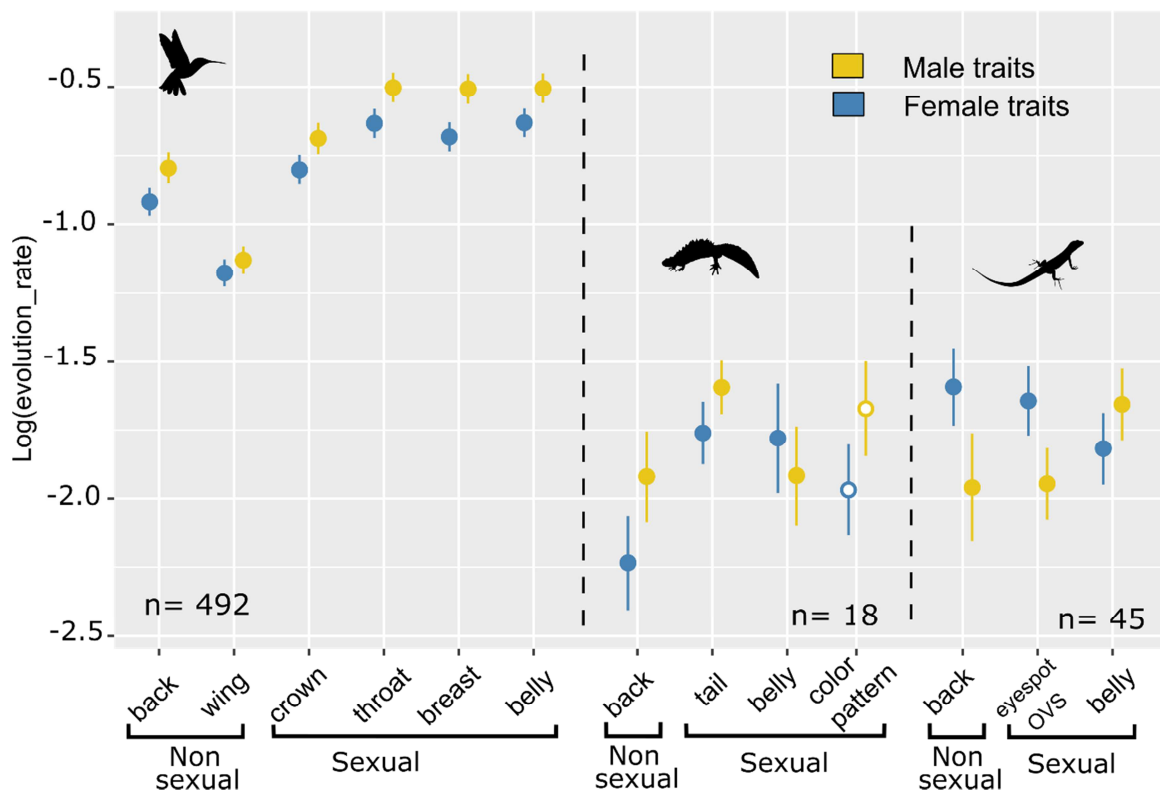
In lizards, the results did not fit our prediction. Depending on the trait, disparity and evolutionary rates were either similar between females and males or higher in females (Figure 1, Tables S7 and S8, model split by body parts). Moreover, the two body parts under sexual selection (belly and OVS\_Eyespot coloration) did not show higher disparity and evolutionary rates than did dorsal coloration (Tables S7 and S8, model split by sex). The



**Figure 1.** Mean color disparity ( $\pm$  Standard Error) measured in the bird, newt and lizard dataset for each body part and sex. Color measurements were standardized by mean intraspecific standard deviation before analysis. Color pattern of newts are not the same type of data and they were analyzed separately.

interaction between body parts and sex was significant for both disparity and evolutionary rates ( $\chi^2=7.3$ ,  $p=0.02$  and  $\chi^2=10.3$ ,  $p=0.006$  respectively).

Additional analyses confirmed that (i) Brownian rates of evolution were qualitatively similar to those of the RRphylo rates (Table S9, Figure S1) and (ii) most patterns found in the bird dataset were replicated in all sub-datasets (Supplementary Material and Table S10).



**Figure 2.** Mean rates of evolution ( $\pm$  Standard Error) measured in the bird, newt and lizard dataset for each body part and sex. Color measurements were standardized by mean intraspecific standard deviation before analysis. The rates of evolution were estimated with RRphylo models. Color pattern of newts are not the same type of data and they were analyzed separately.

## **Discussion**

Our analyses revealed that sexual selection does not always increase the disparity and the rates of evolution of coloration in vertebrates, a result at odd with the vast majority of empirical studies on the subject (Table S1). In birds, where most of the previous studies were made, our results were in agreement with previous findings: we found higher disparity and evolutionary rates in males and for body parts under sexual selection (Seddon et al. 2013; Cooney et al. 2019; Marcondes and Brumfield 2019). This confirms and extends previous results, with an independent dataset comparable in size to the largest published ones (around 500 species), and larger in taxonomic breadth (all birds, not only passerines). However, in newts and lacertids, taxa never investigated until now, the effect of sexual selection was less clear. In newts, body parts under sexual selection had higher disparity and evolutionary rates than body part under non-sexual selection, but there were differences of evolution between sexes only for pattern, and not for colorimetric variables themselves. In lacertids, the results did not match the predictions, as males and body parts under sexual selection had similar or lower disparity and evolutionary rates than females and body parts under non-sexual selection. It thus appears that the commonly held perception that sexual selection favors rapid evolution of body colors is biased by a disproportionate focus on one or a few groups (birds, and possibly some fishes) in which the color repertoire seems particularly responsive.

The fact that we found low disparity and evolutionary rates for sexual colorations with the two types of standardization support the generality of our conclusion. However, the differences we found between standardization in lizards reveal the importance to consider this issue. Lizard coloration, and in particular ventral coloration, is highly variable, and several species included in the analysis show color polymorphism (Huyghe et al. 2012; Speybroeck et al. 2016; Pérez i de Lanuza et al. 2018). In such case, the two type of standardization give a different weight to different things. The standardization by the intraspecific variation emphasizes the potential overlap between species morphs, while the standardization by the mean gives a stronger role to the average change between species. Ideally, these two approaches would be combined using evolution models accounting for the intraspecific variability of the traits. However, this option is only available for some simple model of evolution (Revell and Graham Reynolds 2012), and remain to be developed for branch-specific models.

Why sexually-selected colorations undergo evolutionary acceleration in some clades but not in others may depend on the type of information they convey: on species identity versus on mate quality (Hill 2015). Colorations involved in species recognition should diverge faster, because any divergence (whatever the direction each species takes) is positively selected in closely related taxa to avoid hybridization (Prum 2012). For example, if the ancestral species has a green patch, a change to either yellow or red or blue will be favored. On the other hand, divergence in coloration indicating mate quality is limited by the necessity to provide honest information and by receiver sensory bias (Hill 2011; Biernaskie et al. 2014)- say, assuming that a red patch is a signal of male quality, males that have no red and/or females that reject red males, will be initially counter-selected. However, some studies found high evolutionary rates for sexual traits indicating individual quality (e.g. carotenoids coloration Cooney et al. 2019), suggesting that the differences between the two types of sexual traits may not be so clear. In addition, some color patches could serve for both species recognition and quality assessment, either simultaneously or depending on the species.

In lizards, our results are partially in agreement with this hypothesis. OVS and eyespots colorations have low rates of evolution and are used to assess the quality of males and/or their fighting capacities (Perez et al 2014). Belly coloration, which also have low rates of evolution, is known to be link to alternative reproductive strategies (Calsbeek et al. 2010) and may be an indicator of body condition (Martín et al. 2008; but see De Lanuza et al. 2014). The role of coloration in interspecific recognition was never studied for our species, but this information is known to be convey by the pheromones (Cooper and Pèrez-Mellado 2002). On the other hand, the pattern found in newts contradicts this theory, as the ventral coloration, which has low rate of evolution, is used in species recognition and is not correlated with indicators of condition (Secondi et al. 2010; Kopecký and Šichtař 2014). In birds, it seems very difficult to make generalizations on which sexual colorations serve as signals of species recognition versus intrinsic quality. For instance ventral coloration inform about species identity in chestnut-bellied flycatcher (Uy et al. 2009) but about individual quality in Great Tit (Senar et al. 2008). Nonetheless, the information conveyed by sexual colorations are still unknown for most of the species. All in all, the observation that clades differ in whether sexually-selected colorations evolve faster than non-sexually selected ones cannot at present be directly related to a difference in functions of sexual coloration among the clades – although functional heterogeneity probably introduces noise in the sexual-nonsexual comparisons.

Due to the anisogamy, sexual selection is expected to be stronger in males than in females (Janicke et al. 2016). Yet, we found in both birds and newts datasets that females, like males, exhibit a higher disparity and higher evolutionary rates in some of their sexual colorations compared to their non-sexual colorations. Such pattern was already observed in birds (Marcondes and Brumfield 2019). It could have three non-exclusive origins. Firstly, the evolution of sexual traits in females is linked to the evolution of the same traits in males because they share a large part of their genome (Lande 1980). Cross-sex genetic correlation is often strongly positive ( $>0.8$ ) and may thus drive the evolution of female phenotype in a wide range of taxa (Poissant et al. 2010). Secondly, it is also possible that female sexual colorations are “true” sexual traits. This is a potential explanation for the three clades, as mutual mate choice has been described in several species of newt, lizards and birds present our dataset (Verrell 1986; Wolf et al. 2004; Nolan et al. 2010; Huyghe et al. 2012; Lüdtkke and Foerster 2018). Lastly, the coloration of a body part may have a different function in males and females: it could influence mating choice in males and mediate social interaction in female (Watson and Simmons 2010; Baldauf et al. 2011). Social selection influences the traits used in social interaction, like in territorial defense or resource acquisition, and is predicted to also increase the rates of phenotypic evolution (West-Eberhard 1983; Drury et al. 2018). It is a plausible explanation, especially in birds, where it has been found to have a strong influence on female coloration (Tobias et al. 2012; Dey et al. 2015; Doutrelant et al. 2020).

One limit of our analysis lies in the classification between sexual and non-sexual body-parts, which may be inaccurate for several reasons. First, some colorations may have been attributed to the wrong category because the function of the coloration for a body part may vary among species. In particular, coloration considered as non-sexual in our analysis may actually be sexual in some species. This inaccuracy may be important in birds, as it could explain the general increase of disparity and evolutionary rates observed in males compared to females, while differences between sexes were only predicted for sexual traits. Secondly, it is possible that what we considered as sexual ornament is instead involved in social interactions (see previous paragraph). This issue should have a limited impact on our results for males, because the role of male mating choice and intrasexual competition has been extensively described for the colorations we studied (references Table 1), but it could explain the patterns found in females. Nonetheless, it is difficult to evaluate the influence of social selection in our analysis because currently no large-scale study investigated the relative influence of social and sexual selection on the evolution of coloration.

In this analysis, we only investigated a small part of the phenotypic differences that may exist among species (the rest lies in the size, the shape, the color pattern...), and we thus cannot conclude about the role of sexual selection in global phenotypic diversification of vertebrates. It is thus possible that the disparity/evolutionary rate of the whole phenotype was actually increased by sexual selection in some of the studied taxa, but that this effect was not detected – being concentrated on traits that we did not measure (e.g. pheromones in lizards). To circumvent this limitation, which actually concerns most comparative studies, we need more integrative measures of phenotype that can include diverse types of information (e.g. coloration, shape). Some methods are currently in development (see de Solan et al. 2020 for a method based on artificial neural network), and we thus expect that this limit will disappear with their improvement and popularization.

## Conclusion

Here, we showed that, contrary to what was previously reported in literature, sexual selection does not always increase the rates of evolution or the phenotypic disparity (Arnegard et al. 2010; Klaczko et al. 2015; Marcondes and Brumfield 2019). Indeed, some colorations in newts and lacertids were conserved, despite being sexually selected. This suggests that the relative role of sexual and natural selections might vary between the major groups of vertebrates, although more empirical studies on non-avian models would be needed to validate this hypothesis. This findings, associated with the fact that sexual coloration only evolved in a small fraction of the animal kingdom (Wiens and Tuschhoff 2020), also suggest that the role of sexual selection in the diversity of phenotype observed in nature may have been overestimated. One possible reason is a taxon bias in favor of passerine birds, a group in which sexually selected color patches are strikingly labile and diverse. The next step of our analysis would be to understand what determine the variation of lability and conservation observed for sexual trait. In particular, it would thus be interesting, in a future study, to investigate if traits acting as signals differ in their rates of evolution depending on the type of information they convey. We also suggest that more empirical studies of evolutionary rates and phenotypic diversity of males versus females or sexually versus non-sexually selected traits are needed in a large variety of taxa, and warn against potential publication biases when no effects of sexual selection are detected.

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## References

- Arnegard, M. E., P. B. McIntyre, L. J. Harmon, M. L. Zelditch, W. G. R. Crampton, J. K. Davis, J. P. Sullivan, et al. 2010. Sexual signal evolution outpaces ecological divergence during electric fish species radiation. *The American naturalist* 176:335–56.
- Baeckens, S., J. Martín, R. García-Roa, P. Pafilis, K. Huyghe, and R. Van Damme. 2018. Environmental conditions shape the chemical signal design of lizards. (K. McGraw, ed.) *Functional Ecology* 32:566–580.
- Baldauf, S. A., T. C. M. Bakker, H. Kullmann, and T. Thünken. 2011. Female nuptial coloration and its adaptive significance in a mutual mate choice system. *Behavioral Ecology* 22:478–485.
- Biernaskie, J. M., A. Grafen, and J. C. Perry. 2014. The evolution of index signals to avoid the cost of dishonesty. *Proceedings of the Royal Society B: Biological Sciences* 281:20140876.
- Boul, K. E., W. Chris Funk, C. R. Darst, D. C. Cannatella, and M. J. Ryan. 2007. Sexual selection drives speciation in an Amazonian frog. *Proceedings of the Royal Society B: Biological Sciences* 274:399–406.
- Castiglione, S., G. Tesone, M. Piccolo, M. Melchionna, A. Mondanaro, C. Serio, M. Di Febbraro, et al. 2018. A new method for testing evolutionary rate variation and shifts in phenotypic evolution. (N. Cooper, ed.) *Methods in Ecology and Evolution* 9:974–983.
- Clark, D. L., J. A. Roberts, M. Rector, and G. W. Uetz. 2011. Spectral reflectance and communication in the wolf spider, *Schizocosa ocreata* (Hentz): Simultaneous crypsis and background contrast in visual signals. *Behavioral Ecology and Sociobiology* 65:1237–1247.
- Clavel, J., G. Escarguel, and G. Merceron. 2015. mvMORPH : an R package for fitting multivariate evolutionary models to morphometric data. (T. Poisot, ed.) *Methods in Ecology and Evolution* 6:1311–1319.
- Cooney, C. R., Z. K. Varley, L. O. Nouri, C. J. A. Moody, M. D. Jardine, and G. H. Thomas. 2019. Sexual selection predicts the rate and direction of colour divergence in a large avian radiation. *Nature Communications* 10:1–9.
- Cooper, W. E., and V. Pérez-Mellado. 2002. Pheromonal discriminations of sex, reproductive condition, and species by the lacertid lizard *Podarcis hispanica*. *Journal of Experimental Zoology* 292:523–527.
- Darwin, C. 1871. *The descent of man, and selection in relation to sex.* (John Murray, ed.). London, UK.
- de Solan, T., J. P. Renoult, P. Geniez, P. David, and P. A. Crochet. 2020. Looking for Mimicry in a Snake Assemblage Using Deep Learning. *The American naturalist* 196:74–86.
- Dey, C. J., M. Valcu, B. Kempenaers, and J. Dale. 2015. Carotenoid-based bill coloration functions as a social, not sexual, signal in songbirds (Aves: Passeriformes). *Journal of Evolutionary Biology* 28:250–258.
- Doutrelant, C., A. Fargevieille, and A. Grégoire. 2020. Evolution of female coloration: What have we learned from birds in general and blue tits in particular. Pages 124–181 in *Advances in the Study of Behavior*. Academic Press Inc.
- Doutrelant, C., M. Paquet, J. P. Renoult, A. Grégoire, P. A. Crochet, and R. Covas. 2016. Worldwide patterns of bird colouration on islands. (G. Grether, ed.) *Ecology Letters*. Blackwell Publishing Ltd.
- Drury, J. P., J. A. Tobias, K. J. Burns, N. A. Mason, A. J. Shultz, and H. Morlon. 2018. Contrasting impacts of competition on ecological and social trait evolution in songbirds. *PLoS Biology*



16:e2003563.

- Galán, P. 2008. Ontogenetic and sexual variation in the coloration of the lacertid lizards *Iberolacerta monticola* and *Podarcis bocagei*. Do the females prefer the greener males? *Animal Biology* 58:173–198.
- García-Porta, J., I. Irisarri, M. Kirchner, A. Rodríguez, S. Kirchhof, J. L. Brown, A. MacLeod, et al. 2019. Environmental temperatures shape thermal physiology as well as diversification and genome-wide substitution rates in lizards. *Nature Communications* 10:1–12.
- Gomez, D., and M. Théry. 2007. Simultaneous crypsis and conspicuousness in color patterns: comparative analysis of a neotropical rainforest bird community. *The American naturalist* 169:S42–S61.
- Gonzalez-Voyer, A., and N. Kolm. 2011. Rates of phenotypic evolution of ecological characters and sexual traits during the Tanganyikan cichlid adaptive radiation. *Journal of Evolutionary Biology* 24:2378–2388.
- Guillermé, T. 2018. dispRity: A modular R package for measuring disparity. (T. Poisot, ed.) *Methods in Ecology and Evolution* 9:1755–1763.
- Hart, N. S. 2004. Microspectrophotometry of visual pigments and oil droplets in a marine bird, the wedge-tailed shearwater *Puffinus pacificus*: Topographic variations in photoreceptor spectral characteristics. *Journal of Experimental Biology* 207:1229–1240.
- Hart, N. S., J. C. Partridge, I. C. Cuthill, and A. T. D. Bennett. 2000. Visual pigments, oil droplets, ocular media and cone photoreceptor distribution in two species of passerine bird: The blue tit (*Parus caeruleus* L.) and the blackbird (*Turdus merula* L.). *Journal of Comparative Physiology - A Sensory, Neural, and Behavioral Physiology* 186:375–387.
- Hill, G. E. 2011. Condition-dependent traits as signals of the functionality of vital cellular processes. *Ecology Letters* 14:625–634.
2015. Sexiness, Individual Condition, and Species Identity: The Information Signaled by Ornaments and Assessed by Choosing Females. *Evolutionary Biology* 42:251–259.
- Hoekstra, H. E., J. M. Hoekstra, D. Berrigan, S. N. Vignieri, A. Hoang, C. E. Hill, P. Beerli, et al. 2001. Strength and tempo of directional selection in the wild. *Proceedings of the National Academy of Sciences of the United States of America* 98:9157–9160.
- Huyghe, K., B. Vanhooydonck, A. Herrel, Z. Tadić, and R. Van Damme. 2012. Female lizards ignore the sweet scent of success: Male characteristics implicated in female mate preference. *Zoology* 115:217–222.
- Janicke, T., I. K. Häderer, M. J. Lajeunesse, and N. Anthes. 2016. Evolutionary Biology: Darwinian sex roles confirmed across the animal kingdom. *Science Advances* 2:e1500983.
- Jetz, W., G. H. Thomas, J. B. Joy, K. Hartmann, and A. O. Mooers. 2012. The global diversity of birds in space and time. *Nature* 491:444–448.
- Kaliontzopoulou, A., C. Pinho, D. J. Harris, and M. A. Carretero. 2011. When cryptic diversity blurs the picture: a cautionary tale from Iberian and North African *Podarcis* wall lizards. *Biological Journal of the Linnean Society* 103:779–800.
- Kim, T. W., and J. C. Choe. 2003. The effect of food availability on the semilunar courtship rhythm in the fiddler crab *Uca lactea* (de Haan) (Brachyura: Ocypodidae). *Behavioral Ecology and Sociobiology* 54:210–217.
- Kingsolver, J. G., and S. E. Diamond. 2011. Phenotypic selection in natural populations: what limits directional selection? *The American naturalist* 177:346–57.
- Kingsolver, J. G., and D. W. Pfennig. 2007. Patterns and Power of Phenotypic Selection in Nature.

- BioScience 57:561–572.
- Klaczko, J., T. Ingram, and J. Losos. 2015. Genitals evolve faster than other traits in *Anolis* lizards. *Journal of Zoology* 295:44–48.
- Kopecký, O., and J. Šichtař. 2014. Sex differences in ventral side colouration of Alpine newts. *Israel Journal of Ecology & Evolution* 60:29–34.
- Korenyak, D. A., and V. I. Govardovskii. 2013. Photoreceptors and visual pigments in three species of newts. *Journal of Evolutionary Biochemistry and Physiology* 49:399–407.
- Kratsch, C., and A. C. McHardy. 2014. RidgeRace: ridge regression for continuous ancestral character estimation on phylogenetic trees. *Bioinformatics* 30:i527–i533.
- Lande, R. 1980. Sexual dimorphism, sexual selection, and adaptation in polygenic characters. *Evolution* 34:292–305.
- Ligon, R. A., C. D. Diaz, J. L. Morano, J. Troscianko, M. Stevens, A. Moskeland, T. G. Laman, et al. 2018. Evolution of correlated complexity in the radically different courtship signals of birds-of-paradise. *PLoS Biology* 16:e2006962.
- Lüdtke, D. U., and K. Foerster. 2018. Choosy males court both large, colourful females and less colourful but responsive females for longer. *Animal Behaviour* 146:1–11.
- Maia, R., C. M. Eliason, P.-P. Bitton, S. M. Doucet, and M. D. Shawkey. 2013. pavo : an R package for the analysis, visualization and organization of spectral data. (A. Tatem, ed.) *Methods in Ecology and Evolution* 4:906–913.
- Marcondes, R. S., and R. T. Brumfield. 2019. Fifty shades of brown: Macroevolution of plumage brightness in the Furnariida, a large clade of drab Neotropical passerines. *Evolution* 73:704–719.
- Martin, M. D., and T. C. Mendelson. 2014. Changes in sexual signals are greater than changes in ecological traits in a dichromatic group of fishes. *Evolution* 68:3618–3628.
- Martin, M., J. F. Le Galliard, S. Meylan, and E. R. Loew. 2015. The importance of ultraviolet and near-infrared sensitivity for visual discrimination in two species of lacertid lizards. *Journal of Experimental Biology* 218:458–465.
- Masta, S. E., and W. P. Maddison. 2002. Sexual selection driving diversification in jumping spiders. *Proceedings of the National Academy of Sciences of the United States of America* 99:4442–4447.
- Mayr, E. 1972. Sexual selection and natural selection. Pages 87–104 in *Sexual selection and the descent of man: The darwinian pivot*.
- Nolan, P. M., F. Stephen Dobson, M. Nicolaus, T. J. Karels, K. J. McGraw, and P. Jouventin. 2010. Mutual Mate Choice for Colorful Traits in King Penguins. *Ethology* 116:635–644.
- Oliver, J. C., K. A. Robertson, and A. Monteiro. 2009. Accommodating natural and sexual selection in butterfly wing pattern evolution. *Proceedings of the Royal Society B: Biological Sciences* 276:2369–2375.
- Osorio, D., and M. Vorobyev. 2005. Photoreceptor spectral sensitivities in terrestrial animals: Adaptations for luminance and colour vision. *Proceedings of the Royal Society B: Biological Sciences*. Royal Society.
- Pagel, M., and A. Meade. 2007. BayesTraits - Available at <http://www.evolution.rdg.ac.uk/BayesTraitsV3.0.2>.
- Pérez i de Lanuza, G., J. Ábalos, A. Bartolomé, and E. Font. 2018. Through the eye of a lizard: Hue discrimination in a lizard with ventral polymorphic coloration. *Journal of Experimental Biology* 221.

- Pérez i de Lanuza, G., E. Font, and J. L. Monterde. 2013. Using visual modelling to study the evolution of lizard coloration: Sexual selection drives the evolution of sexual dichromatism in lacertids. *Journal of Evolutionary Biology* 26:1826–1835.
- Poissant, J., A. J. Wilson, and D. W. Coltman. 2010. Sex-specific genetic variance and the evolution of sexual dimorphism: a systematic review of cross-sex genetic correlations. *Evolution* 64:97–107.
- Price, N., S. Green, J. Troschianko, T. Tregenza, and M. Stevens. 2019. Background matching and disruptive coloration as habitat-specific strategies for camouflage. *Scientific Reports* 9:1–10.
- Prum, R. O. 2012. Aesthetic evolution by mate choice: Darwin’s really dangerous idea. *Philosophical Transactions of the Royal Society B: Biological Sciences*. Royal Society.
- Pryke, S. R., and S. C. Griffith. 2007. The relative role of male vs. female mate choice in maintaining assortative pairing among discrete colour morphs. *Journal of Evolutionary Biology* 20:1512–1521.
- Revell, L. J., and R. Graham Reynolds. 2012. A new bayesian method for fitting evolutionary models to comparative data with intraspecific variation. *Evolution* 66:2697–2707.
- Rosenblum, E. B. 2006. Convergent evolution and divergent selection: lizards at the White Sands ecotone. *The American naturalist* 167:1–15.
- Rousset, F. 2014. spaMM: Mixed models, particularly spatial GLMMs.
- Sacchi, R., A. J. Coladonato, M. Ghitti, M. Mangiacotti, S. Scali, M. Bovo, and M. Zuffi. 2018. Morph-specific assortative mating in common wall lizard females. *Current Zoology* 64:449–453.
- Schanda, J. 2007. CIE Colorimetry. Pages 25–78 in *Colorimetry*. John Wiley & Sons, Inc., Hoboken, NJ, USA.
- Secondi, J., V. Dupont, A. Davranche, N. Mondy, T. Lengagne, and M. Théry. 2017. Variability of surface and underwater nocturnal spectral irradiance with the presence of clouds in urban and peri-urban wetlands. (T. Longcore, ed.) *PLOS ONE* 12:e0186808.
- Secondi, J., A. Johanet, O. Pays, F. Cazimajou, Z. Djalout, and C. Lemaire. 2010. Olfactory and visual species recognition in newts and their role in hybridization. *Behaviour* 147:1693–1712.
- Seddon, N., C. A. Botero, J. A. Tobias, P. O. Dunn, H. E. A. MacGregor, D. R. Rubenstein, J. A. C. Uy, et al. 2013. Sexual selection accelerates signal evolution during speciation in birds. *Proceedings of the Royal Society B: Biological Sciences* 280:20131065–20131065.
- Senar, J. C., J. J. Negro, J. Quesada, I. Ruiz, and J. Garrido. 2008. Two Pieces of Information in a Single Trait? The Yellow Breast of the Great Tit (*Parus major*) Reflects Both Pigment Acquisition and Body Condition. *Behaviour* 145:1195–1210.
- Shine, R., and S. Shetty. 2001. The influence of natural selection and sexual selection on the tails of sea-snakes (*Laticauda colubrina*). *Biological Journal of the Linnean Society* 74:121–129.
- Speybroeck, J., W. Beukema, B. Bok, J. Van Der Voort, and I. Velikov. 2016. *Field Guide to the Amphibians and Reptiles of Britain and Europe*. Bloomsbury Publishing.
- Starr, C., R. Taggart, C. Evers, and L. Starr. 2014. *Biology: The Unity and Diversity of Life* (Nelson Education.).
- Stuart-Fox, D. M., and T. J. Ord. 2004. Sexual selection, natural selection and the evolution of dimorphic coloration and ornamentation in agamid lizards. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 271:2249–2255.
- Tobias, J. A., R. Montgomerie, and B. E. Lyon. 2012. The evolution of female ornaments and

- weaponry: Social selection, sexual selection and ecological competition. *Philosophical Transactions of the Royal Society B: Biological Sciences* 367:2274–2293.
- Tobler, M., M. Palacios, L. J. Chapman, I. Mitrofanov, D. Bierbach, M. Plath, L. Arias-Rodriguez, et al. 2011. Evolution in extreme environments: replicated phenotypic differentiation in livebearing fish inhabiting sulfidic springs. *Evolution* 65:2213–2228.
- Uy, J. A. C., R. G. Moyle, and C. E. Filardi. 2009. Plumage and song differences mediate species recognition between incipient Flycatcher species of the Solomon Islands. *Evolution* 63:153–164.
- Verrell, P. A. 1986. Male Discrimination of Larger, More Fecund Females in the Smooth Newt, *Triturus vulgaris*. *Journal of Herpetology* 20:416.
- Watson, N. L., and L. W. Simmons. 2010. Mate choice in the dung beetle *Onthophagus sagittarius*: are female horns ornaments? *Behavioral Ecology* 21:424–430.
- West-Eberhard, M. J. 1983. Sexual selection, social competition, and speciation. *Quarterly Review of Biology* 58:155–183.
- Wiens, J. J., and E. Tuschhoff. 2020. Songs versus colours versus horns: what explains the diversity of sexually selected traits? *Biological Reviews* 95:847–864.
- Wolf, W. L., J. M. Casto, V. Nolan, and E. D. Ketterson. 2004. Female ornamentation and male mate choice in dark-eyed juncos. *Animal Behaviour* 67:93–102.

## Annexes: Supplementary Methods

### Newt phylogeny

The phylogeny of the European newts is still poorly resolved, partly because of past gene flow among some of the *Lissotriton* lineages (Pabijan et al. 2017), partly because multilocus sequence data are not available for all species of *Lissotriton*. To solve this problem, we built a dataset of 70 nuclear genes for 69 individuals belonging to the 13 lineages of *Lissotriton* (list of the sequenced genes Table S4). Data for nine of the thirteen lineages have already been published (Pabijan et al. 2017). We concatenated the sequences and performed a Bayesian phylogenetic analysis with a relaxed molecular clock approach, implemented in the software Mr. Bayes. We used a general time-reversible model of nucleotide substitution allowing for gamma-distributed substitution rate variation among sites and invariant sites (GTR+G+I; Yang 1994). We ran two analysis with four Markov Chain Monte Carlo (MCMC) chains for  $5 \times 10^6$  generations, sampling every 1000 generations after discarding the first 25% of the chains as burn-in. We verified chains stationarity (stable joint probability distribution across generations) and convergence of independent chains by verifying their split frequency distributions throughout the chains and by checking that the effective sample sizes of model parameter estimates were greater than 200 using the TRACER software (Rambaut and Drummond 2004). We saved 100 random trees of the posterior distribution. Individuals of the same lineage were grouped together in all the trees. For each tree, we sampled one randomly chosen individual of each lineage, in order to create a specie tree. We then added the five remaining non-*Lissotriton* species. Their positions in the tree and their divergence times were obtained from bibliographic sources (Zhang et al. 2008; Wielstra et al. 2019).

### Classification into sexual and non-sexual traits

Using literature sources, we classified all the traits in the sexual and non-sexual category (classification and literature summarized Table 1). We classified in the non-sexual traits category the coloration of the back of newts, lizards and birds, plus the coloration of the wing of birds. For the sexual trait category, we included the ventral and lateral coloration of lizards, the ventral and caudal coloration of newts, and the coloration of the crown and all the ventral patches (belly, breast, throat) of birds. For the newts, we considered the caudal

coloration and ventral patterns of coloration as sexual traits, although their role was never properly investigated, because these traits are all sexually dimorphic, and the tail plays, in general, an important role in male courtship (Halliday 1975).

The selective pressures acting on sexual traits are often different in males and female (Dale et al. 2015). Yet, numerous studies found a correlation in ornaments evolution between sexes (Poissant et al. 2010; Potti and Canal 2011). Likewise, we detected in positive correlation between the rate of males and females in all dataset ( $p < 0.001$  every time, Figure S2). Traits were thus classified into sexual categories in both sex, even if their roles in mating choice or intrasexual competition was described in one sex only.

### **Influence of sampling origin in birds**

Differences between non-sexual and sexual traits for the bird dataset could have been influenced by the sampling origin. Sexual selection is expected to be reduced in islands (discussion Doutrelant et al. 2016), and non-sexual traits are expected to diverge between species sampled around the world, while they should converge for species sampled in the same community. Such pattern is not expected for sexual traits, as species living in the same environment often show strong variation in sexual attributes (Crampton et al. 2011).

We could not include a factor “sampling origin” in the linear Mixed-effect model with all species, because it would have led to a three-way interaction (sex:body\_part:sampling\_origin) which would be uninterpretable. Instead, we isolated the disparity measurements and the RRphylo rates of the island species, mainland species and Amazonian forest species, and for each origin separately, we built two Linear Mixed-effect Models (one for disparity and one for evolutionary rates) with the same effects than for the disparity analysis. We used the disparity and evolutionary rates measurements based on the colorimetric values standardized by the mean intraspecific standard deviation. The species with two sampling origins, *Amazona farinosa* and *Butorides striata*, were removed before analysis.

## Annexes: Supplementary results

### Results of models based on colorimetric variables standardized by the mean value

In birds, the results were generally similar with those obtained with the standardization with mean intraspecific standard deviation. The likelihood-ratio test indicated that the interaction between body parts and sex was non-significant for both for disparity and evolutionary rates ( $\chi^2=3.6$ ,  $p=0.60$  and  $\chi^2=8.7$ ,  $p=0.12$  respectively). Males had higher disparities and evolutionary rates than females for all body parts (Figure S3 and S4, Table S10). In addition, sexually selected body parts had a higher disparity and evolutionary rates than non-sexually selected body parts.

In newts, the results were partially similar than those obtained with the other standardization. The likelihood-ratio test indicated a significant interaction between body parts and sex for disparity, but not for evolutionary rates ( $\chi^2=5.86$ ,  $p=0.05$  and  $\chi^2=5.2$ ,  $p=0.07$  respectively). For all body parts, evolutionary rates were similar between sexes (Figure S3 and S4, Tables S10 and S11). However, both sexes had a similar disparity only for the belly (sexual trait), while males had higher disparity than females for the back (non-sexual trait), and lower disparity than females for the tail (sexual trait, Table S11). Moreover, we did not recover the higher disparity and evolutionary rates for body part under sexual selection we found with the other standardization. Male disparity was similar for body parts under sexual and non-sexual selection, while female had a higher disparity for the tail than for both back and belly. Both sexes, showed higher evolutionary rates for the tail compared to belly and back. Finally, the PCA scores describing sexually-selected patterns indicated a higher disparity for males compared to females, but similar evolutionary rates (disparity:  $t=4.0$ ,  $p<0.001$ ; evolutionary rates:  $t=-0.43$ ,  $p=0.66$ ).

In lizards, the results showed some differences with those obtained while standardizing with mean intraspecific standard deviation. The interaction between body parts and sex was significant for evolutionary rates but not for disparity ( $\chi^2=4.0$ ,  $p=0.13$  and  $\chi^2=8.1$ ,  $p=0.02$  respectively). The main difference was that males had higher disparity than females for all body parts. In addition, males had higher evolutionary rates than females for the belly (sexual traits), but not for the back and the OVS\_Eyespot (a non-sexual and sexual trait; Figure S3 and S4, Table S10 and S12). However, the two body parts under sexual selection show similar or lower disparity and evolutionary rates than did dorsal coloration.

### **Influence of sampling origin in birds**

The higher disparity detected in males compared to females in the whole dataset was recovered for the mainland and Amazonian forest species, but not for island species (Figure S5, Tables S13). Moreover, the higher disparity for body part under sexual selection compared to body part under non-sexual selection was confirmed for Amazonian forest species. In island and mainland species, the body part under sexual selection had similar disparity than dorsal coloration, but a higher disparity than wing coloration.

The higher rates of evolution detected for sexual coloration in the whole dataset analysis was recover for the species of mainland and of the Amazonian forest, but only two sexual traits had higher evolutionary rates that non-sexual ones in insular species (Figure S6, Table S13). In addition, the increase of evolutionary rates in males compared to females detected in the whole dataset was confirmed for all origins.



Article	Taxa (number of sp. included)	Trait measured	Index used	Test realised		Result	Methodological issue
				Male vs female	Sexual vs nonsexual traits		
Friedman and Remes 2015	Maluridae (Bird - 24)	Plumage coloration (11 patches)	Evolutionary rates ( $\sigma^2$ BM)	x	x	Higher evolutionary rates in males and for patches used in social and sexual signalling	Colour variables not standardized
Marcondes and Brumfield 2019	Furnariida (Bird - 586)	Plumage coloration (7 patches)	Evolutionary rates ( $\sigma^2$ BM)	x	x	Higher evolutionary rates for patches used in social and sexual signalling, but no difference between sexes	
Cooney et al. 2019	Tyrannida (Bird - 372)	Plumage coloration (10 patches)	Evolutionary rates (BayesTrait variable rates models)	x	x	Higher evolutionary rates in males and for patches used in social and sexual signalling	
Seddon et al. 2013	Passerina (Bird - 168)	Plumage coloration (6 patches)	Evolutionary rates and disparity ( $\sigma^2$ BM/OU)	x	x	Evolutionary rates increase in males with dichromatism for patches used in social and sexual signalling. Disparity increases in males with dichromatism.	
Shultz and Burns 2017	Thraupidae (Bird - 351)	Plumage coloration (9 patches)	Evolutionary rates and disparity (intermodal coloration distance)	x		Higher evolutionary rates and disparity in males	
Price and Eaton 2014	Icteridae (Bird - 37)	Plumage coloration (22 patches)	Evolutionary rates (intermodal coloration distance)	x	x	Higher evolutionary rates in female but higher disparity in males for extant species	
Oliver et al. 2009	Satyrinae (Butterflies – 54)	Color pattern	Evolutionary rates (discrete evolutionary rates)	x	x	Higher evolutionary rates for patterns under sexual selection in males	
Martin and Mendelson 2014	Etheostoma (Fish - 16)	Sexual coloration, body shape (42 patches)	Divergence between species pairs		x	Higher divergence for sexual traits	
Arnegard et al. 2010	Paramormyrops (Fish - 21)	Sexual electric signals, body shape and trophic position	Evolutionary rates (mahalanobis distances regressed on time)		x	Higher evolutionary rate for sexual traits	Comparison of species pairs without correction for non-independence
Gonzalez-Voyer and Kolm 2011	Cichlidae (Fish - 49)	Sexual dimorphism and dichromatism, ecological traits	Evolutionary rates ( $\alpha$ OU) and disparity		x	Higher evolutionary rate and disparity for sexual traits	Traits in different scales compared without standardization
Klaczko et al. 2015	Anolis (Reptile - 25)	Genitalia shape, dewlap and limb measurements	Evolutionary rates ( $\sigma^2$ BM)		x	Higher evolutionary rate for sexual traits	

**Table S1.** List of the studies comparing the rates of evolution and/or disparity between sexes or between sexual and non-sexual traits

- Arnegard, M. E., P. B. McIntyre, L. J. Harmon, M. L. Zelditch, W. G. R. Crampton, J. K. Davis, J. P. Sullivan, et al. 2010. Sexual signal evolution outpaces ecological divergence during electric fish species radiation. *The American naturalist* 176:335–56.
- Cooney, C. R., Z. K. Varley, L. O. Nouri, C. J. A. Moody, M. D. Jardine, and G. H. Thomas. 2019. Sexual selection predicts the rate and direction of colour divergence in a large avian radiation. *Nature Communications* 10:1–9.
- Crampton, W. G. R., N. R. Lovejoy, and J. C. Waddell. 2011. Reproductive character displacement and signal ontogeny in a sympatric assemblage of electric fish. *Evolution* 65:1650–1666.
- Dale, J., C. J. Dey, K. Delhey, B. Kempenaers, and M. Valcu. 2015. The effects of life history and sexual selection on male and female plumage colouration. *Nature* 527:367–370.
- Doutrelant, C., M. Paquet, J. P. Renoult, A. Grégoire, P. A. Crochet, and R. Covas. 2016. Worldwide patterns of bird colouration on islands. (G. Grether, ed.) *Ecology Letters*. Blackwell Publishing Ltd.
- Friedman, N. R., and V. Remeš. 2015. Rapid evolution of elaborate male coloration is driven by visual system in Australian fairy-wrens (Maluridae). *Journal of Evolutionary Biology* 28:2125–2135.
- Gonzalez-Voyer, A., and N. Kolm. 2011. Rates of phenotypic evolution of ecological characters and sexual traits during the Tanganyikan cichlid adaptive radiation. *Journal of Evolutionary Biology* 24:2378–2388.
- Halliday, T. R. 1975. An observational and experimental study of sexual behaviour in the smooth newt, *Triturus vulgaris* (Amphibia: Salamandridae). *Animal Behaviour* 23:291–322.
- Klaczko, J., T. Ingram, and J. Losos. 2015. Genitals evolve faster than other traits in *Anolis* lizards. *Journal of Zoology* 295:44–48.
- Marcondes, R. S., and R. T. Brumfield. 2019. Fifty shades of brown: Macroevolution of plumage brightness in the Furnariida, a large clade of drab Neotropical passerines. *Evolution* 73:704–719.
- Martin, M. D., and T. C. Mendelson. 2014. Changes in sexual signals are greater than changes in ecological traits in a dichromatic group of fishes. *Evolution* 68:3618–3628.
- Oliver, J. C., K. A. Robertson, and A. Monteiro. 2009. Accommodating natural and sexual selection in butterfly wing pattern evolution. *Proceedings of the Royal Society B: Biological Sciences* 276:2369–2375.
- Pabijan, M., P. Zieliński, K. Dudek, M. Stuglik, and W. Babik. 2017. Isolation and gene flow in a speciation continuum in newts. *Molecular Phylogenetics and Evolution* 116:1–12.
- Poissant, J., A. J. Wilson, and D. W. Coltman. 2010. Sex-specific genetic variance and the evolution of sexual dimorphism: a systematic review of cross-sex genetic correlations. *Evolution* 64:97–107.
- Potti, J., and D. Canal. 2011. Heritability and genetic correlation between the sexes in a songbird sexual ornament. *Heredity* 106:945–954.
- Price, J. J., and M. D. Eaton. 2014. Reconstructing the evolution of sexual dichromatism: current color diversity does not reflect past rates of male and female change. *Evolution* 68:2026–2037.

- Rambaut, A., and A. Drummond. 2004. Tracer v1.7 Available at <https://github.com/beat-dev/tracer/>.
- Seddon, N., C. A. Botero, J. A. Tobias, P. O. Dunn, H. E. A. MacGregor, D. R. Rubenstein, J. A. C. Uy, et al. 2013. Sexual selection accelerates signal evolution during speciation in birds. *Proceedings of the Royal Society B: Biological Sciences* 280:20131065–20131065.
- Shultz, A. J., and K. J. Burns. 2017. The role of sexual and natural selection in shaping patterns of sexual dichromatism in the largest family of songbirds (Aves: Thraupidae). *Evolution* 71:1061–1074.
- Wielstra, B., E. McCartney-Melstad, J. W. Arntzen, R. K. Butlin, and H. B. Shaffer. 2019. Phylogenomics of the adaptive radiation of Triturus newts supports gradual ecological niche expansion towards an incrementally aquatic lifestyle. *Molecular Phylogenetics and Evolution* 133:120–127.
- Yang, Z. 1994. Estimating the pattern of nucleotide substitution. *Journal of Molecular Evolution* 39:105–111.
- Zhang, P., T. J. Papenfuss, M. H. Wake, L. Qu, and D. B. Wake. 2008. Phylogeny and biogeography of the family Salamandridae (Amphibia: Caudata) inferred from complete mitochondrial genomes. *Molecular Phylogenetics and Evolution* 49:586–597.

**Table S2.** List of the species for the birds, newts and Lacertids dataset, associated with the location of the individuals sampled. For the bird dataset, is also precised if the species originates of the Amazonian forest, of island or of mainland. † indicates lacertids species for which the dorsal coloration was considered as a sexual characters.

This table is not available in the printed version of the thesis due to its size, but can be found in the file MS4 Sexual selection and evolution rates at: <https://1drv.ms/f/s!AhWgyBtt5X3FamXDhTlzauD5uXo>

**Table S3.** Description of the coloration pattern traits included in Principal Component Analysis, and their correlations with the first three components. In bold the strongest correlation for each axis.

Traits	Description and trait coding	Axis 1	Axis 2	Axis 3
Belly size	Size of melanic spots on belly: (0) no points, (1) small spots, (2) medium spots, (3) small and large spots, (4) large spots	<b>0.83</b>	0.29	0.11
Belly number	Density of dark spotting on belly: (0) no points, (1) few spots, (2) many spots	<b>0.75</b>	0.34	<b>0.50</b>
Throat size	Size of melanic spots on throat: (0) no points, (1) small spots, (2) medium spots, (3) small and large spots, (4) large spots	<b>0.88</b>	0.07	-0.36
Throat number	Spotting on on throat: (0) no points, (1) few spots, (2) many spots	<b>0.86</b>	0.10	-0.10
Flank size	Size of melanic spots on flank: (0) no spots, (1) small spots, (2) medium spots, (3) small and large spots , (4) large spots, (5) continuous line	<b>0.64</b>	<b>-0.62</b>	-0.28
Flank number	Spotting on flanks: (0) no spot, (1) few spots, (2) many spots, (3) continuous line	0.28	<b>-0.85</b>	0.40
Percent of the variance accounted by the PCA		54%	22%	11%

**Table S4.** List of the individuals and sequenced genes used for the phylogeny of *Lissotriton*.

This table is not available in the printed version of the thesis due to its size, but can be found in the file MS4 Sexual selection and evolution rates at:  
<https://1drv.ms/f/s!AhWgyBtt5X3FamXDhTlZauD5uXo>

**Table S5.** Summary of the body parts and color variables used in each dataset, and estimation of the number of RRphylo models fitted.

Dataset	Body parts measured	Color variables measured	Number of RRphylo model fitted
Bird dataset	Back, wing, crown, throat, breast, belly	5 variables: - Luminance - Quantum catch of the cone u, s, m, l	6000 (2 sexes x 6 body parts x 5 color variables x 100 phylogenetic trees)
Newt dataset	Back, belly, tail	4 variables: - Luminance - Quantum catch of the cone s, m, l	2400 (2 sexes x 3 body parts x 4 color variables x 100 phylogenetic trees)
Lizard dataset	Back, throat, belly	5 variables: - Luminance - Quantum catch of the cone u, s, m, l	3000 (2 sexes x 3 body parts x 5 color variables x 100 phylogenetic trees)

**Table S6.** Raw summary of the Linear mixed effects models made on the disparity and Rrphylo evolution rates, standardized by the intraspecific standard deviation, for the lizards, birds and newts dataset. Results are shown only for the best model after comparison between model with and without sex:body\_part interaction. In the three dataset, the intercept correspond to the back for females.

This table is not available in the printed version of the thesis due to its size, but can be found in the file MS4 Sexual selection and evolution rates at:  
<https://1drv.ms/f/s!AhWgyBtt5X3FamXDhTlzauD5uXo>

**Table S7.** Raw summary of the Linear mixed effects models testing separately the effect of sexes and body parts on the disparity measurements made on the lizards and birds dataset and standardized by the intraspecific standard deviation. In the models separated by sexes, the intercept correspond to the back. In the models separated by body parts, the intercept correspond to females.

This table is not available in the printed version of the thesis due to its size, but can be found in the file MS4 Sexual selection and evolution rates at:  
<https://1drv.ms/f/s!AhWgyBtt5X3FamXDhTlzauD5uXo>

**Table S8.** Raw summary of the Linear mixed effects models testing separately the effect of sexes and body parts on the Rrphylo rate of evolution measured in lizards dataset and standardized by the intraspecific standard deviation. In the models separated by sexes, the intercept correspond to the back. In the models separated by body parts, the intercept correspond to females.

This table is not available in the printed version of the thesis due to its size, but can be found in the file MS4 Sexual selection and evolution rates at:  
<https://1drv.ms/f/s!AhWgyBtt5X3FamXDhTlZauD5uXo>

**Table S9.** Raw summary of the full Linear mixed effects model (body part effect, sex effect and their interaction) made on the Brownian evolution rates estimated on the birds dataset.

This table is not available in the printed version of the thesis due to its size, but can be found in the file MS4 Sexual selection and evolution rates at:  
<https://1drv.ms/f/s!AhWgyBtt5X3FamXDhTlZauD5uXo>

**Table S10.** Raw summary of the Linear mixed effects models made on the disparity and Rrphylo evolution rates, standardized by the mean, for the lizards, birds and newts dataset. Results are shown only for the best model after comparison between model with and without sex:body\_part interaction. In the three dataset, the intercept correspond to the back for females.

This table is not available in the printed version of the thesis due to its size, but can be found in the file MS4 Sexual selection and evolution rates at:  
<https://1drv.ms/f/s!AhWgyBtt5X3FamXDhTlZauD5uXo>

**Table S11.** Raw summary of the Linear mixed effects models testing separately the effect of sexes and body parts on the disparity measurements made on the newts dataset and standardized by the mean. In the models separated by sexes, the intercept correspond to the back. In the models separated by body parts, the intercept correspond to females.

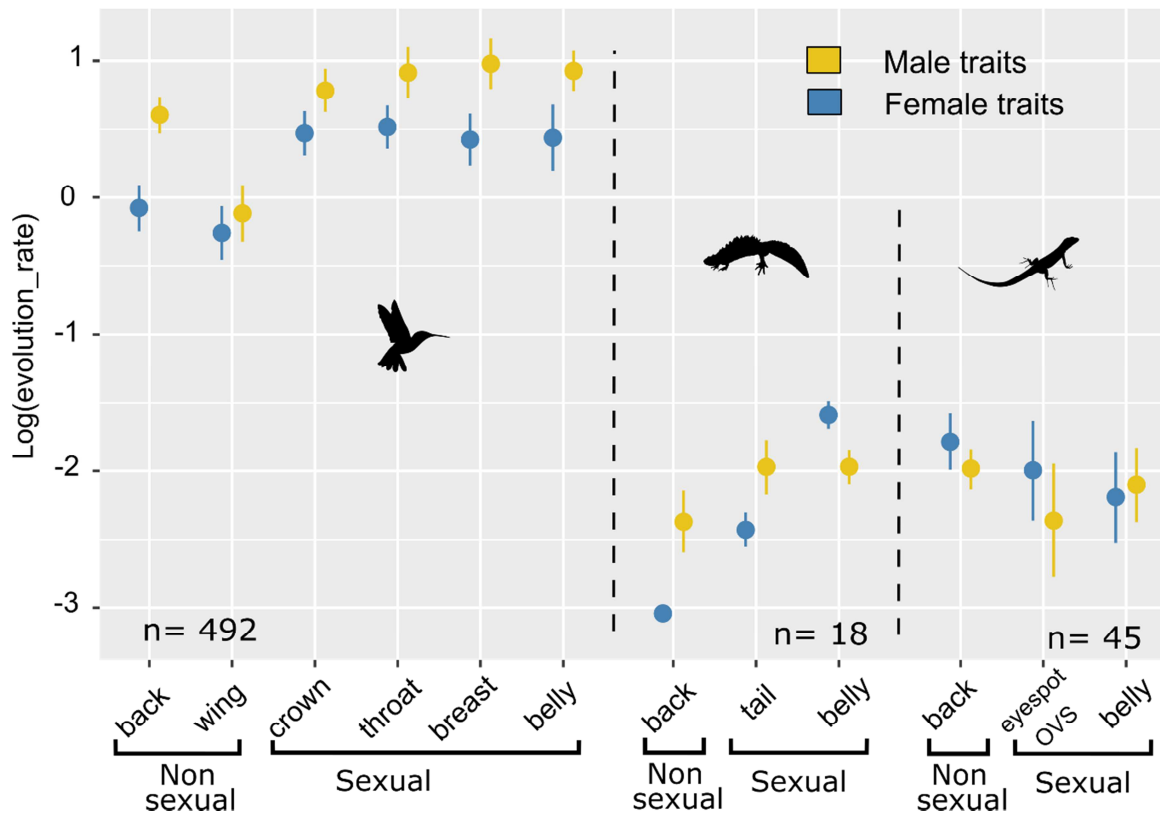
This table is not available in the printed version of the thesis due to its size, but can be found in the file MS4 Sexual selection and evolution rates at:  
<https://1drv.ms/f/s!AhWgyBtt5X3FamXDhTlZauD5uXo>

**Table S12.** Raw summary of the Linear mixed effects models testing separately the effect of sexes and body parts on the Rrphylo rate of evolution measured in lizards dataset and standardized by the mean. In the models separated by sexes, the intercept correspond to the back. In the models separated by body parts, the intercept correspond to females.

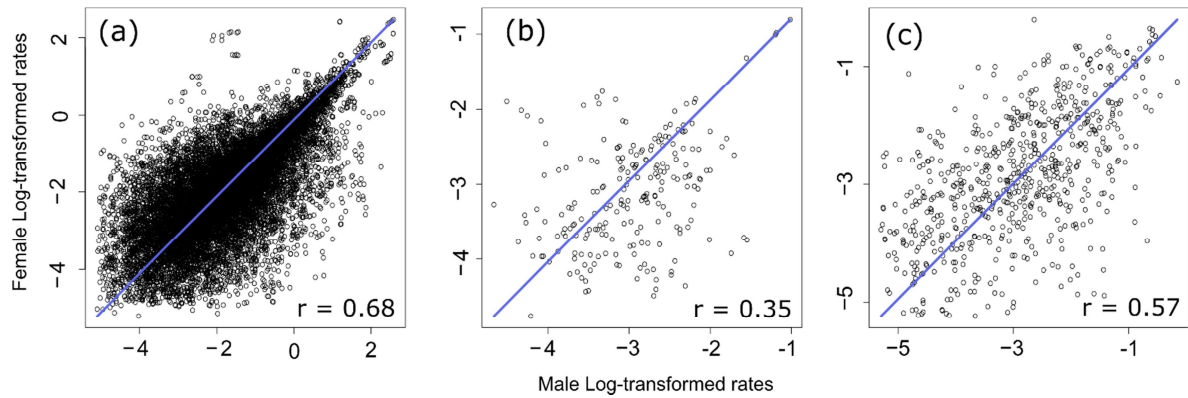
This table is not available in the printed version of the thesis due to its size, but can be found in the file MS4 Sexual selection and evolution rates at:  
<https://1drv.ms/f/s!AhWgyBtt5X3FamXDhTlZauD5uXo>

**Table S13.** Comparison of the raw summary obtained from the Linear mixed effects models built with the full bird dataset or with only mainland, island and Amazonian forest species. These models included all the fixed effects (body part effect, sex effect and their interaction) and were made with disparity and RRphylo rates of evolution.

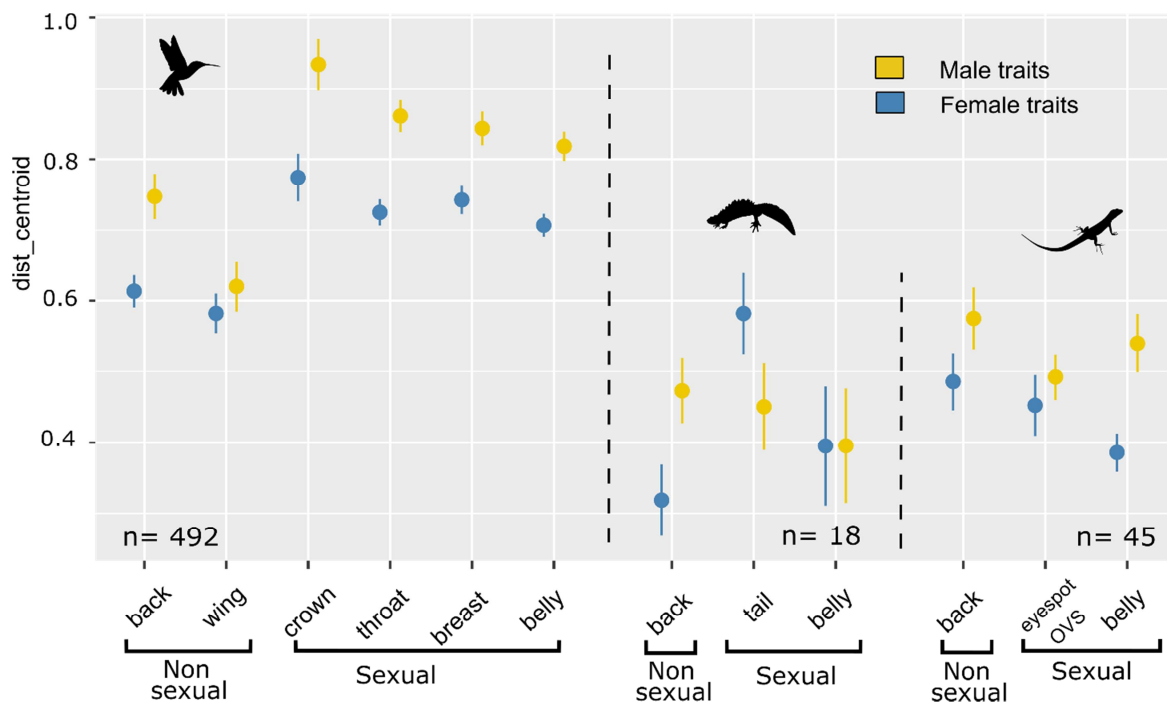
This table is not available in the printed version of the thesis due to its size, but can be found in the file MS4 Sexual selection and evolution rates at: <https://1drv.ms/f/s!AhWgyBtt5X3FamXDhTlzauD5uXo>



**Figure S1.** Boxplot of log-transformed rate of evolution for the sexual and non-sexual coloration, estimated with Brownian motion models. Color measurements were standardized by mean intraspecific standard deviation before analysis. Comparison between traits categories obtained from linear mixed effect models (see Methods section). \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ .

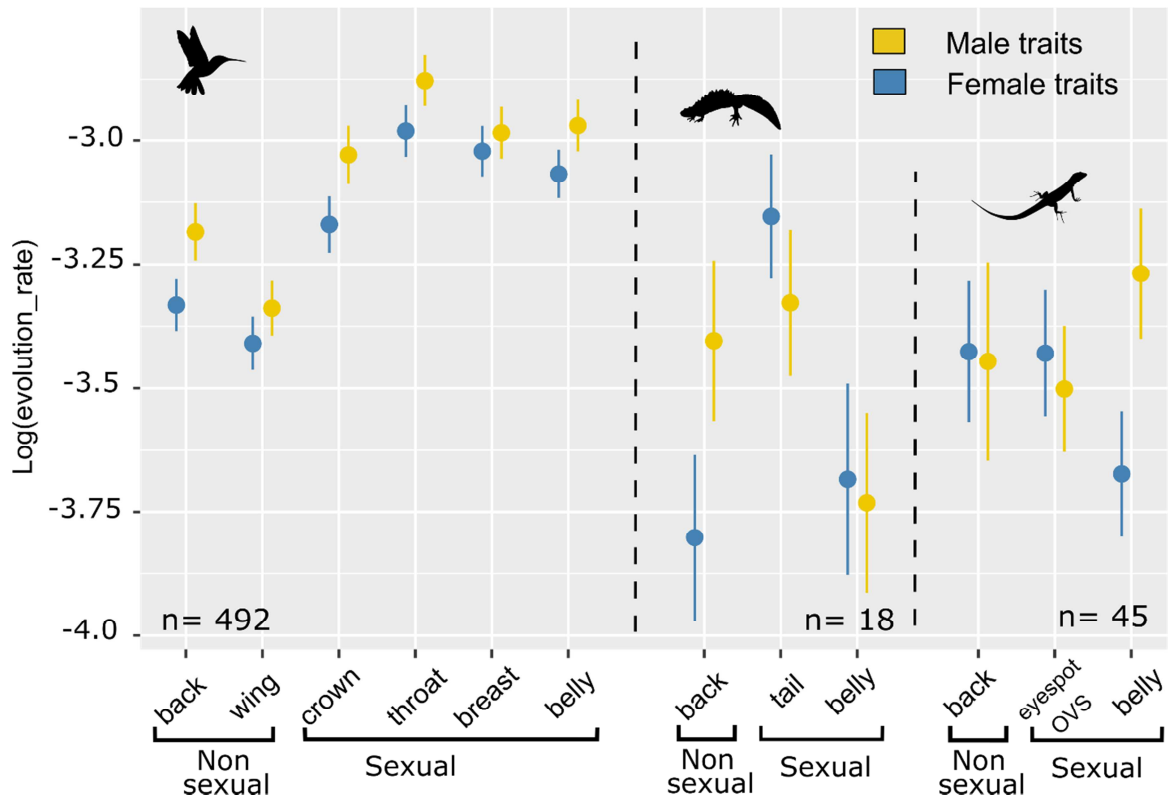


**Figure S2.** Relation between the RRphylo evolutionary rates of males and females for the bird (a), the newt (b) and the lizard dataset (c). The diagonal line is displayed in blue. Pearson correlation coefficients are indicated at the bottom, in each case the test of association was significant ( $p < 0.001$ ).

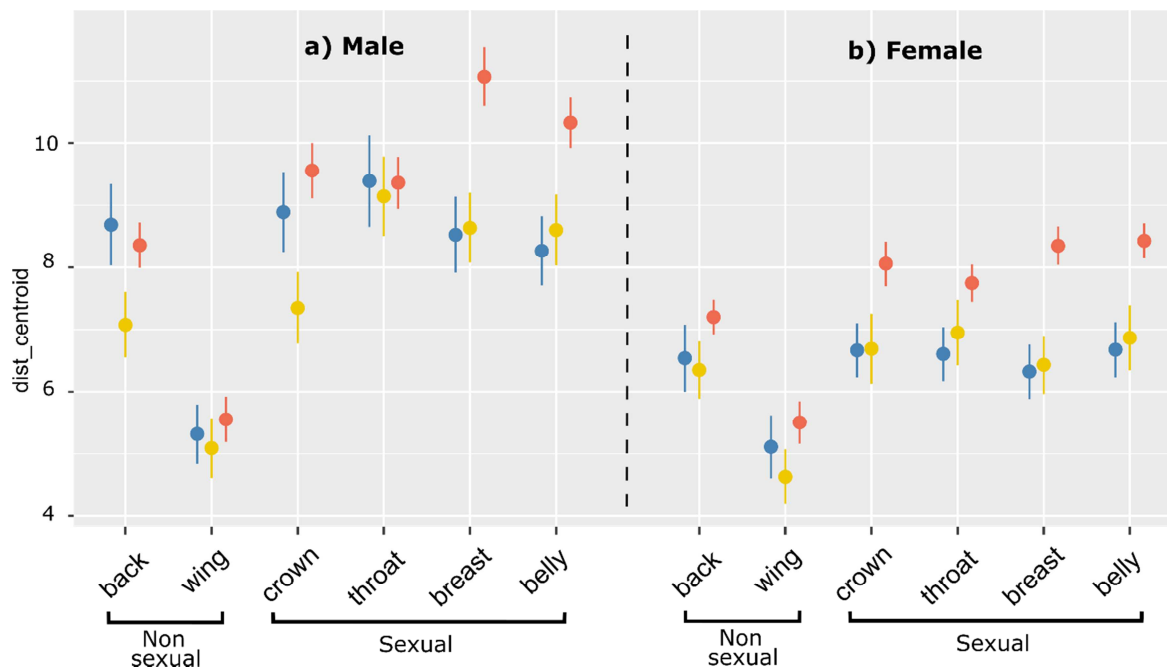


**Figure S3.** Mean color disparity ( $\pm$  Standard Error) measured in the bird, newt and lizard dataset for each body part and sex. Color measurements were standardized by mean value of the colorimetric variable before analysis. Color pattern of newts are not the same type of data and they were analyzed separately.

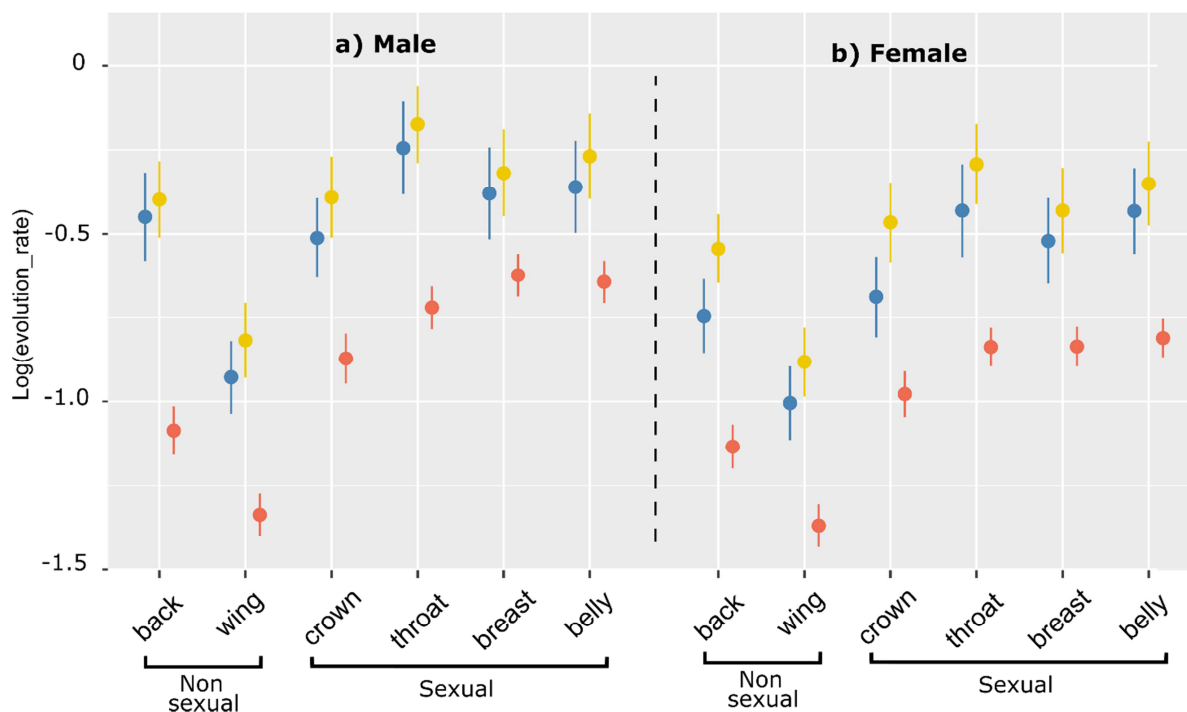




**Figure S4.** Mean rates of evolution ( $\pm$  Standard Error) measured in the bird, newt and lizard dataset for each body part and sex. Color measurements were standardized by the mean value of the colorimetric variable before analysis. The rates of evolution were estimated with RRphylo models. Color pattern of newts are not the same type of data and they were analyzed separately.



**Figure S5.** Mean color disparity ( $\pm$  Standard Error) measured in the bird of the mainland (blue), island (yellow) and Amazonian forest (red) for each body part and sex. Color measurements were standardized by mean intraspecific standard deviation before analysis.



**Figure S6.** Mean rates of evolution ( $\pm$  Standard Error) measured the bird of the mainland (blue), island (yellow) and Amazonian forest (red) for each body part and sex. Color measurements were standardized by mean intraspecific standard deviation before analysis. The evolutionnary rates were estimated with RRphylo models.



# **CHAPTER FOUR**

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**Coloration evolution and species diversification**



# **Manuscript E**

## **No effect of color polymorphism on the speciation rates of lacertids**

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Barry Sinervo, Pierre-andre Crochet**

Manuscript in preparation



### **Abstract**

Sexual selection and color polymorphism are both thought to increase rates of speciation. Sexual selection is predicted to facilitate the reproductive isolation between species, while polymorphism should favor rapid evolution of new lineages in populations where a morph is lost. Here, we investigated the influence of a proxy of sexual selection intensity, the presence/absence of sexual colorations, and of color polymorphism on the diversification of Lacertids. We used several state-dependent diversification methods, and showed that, regardless of the methods, sexual colorations and color polymorphism were not related to species diversification. While the lack of correlation between sexual colorations and speciation rates is in line with most of the literature testing this hypothesis, the results for color polymorphism contradict previous studies, and call into question the hypothesis of morphic speciation.

**Key words:** coloration, sexual selection, polymorphism, speciation, reptile





## **Introduction**

Species diversification is not a constant process across time and taxonomic groups (Rabosky et al. 2007; Diaz et al. 2019). For example, in birds, some recent orders like Passerines contain thousands of species, while old orders, such as Opisthocomiformes, contain only a few. Numerous studies tried to explain these variations of speciation rates on the basis of the ecology or phenotypic characteristics of organisms (Cardillo et al. 2003; Arbuckle and Speed 2015). However, among the traits investigated for their influence on diversification processes, one particular category, the sexual traits, has attracted a lot of attention. The idea of an increase of speciation rates with sexual selection intensity stems from the observation that sexual trait divergence can generate reproductive isolation between parapatric (Boul et al. 2007) or allopatric lineages (Price 1998; Panhuis et al. 2001), and strengthen reproductive barriers during secondary contact (Svedin et al. 2008). However, until now, studies linking sexual selection and speciation rates led at most to mixed results (Barraclough et al. 1995; Seehausen 2000; Kraaijeveld et al. 2011; Huang and Rabosky 2014; Cooney et al. 2017).

The heterogeneity of speciation rates between lineages may also be explained by a second, much less studied, factor: polymorphism. Polymorphic species - species with several distinct heritable morphs co-occurring within a population – are expected to have higher speciation rates for two reasons. First, polymorphic species can undergo a “morphic speciation” (West-Eberhard 1986). This occurs when a population of a polymorphic species loses a morph during the colonization of a new environment, a loss that may be followed by a rapid divergence of the remaining morphs. The loss of one morph is assumed to release the genetic constraint acting on the other morphs, allowing their rapid phenotypic specialization (character release, West-Eberhard 1986). Genetic constraints appear in polymorphic populations when an allele increases the fitness of one morph but decreases the fitness of the other morphs, preventing its spread in the population. In addition, the loss of a morph may affect the fitness of the remaining morphs because of a change in the competitive or predation environment. Predation often results in selection with negative frequency dependence (Olendorf et al. 2006), and the loss of a morph may drastically change the predation pressure acting on the remaining morphs (Bond 2007). Similarly, morphs often show different suits of competitive traits, and their fitness can depend on the frequency of other morphs in the population (Sinervo and Lively 1996). These changes of selective pressures, associated with the adaptation to the new environment, may lead to a rapid phenotypic divergence of the morph(s) present in a colonizing population (Corl et al. 2010a).

Apart from the morphic speciation hypothesis, polymorphism may also increase speciation through ecological specialization. Specialization can facilitate range expansion through an increase of the colonization abilities of the species and the persistence of populations in variable environments (Forsman et al. 2008; Takahashi and Noriyuki 2019). All these factors are known to increase the speciation rates (Kennedy et al. 2017).

Until now, the link between speciation rates and polymorphism was only investigated in three studies. The first one found an increase of speciation rates with polymorphism in four out of six groups of birds, but no differences in the two remaining groups (Hugall and Stuart-Fox 2012). The two other studies were made on a lizard species at the intraspecific scale (Corl et al. 2010b, 2012). They found that polymorphic populations were often ancestral, and that populations that lost a morph after colonizing a new environment showed genetic incompatibilities with the ancestral population and rapid phenotypic changes. Taken together, these findings support the hypothesis of increased speciation rates in polymorphic species.

Lacertids is a group of Squamata containing more than 300 species distributed across Europe, Africa and Asia. Most of these species display conspicuous sexual colorations, with blue or green ocelli on lateral body parts and bright colorations on their belly, which are known to play a role in mate choice and intrasexual competition (Names et al. 2019; Badiane et al. 2020). Important differences in coloration are often observed between closely related species (e.g. the white and yellow ventrum of *Congolacerta vauereselli* and *C. asukului*). Moreover some lineages, like the *Podarcis*, exhibit several distinct morphs in their sexual coloration, while other are monomorphic or even lack sexual colorations, like the *Lacerta* and the *Acanthodactylus* respectively (Speybroeck et al. 2016). Color polymorphism has been extensively studied in one species, *Podarcis muralis*, where distinct breeding strategies and life history traits were found to be associated to each morph (Calsbeek et al. 2010; Galeotti et al. 2013). The presence of specialized morphs and the lability of polymorphism made of lacertids a proper model to investigate the hypothesis of morphic speciation.

Here, we investigated the evolution of sexual colorations and color polymorphism in lacertids. We tested whether the presence and the polymorphism of sexual colorations on the ventral or lateral body parts were associated with an increase in speciation rates. We hypothesized that the absence of sexual colorations indicated a low intensity of sexual selection, and we thus expected a decrease of speciation rates in species lacking sexual coloration. Furthermore, following the morphic speciation hypothesis, we expected that

color polymorphism was more often lost than gained during the evolution of Lacertids. We also expected an increase of speciation rates in polymorphic species.

## **Material and Methods**

### **Data acquisition**

We acquired the list of all the lacertids species from the reference website (<http://reptile-database.reptarium.cz/>). We removed from this list the parthenogenetic species, as we did not expect their speciation events to be linked to their coloration (list of insular and parthenogenetic species in Supplementary Table S1). For the same reason, we removed the insular species, none of them having radiated within island (except a few species of *Gallotia*). We also removed four species for which no accurate description was available and five species with an uncertain taxonomic status. Insularity was assessed using the distribution information available in the reptile-database website. At the end, we retained 294 species for the diversification analysis.

We scored the presence of sexual colorations and polymorphism from multiple sources, such as publications and naturalist field books, but also using photographs taken by the authors in the field (sources listed in Table S1). A species was considered as having sexual colorations if either (1) its ventral side, or a part of its ventral side (throat, belly and legs) was not white, or (2) its flanks displayed several ocelli or spots with a distinct color from the rest of dorsal/flank side, or (3) it displayed a specific color during the mating season. Furthermore, we considered a species as polymorphic if, within a population, the individuals of a same sex displayed several distinct colors. We did not score coloration as polymorphic if the morphs were not clearly distinguished, nor if the distinct morphs were associated with age. Data of sexual colorations and polymorphism are provided in Supplementary Table S1.

We used the phylogeny provided by Garcia-Porta et al. (2019) as backbone phylogeny. We subsequently added the 92 non-insular and non-parthenogenetic species that were not sampled by Garcia-Porta. These species were added to the backbone tree using the function `add.species.to.genus` from the `phytools` package (Revell 2012). This function randomly inserts new species within a predefined genus. If the phylogenetic position was known from previous work, the species was added with its sister taxa at a random divergence date. If no phylogenetic information was available, it was randomly added among the species of the

same genus. We repeated this operation one hundred times in order to account for phylogenetic uncertainty in the following analysis.

### Trait evolution and ancestral state reconstruction

We investigated the evolution patterns of sexual colorations and polymorphism using the Blomberg's  $K$ .  $K < 1$  indicates traits with labile evolution, while  $K > 1$  indicates phylogenetic conservatism. It was measured for each of the 100 phylogeny using the `phylosig` function of the `phytools` package (Revell 2012). We also reconstructed the ancestral state of the coloration with the `make.simmap` function. This function simulates stochastic character histories using the state of the character on the tips and a continuous-time reversible Markov model. For this analysis, coloration was coded with three states: (1) no sexual coloration, (2) sexual colorations, (3) sexual colorations and polymorphism. Transition rates between each state were allowed to differ. We repeated the ancestral state reconstruction (ASR) on the 100 phylogenetic trees, with 100 simulations for each analysis.

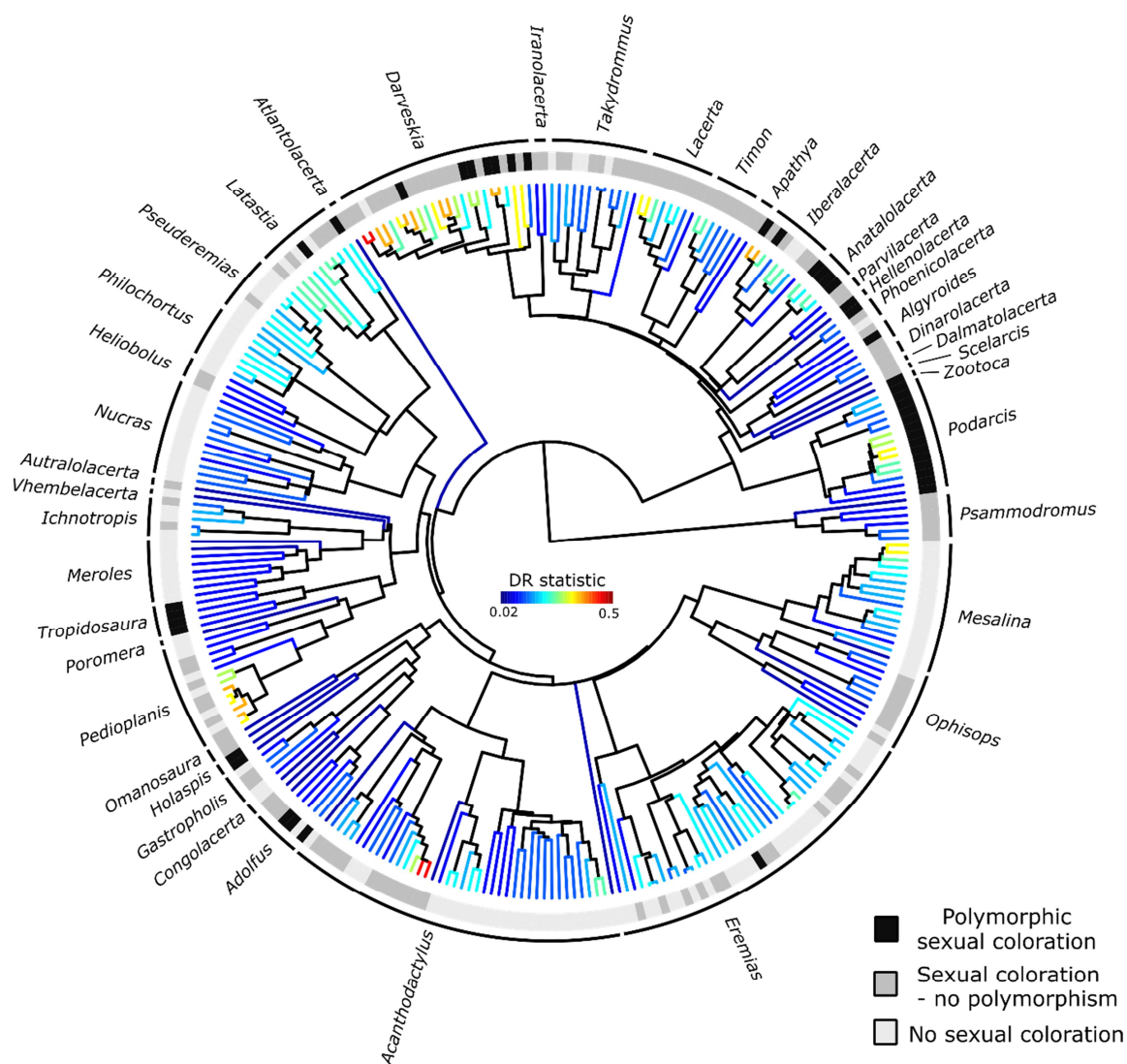
### Character associated diversification analysis

We used state-dependent diversification methods to compare the rates of speciation between species with and without sexual colorations, and between species with sexual colorations but no color polymorphism and species with sexual polymorphic colorations. We excluded the species lacking sexual colorations of the polymorphism analysis because sexual colorations are required before the evolution of polymorphism (no transition between the lack of sexual coloration and color polymorphism, see Results of the ASR). However, we tested the influence of this exclusion by repeating the polymorphism analysis with all the species.

We fitted State Dependent Diversification models (SDD) to test the influence of sexual colorations and color polymorphism on the diversification rates of Lacertid. For each trait, we implemented four models: two models measuring character dependent diversification, plus the corresponding two null model estimating Character Independent Diversification (CID). The first character dependent model we fitted was a Binary-State Speciation and Extinction model (BiSSE, Maddison et al. 2007). This model estimates one rate of speciation and extinction for each character state. In addition, we implemented three models from the Hidden State Speciation and Extinction framework (HiSSE; Beaulieu and O'Meara 2016). The hidden states allow the diversification rates to vary within each state, which is not possible

in BiSSE. We fitted a HiSSE model, a state dependent model with two hidden states. We also fitted two hidden state null-models called CID-2 and CID-4 (Beaulieu and O’Meara 2016). They respectively have the same number of parameters than BiSSE and HiSSE models, but differ in having their diversification parameter independent of the observed character state (i.e. diversification parameter set equal for each observed state). We selected the best-fit model among these four models based on the Akaike Information Criterion corrected for sample size (AICc, models were preferred when  $\Delta AICc > 2$ ). All the models were made using the R package hisse (Beaulieu 2017). To account for phylogenetic uncertainty, we fitted these four models for each trait and phylogenetic tree (800 models in total), and assessed the best-fitting model each time.

SDD models are prone to type-1 errors, and often detect differences in diversification rates even for neutral traits (Rabosky and Goldberg 2015, 2017). We thus performed two additional analyses, with lower type-1 errors, to confirm the results found previously. First, we used the speciation/extinction analysis of BAMM (Rabosky 2014) to detect shifts of the speciation rates in trees. This analysis allows the speciation rates to vary across time. We then applied the STructured RAte Permutations on Phylogenies analysis (STRAPP, Rabosky and Huang 2016) to test if the speciation rates measured with BAMM were different between species with and without sexual colorations and color polymorphism. This method focus only on the rates of speciation at the tips, and compares against a null distribution the Mann–Whitney U-test statistic measuring the relationship between the binary character state and speciation rates. Secondly, we inferred the speciation rates with the Diversification Rates statistic (DR statistic, Jetz et al. 2012). For a given species, the DR statistic is computed as a weighted average of the inverse branch lengths connecting the focal species to the root of the phylogeny (i.e. all the branches between the tip and the root). We then used a non-parametric test, FiSSE (Fast, intuitive State-dependent Speciation Extinction analysis, Rabosky and Goldberg 2017), to investigate the effect of sexual colorations and polymorphism on diversification rates. For a given tree, this test first measures the difference of mean DR statistic between species with different character state, and then compare this difference to a null distribution. We repeated these analyses for each trait and phylogenetic trees.



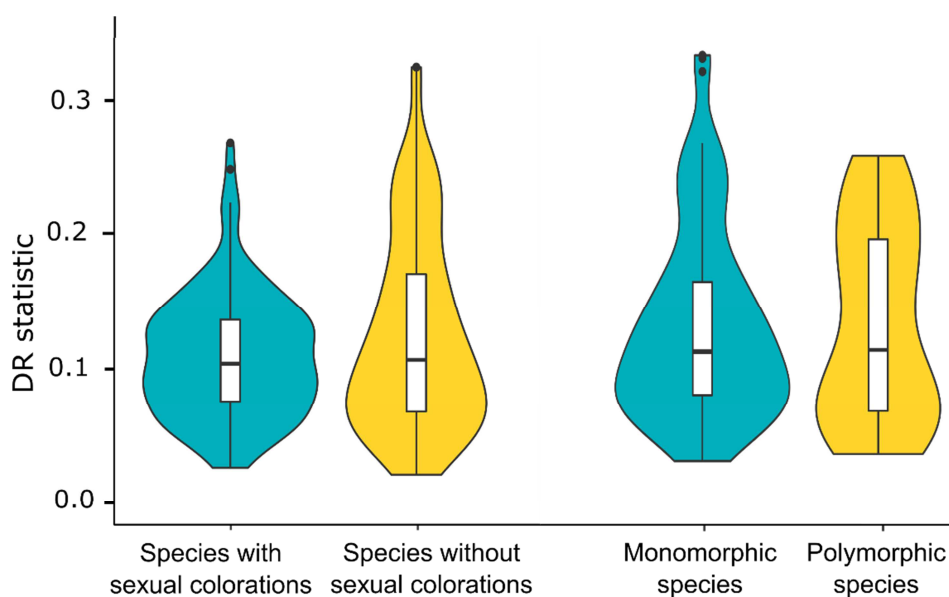
**Figure 1.** Phylogenetic relationships of the 295 Lacertids species included in the diversification analysis. This tree was randomly chosen among the hundred trees produced for the analysis to account for phylogenetic uncertainty (see methods). States of sexual coloration and polymorphism characters are indicated at the tips. Tip branches are colored according to the mean DR statistic measured across all trees (an estimate of the branch-specific diversification rate).

## Results

Sexual colorations are frequently observed in lacertids (present in 63% of the species analyzed, Figure 1), while color polymorphism are more rare (15% of the species analyzed). The evolution of both traits is highly labile (mean Blomberg's  $K$  across phylogenies = 0.20 and 0.25 respectively; significantly different from Brownian model: mean  $p$ -value = 0.06 and 0.08 respectively). The ancestral state reconstruction suggested more acquisition than loss in the evolution of both sexual colorations and color polymorphism (Figure S1, 36 acquisitions vs 31 losses for sexual colorations and 18 acquisitions vs 14 losses for color polymorphism). Transitions from no sexual colorations to polymorphism, and the reverse, were negligible (1.1 and 0.3 respectively). Finally, the ancestor of lacertids probably displayed both sexual colorations and polymorphism (Figure S1).

For polymorphism, the results presented below are for analyses performed after exclusion of the species lacking sexual colorations. When included, these species had a low impact on the analyses, and did not change the overall conclusion (see Supplementary Methods).

The best fitting models for the evolution of sexual coloration were CID 2 or CID 4 for the majority of trees (Figure 2). Models of characters associated diversification obtained the lowest AICC only in two trees out of the hundred trees. Similarly, we found a low support for an influence of color polymorphism on the diversification of specie with sexual coloration. CID 2 and CID 4 were the best fitting models for 70 and 5 trees respectively, while HiSSE was



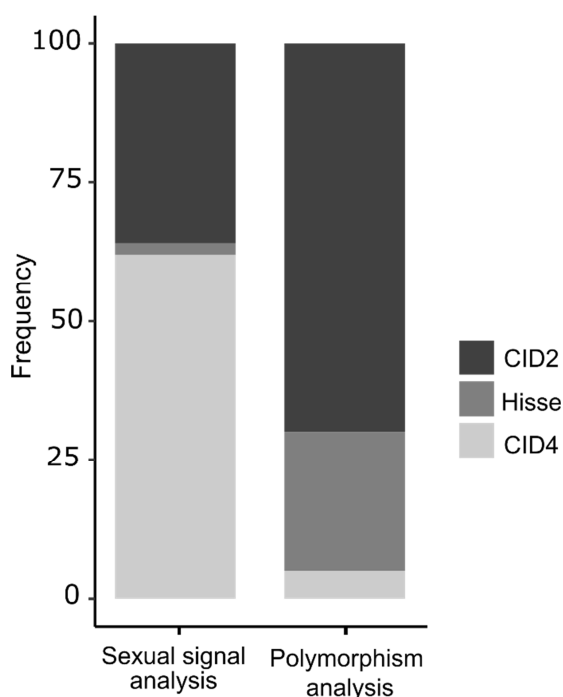
**Figure 3.** Violin plots of the DR statistic values for the species with and without sexual coloration, and with and without color polymorphism.



the best fitting model for 25 trees only (Figure 2).

The BAMM analysis detected a shift of speciation rates only in 21 trees out of the hundred trees. Confirming this observation, the STRAPP tests revealed that speciation rates were independent of the presence of sexual colorations and color polymorphism ( $p > 0.5$  for all tests).

Finally, when measured with the DR statistic, species with sexual colorations had slightly higher speciation rates than the species without sexual colorations (mean rates across tree:  $0.13 \pm 0.07$  and  $0.11 \pm 0.05$  respectively, Figure 1 and 3). However, there was low differences between the speciation rates of species with sexual colorations but without color polymorphism and species with sexual polymorphic colorations (mean rates across tree:  $0.12 \pm 0.07$  and  $0.13 \pm 0.07$  respectively, Figure 1 and 3). In accordance with this observation, FiSSE analysis did not detect differences of speciation rates between species with or without sexual colorations and color polymorphism ( $p > 0.5$  for all tests).



**Figure 2.** Frequency of the best fitting diversification models for the sexual coloration and color polymorphism analysis repeated across one hundred trees. Model with character dependent diversification were considered as best fitting when  $\Delta AICc > 2$ . BiSSE models were included in both analyses, but never had the lowest AICc.

## **Discussion**

Our analysis revealed that neither the presence of sexual colorations nor color polymorphism increases the rates of speciation in Lacertids. We also showed that sexual colorations and color polymorphism are labile across taxonomy, and were gained and lost several times during the diversification of Lacertids. The lack of effect of sexual colorations on speciation is in agreement with the numerous studies that failed to link speciation and proxies of sexual selection (Huang and Rabosky 2014; Gomes et al. 2016; Cooney et al. 2017). However, our results for color polymorphism is in contradiction with the two previous studies that tested the morphic speciation hypothesis (Corl et al. 2010b, 2012; Hugall and Stuart-Fox 2012).

The observation that the presence of sexual coloration has a low effect on diversification rates, although in line with previous studies, contrasts with the accumulation of evidence showing an influence of sexual selection on pre- and post-zygotic isolation (Boughman 2001; Masta and Maddison 2002; Boul et al. 2007; Svedin et al. 2008). However, there is not necessarily a link between processes acting at short time scale and diversification rates observed at large phylogenetic scale. This decoupling could arise because different processes influence the creation of new species and their persistence over time. In agreement with this idea, some authors found that sexual selection increases the risk of extinction in birds (McLain et al. 1995; Doherty et al. 2003; but see Cooney et al. 2017 for opposite results). However, we could not test this hypothesis in this study because SDD models provide inaccurate measures of extinctions (Beaulieu and O'Meara 2016). The lack of correlation between speciation and the presence of sexual colorations could also come from the fact that speciation does not increase with the intensity of sexual selection but rather with the rates of sexual trait evolution. These two indices are not necessarily correlated, as a sexual trait may be at the same time very conserved across species and under strong sexual selection within each (de Solan et al, in press). The few studies investigating this question found support for this hypothesis (Cardoso and Mota 2008; Gomes et al. 2016) but they were made on bird and tests on other taxonomic groups are needed.

A large variety of factors, besides those examined in this study, may explain the variations of diversification observed in Lacertids (for instance diet, Tran 2016; habitat specialization Liedtke et al. 2016 or reproduction mode, Lynch 2009). Nonetheless, a likely explanation may be an increase of speciation due to adaptive radiation. The two most diverse genera (*Acanthodactylus* and *Eremias*) represent 22% of all the Lacertid species and live in arid

areas of Middle East or North Africa. Increase of lizard diversity in arid areas was already reported in biogeographic studies (Powney et al. 2010; Lewin et al. 2016), and is in agreement with the spatial patterns of species richness (Roll et al. 2017). This pattern could be the result of the strong relation between the environment and ecological and life history traits of lizards (e.g. increase in activity time and fecundity with temperature; Adolph and Porter 1993) that may have facilitated speciation in arid areas (discussed in Powney et al. 2010). In addition, arid areas present a low diversity of other vertebrate species (Jenkins et al. 2013), which may lead to a decrease of interspecific competition for Lacertids species living in this habitat (i.e. ecological release, Terborgh and Faaborg 1973). Such situation would ease the colonization of new ecological niches in which Lacertids could radiate (Losos and Queiroz 1997; Kennedy et al. 2012). Probably due to strong ecological pressures, the species living in arid area have a white belly and yellowish dorsal coloration, and often lack sexual colorations. This effect may have influenced the analysis on sexual coloration, but is unlikely to affect the conclusion of our analysis on polymorphism, as we did not detect difference of speciation rate after the exclusion of species lacking sexual coloration.

One main limitation of our work lies in the data we used to score the coloration. Neither photographs nor literature sources can account for variation of ultraviolet (UV) coloration. Lacertids frequently use UV coloration for mate choice or to signal their fighting capacity (Olsson et al. 2011; De Lanuza et al. 2014). Thus, in principle, we could have underestimated the frequency of sexual coloration and polymorphism in our dataset. However, known UV signals in lizards are always displayed on a patch with a visible different color than the rest of the body (blue throat or green ocelli for instance), so it is unlikely that this issue affects our detection of sexual coloration, although it may have done so for polymorphism. To the authors' knowledge, there is currently no example of polymorphism limited only to UV coloration. A second methodological limitation may stem from the strong geographical bias prevailing in the taxonomic researches. In the last twenty years, an intensive work was made to unravel the phylogeny of the Lacertids of the northern hemisphere, allowing the description of numerous species. On the other hand, almost no taxonomic studies based on genetic data was made on Lacertids of the equatorial region. The diversity of species living in this region is thus probably strongly underestimated. It is difficult to predict in which ways this issue may influence our results, because species with and without sexual colorations and color polymorphism are present in the equatorial region.

Here, we showed that the evolution of coloration did not affect the diversification processes of Lacertids. Indeed, we found that the rates of speciation were not influenced by the presence of sexual colorations nor by color polymorphism. These results call into question

the morphic speciation hypothesis, as it is currently supported at large taxonomic scale by only one study on birds. The factors shaping the diversification of Lacertids may rather be environmental (Garcia-Porta et al. 2019). However, the links between environmental variables and species diversification are still unclear on a larger phylogenetic scale (Marin et al. 2018), and it would be interesting in the future to focus on the effect of temperature and precipitation on the diversification of reptiles, or more generally of ectothermic species.

## References

- Adolph, S. C., and W. P. Porter. 1993. Temperature, activity, and lizard life histories. *American Naturalist* 142:273–295.
- Arbuckle, K., and M. P. Speed. 2015. Antipredator defenses predict diversification rates. *Proceedings of the National Academy of Sciences of the United States of America* 112:13597–13602.
- Badiane, A., M. Martin, S. Meylan, M. Richard, B. Decencière Ferrandière, and J.-F. Le Galliard. 2020. Male ultraviolet reflectance and female mating history influence female mate choice and male mating success in a polyandrous lizard. *Biological Journal of the Linnean Society* 130:586–598.
- Barracough, T. G., P. H. Harvey, and S. Nee. 1995. Sexual selection and taxonomic diversity in passerine birds. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 259:211–215.
- Beaulieu, J. M. 2017. Package ‘hisce.’
- Beaulieu, J. M., and B. C. O’Meara. 2016. Detecting hidden diversification shifts in models of trait-dependent speciation and extinction. *Systematic biology* 65:583–601.
- Bond, A. B. 2007. The Evolution of Color Polymorphism: Crypticity, Searching Images, and Apostatic Selection. *Annual Review of Ecology, Evolution, and Systematics* 38:489–514.
- Boughman, J. W. 2001. Divergent sexual selection enhances reproductive isolation in sticklebacks. *Nature* 411:944–948.
- Boul, K. E., W. Chris Funk, C. R. Darst, D. C. Cannatella, and M. J. Ryan. 2007. Sexual selection drives speciation in an Amazonian frog. *Proceedings of the Royal Society B: Biological Sciences* 274:399–406.
- Calsbeek, B., D. Hasselquist, and J. Clobert. 2010. Multivariate phenotypes and the potential for alternative phenotypic optima in wall lizard (*Podarcis muralis*) ventral colour morphs. *Journal of Evolutionary Biology* 23:1138–1147.
- Cardillo, M., J. S. Huxtable, and L. Bromham. 2003. Geographic range size, life history and rates of diversification in Australian mammals. *Journal of Evolutionary Biology* 16:282–288.
- Cardoso, G. C., and P. G. Mota. 2008. Speciation evolution of coloration in the genus *Carduelis*. *Evolution* 62:753–762.
- Cooney, C. R., J. A. Tobias, J. T. Weir, C. A. Botero, and N. Seddon. 2017. Sexual selection, speciation and constraints on geographical range overlap in birds. *Ecology Letters* 20:863–871.
- Corl, A., A. R. Davis, S. R. Kuchta, T. Comendant, and B. Sinervo. 2010a. Alternative mating strategies and the evolution of sexual size dimorphism in the side-blotched lizard, *Uta stansburiana*: a population-level comparative analysis. *Evolution* 64:79–96.
- Corl, A., A. R. Davis, S. R. Kuchta, and B. Sinervo. 2010b. Selective loss of polymorphic mating types is associated with rapid phenotypic evolution during morphic speciation. *Proceedings of the National Academy of Sciences of the United States of America* 107:4254–4259.
- Corl, A., L. T. Lancaster, and B. Sinervo. 2012. Rapid formation of reproductive isolation between two populations of side-blotched lizards, *Uta stansburiana*. *Copeia* 593–602.
- De Lanuza, G. P. i., P. Carazo, and E. Font. 2014. Colours of quality: Structural (but not pigment) coloration informs about male quality in a polychromatic lizard. *Animal Behaviour* 90:73–81.

- Diaz, L. F. H., L. J. Harmon, M. T. C. Sugawara, E. T. Miller, and M. W. Pennell. 2019. Macroevolutionary diversification rates show time dependency. *Proceedings of the National Academy of Sciences of the United States of America* 116:7403–7408.
- Doherty, P. F., G. Sorci, J. A. Royle, J. E. Hines, J. D. Nichols, and T. Boulinier. 2003. Sexual selection affects local extinction and turnover in bird communities. *Proceedings of the National Academy of Sciences of the United States of America* 100:5858–5862.
- Fitzjohn, R. G. 2012. Diversitree: Comparative phylogenetic analyses of diversification in R. *Methods in Ecology and Evolution* 3:1084–1092.
- Forsman, A., J. Ahnesjö, S. Caesar, and M. Karlsson. 2008. A model of ecological and evolutionary consequences of color polymorphism. *Ecology* 89:34–40.
- Galeotti, P., R. Sacchi, D. Pellitteri-Rosa, A. Bellati, W. Cocca, A. Gentilli, S. Scali, et al. 2013. Colour Polymorphism and Alternative Breeding Strategies: Effects of Parent's Colour Morph on Fitness Traits in the Common Wall Lizard. *Evolutionary Biology* 40:385–394.
- Garcia-Porta, J., I. Irisarri, M. Kirchner, A. Rodríguez, S. Kirchhof, J. L. Brown, A. MacLeod, et al. 2019. Environmental temperatures shape thermal physiology as well as diversification and genome-wide substitution rates in lizards. *Nature Communications* 10:1–12.
- Gomes, A. C. R., M. D. Sorenson, and G. C. Cardoso. 2016. Speciation is associated with changing ornamentation rather than stronger sexual selection. *Evolution* 70:2823–2838.
- Huang, H., and D. L. Rabosky. 2014. Sexual Selection and Diversification: Reexamining the Correlation between Dichromatism and Speciation Rate in Birds. *The American Naturalist* 184:E101–E114.
- Hugall, A. F., and D. Stuart-Fox. 2012. Accelerated speciation in colour-polymorphic birds. *Nature* 485:631–634.
- Jenkins, C. N., S. L. Pimm, and L. N. Joppa. 2013. Global patterns of terrestrial vertebrate diversity and conservation. *Proceedings of the National Academy of Sciences of the United States of America* 110:E2603–E2610.
- Jetz, W., G. H. Thomas, J. B. Joy, K. Hartmann, and A. O. Mooers. 2012. The global diversity of birds in space and time. *Nature* 491:444–448.
- Kennedy, J. D., M. K. Borregaard, K. A. Jønsson, B. Holt, J. Fjeldså, and C. Rahbek. 2017. Does the colonization of new biogeographic regions influence the diversification and accumulation of clade richness among the Corvidae (Aves: Passeriformes)? *Evolution* 71:38–50.
- Kennedy, J. D., J. T. Weir, D. M. Hooper, D. T. Tietze, J. Martens, and T. D. Price. 2012. Ecological limits on diversification of the Himalayan core Corvoidea. *Evolution* 66:2599–2613.
- Kraaijeveld, K., F. J. L. Kraaijeveld-Smit, and M. E. Maan. 2011. Sexual selection and speciation: The comparative evidence revisited. *Biological Reviews*. John Wiley & Sons, Ltd.
- Lewin, A., A. Feldman, A. M. Bauer, J. Belmaker, D. G. Broadley, L. Chirio, Y. Itescu, et al. 2016. Patterns of species richness, endemism and environmental gradients of African reptiles 43:2380–2390.
- Liedtke, H. C., H. Müller, M.-O. Rödel, M. Menegon, L. N. Gonwouo, M. F. Barej, V. Gvoždík, et al. 2016. No ecological opportunity signal on a continental scale? Diversification and life-history evolution of African true toads (Anura: Bufonidae). *Evolution* 70:1717–1733.
- Losos, J. B., and K. De Queiroz. 1997. Evolutionary consequences of ecological release in Caribbean Anolis lizards. *Biological Journal of the Linnean Society* 61:459–483.
- Louca, S., and M. W. Pennell. 2020. Extant timetrees are consistent with a myriad of diversification histories. *Nature* 580:502–505.

- Lynch, V. J. 2009. Live-birth in vipers (Viperidae) is a key innovation and adaptation to global cooling during the Cenozoic. *Evolution* 63:2457–2465.
- Maddison, W. P. M., and R. G. Fitzjohn. 2015. The unsolved challenge to phylogenetic correlation tests for categorical characters. *Systematic Biology* 64:127–136.
- Maddison, W. P., P. E. Midford, and S. P. Otto. 2007. Estimating a Binary Character's Effect on Speciation and Extinction. *Systematic Biology* 56:701–710.
- Marin, J., G. Rapacciuolo, G. C. Costa, C. H. Graham, T. M. Brooks, B. E. Young, V. C. Radeloff, et al. 2018. Evolutionary time drives global tetrapod diversity. *Proceedings of the Royal Society B: Biological Sciences* 285:20172378.
- Masta, S. E., and W. P. Maddison. 2002. Sexual selection driving diversification in jumping spiders. *Proceedings of the National Academy of Sciences of the United States of America* 99:4442–4447.
- McLain, D. K., M. P. Moulton, and T. P. Redfearn. 1995. Sexual Selection and the Risk of Extinction of Introduced Birds on Oceanic Islands. *Oikos* 74:27.
- Names, G., M. Martin, A. Badiane, and J. F. Le Galliard. 2019. The relative importance of body size and UV coloration in influencing male-male competition in a lacertid lizard. *Behavioral Ecology and Sociobiology* 73:1–14.
- Olendorf, R., F. H. Rodd, D. Punzalan, A. E. Houde, C. Hurt, D. N. Reznick, and K. A. Hughes. 2006. Frequency-dependent survival in natural guppy populations. *Nature* 441:633–636.
- Olsson, M., S. Andersson, and E. Wapstra. 2011. UV-Deprived Coloration Reduces Success in Mate Acquisition in Male Sand Lizards (*Lacerta agilis*). *PLoS ONE* 6:e19360.
- Panhuis, T. M., R. Butlin, M. Zuk, and T. Tregenza. 2001. Sexual selection and speciation. *Trends in Ecology & Evolution* 16:364–371.
- Powney, G. D., R. Grenyer, C. D. L. Orme, I. P. F. Owens, and S. Meiri. 2010. Hot, dry and different: Australian lizard richness is unlike that of mammals, amphibians and birds. *Global Ecology and Biogeography* 19:386–396.
- Price, T. 1998. Sexual selection and natural selection in bird speciation. (A. E. Magurran & R. M. May, eds.) *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* 353:251–260.
- Rabosky, D. L. 2014. Automatic Detection of Key Innovations, Rate Shifts, and Diversity-Dependence on Phylogenetic Trees. *PLoS ONE* 9:e89543.
- Rabosky, D. L., S. C. Donnellan, A. L. Talaba, and I. J. Lovette. 2007. Exceptional among-lineage variation in diversification rates during the radiation of Australia's most diverse vertebrate clade. *Proceedings of the Royal Society B: Biological Sciences* 274:2915–2923.
- Rabosky, D. L., and E. E. Goldberg. 2015. Model Inadequacy and Mistaken Inferences of Trait-Dependent Speciation. *Systematic Biology* 64:340–355.
- Rabosky, D. L., and E. E. Goldberg. 2017. FiSSE: A simple nonparametric test for the effects of a binary character on lineage diversification rates. *Evolution* 71:1432–1442.
- Rabosky, D. L., and H. Huang. 2016. A Robust Semi-Parametric Test for Detecting Trait-Dependent Diversification. *Systematic Biology* 65:181–193.
- Revell, L. J. 2012. phytools: An R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution* 3:217–223.
- Roll, U., A. Feldman, M. Novosolov, A. Allison, A. M. Bauer, R. Bernard, M. Böhm, et al. 2017. The global distribution of tetrapods reveals a need for targeted reptile conservation. *Nature Ecology and Evolution* 1:1677–1682.

- Seehausen, O. 2000. Explosive speciation rates and unusual species richness in haplochromine cichlid fishes: Effects of sexual selection. *Advances in Ecological Research*. Academic Press Inc.
- Sinervo, B., and C. M. Lively. 1996. The rock-paper-scissors game and the evolution of alternative male strategies. *Nature* 380:240–243.
- Speybroeck, J., W. Beukema, B. Bok, J. Van Der Voort, and I. Velikov. 2016. *Field Guide to the Amphibians and Reptiles of Britain and Europe*. Bloomsbury Publishing.
- Svedin, N., C. Wiley, T. Veen, L. Gustafsson, and A. Qvarnström. 2008. Natural and sexual selection against hybrid flycatchers. *Proceedings of the Royal Society B: Biological Sciences* 275:735–744.
- Takahashi, Y., and S. Noriyuki. 2019. Colour polymorphism influences species' range and extinction risk. *Biology Letters* 15:20190228.
- Terborgh, J., and J. Faaborg. 1973. Turnover and Ecological Release in the Avifauna of Mona Island, Puerto Rico. *The Auk* 90:759–779.
- Tran, L. A. P. 2016. Interaction between digestive strategy and niche specialization predicts speciation rates across herbivorous mammals. *American Naturalist* 187:468–480.
- West-Eberhard, M. J. 1986. Alternative adaptations, speciation, and phylogeny. (A review). *Proceedings of the National Academy of Sciences of the United States of America* 83:1388–1392.



## Supplementary Methods

### Results for polymorphism analysis applied to all species

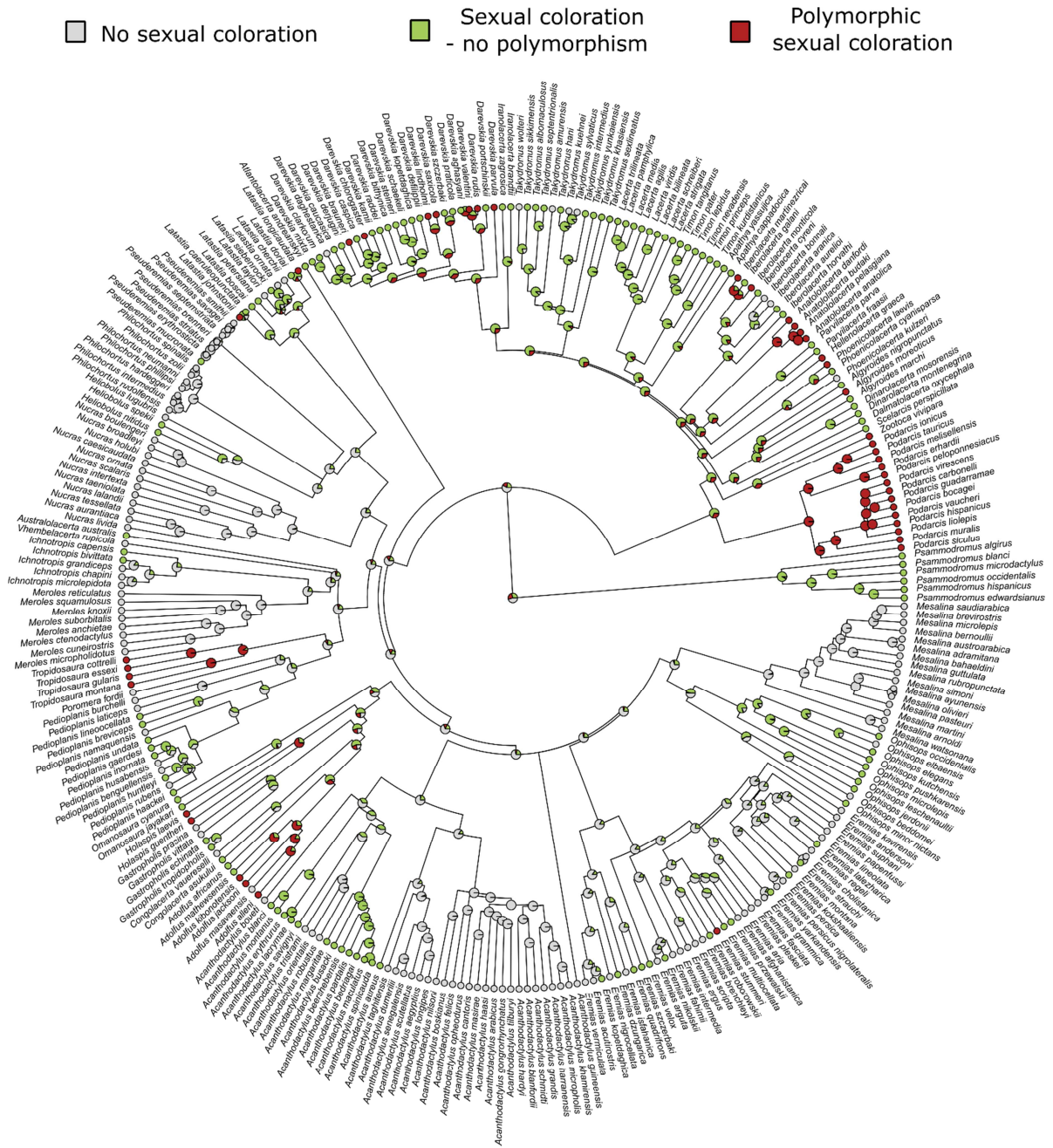
When all the species were included in the analysis, color polymorphism was found to increase the rates of speciation, HiSSE model being the best-fitting model in 88 trees (Figure 2). The mean speciation rates estimated by HiSSE across the trees was of  $0.93 \pm 4.3$  and  $0.15 \pm 0.04$  for the two hidden state of polymorphic species, and of  $0.01 \pm 0.01$  and  $0.11 \pm 0.03$  for the hidden states of non-polymorphic species. The mean extinction rates were very low (often inferior to 0.00001) for both polymorphic and non-polymorphic species. This characteristic, and the larges variations of speciation rates found for some states, may reflects problems of parameters estimation, appearing because some hidden states are estimated to be vanishingly rare in the dataset. Indeed, depending on the phylogenetic tree, the frequency of hidden states was often less than 0.1, and the transition between states was close to 0.

The results STRAPP and FiSSE analysis were not changed by the inclusion of species without sexual colorations. Both analyses indicated that speciation rates were independent of the presence of color polymorphism ( $p > 0.5$  for all tests).

**Table S1.** Polymorphism and sexual coloration data used in the analysis, with the literature sources, and rational for the exclusion of species.

This table is not available in the printed version of the thesis due to its size, but can be found in the file MS5 Polymorphism and speciation at:

<https://1drv.ms/f/s!AhWgyBtt5X3FamXDhTlzauD5uXo>



**Figure S1.** Ancestral state reconstruction (ASR) of Lacertids coloration estimated across 100 phylogenetic trees. The ASR was made with the `make.simmap` function of the `phytools` package, using a continuous-time reversible Markov model. Transition rates were allowed to differ between states.



# **GENERAL DISCUSSION**



In this discussion, I will first come back to what we have learned about the evolution of vertebrate coloration. Next, I will detail the challenges and the methodological advances in the research on coloration. Finally, I will propose some personal ideas about interesting questions that remain to explore in the study of coloration evolution.

## **What have we learned about coloration evolution in vertebrates?**

We addressed in this thesis a wide variety of questions about coloration evolution, at both intra- and inter-specific scale and with various model species.

In **chapter one**, we investigated in manuscript A the effect several selective pressures on the intraspecific variation of the dorsal, ventral and caudal coloration in two species of newts. We tested the effect of large scale climatic and altitudinal gradients as well as local environmental conditions. In addition, we tested the influence of sympatry on coloration. The two newt species were an interesting model to investigate the existence of reproductive character displacement (RCD), because they share a large part of their distribution range and have an imperfect interspecific recognition. We found that the coloration of body parts visible at distance (back and tail) converged in the sympatric zone due to environmental selection. On the other hand, sexual colorations present on body parts visible at short range (belly) tended to diverge in sympatry, likely in response to selection for interspecific recognition. We also showed that environmental variables did not all have a similar influence, with large-scale gradients having a stronger effect than local conditions on dorsal and caudal coloration. Furthermore, this study provides evidence for a role of mating choice in the evolution of coloration. Indeed, the divergence of colorations detected in sympatry was consistent with a pattern of RCD, and was present in both sexes. RCD is usually detected in males only, and this difference is probably the result of the mutual mate choice that occurs in newts.

In **chapter two**, we studied the influence of a defensive strategy, mimicry, on coloration evolution. *Manuscript B* focused on the evolution of Batesian mimicry in western Palearctic snakes. Testing the mimicry hypothesis is a challenging task, in particular at large scale; because it is difficult to obtain a measure of resemblance that account for the all the components of the phenotype. In this study, we developed a new method, based on non-standardized photographs and deep convolutional neural networks, which estimates the resemblance between organisms using their shapes, colorations and the patterns. With this

method, we could test, for the first time, the hypothesis of mimicry for all the species of a community. We showed that mimicry in western Palearctic snakes was present, but rather rare and imperfect (at least from a human point of view). This study also revealed that mimicry could occur even if the model lacks conspicuous coloration. Almost all the mimicry systems described until now are characterized by striking resemblance between model and mimic and bright aposematic coloration (e.g. coral snakes, *Heliconius* butterflies...), and these results show that other types of mimicry are possible. More generally, this study highlighted the utility of methods that use easily findable data (here photographs coming from web) to address questions at scales that could otherwise not be investigated. This first study helped us to develop the theory we present in *manuscript C*. We observed, when reviewing the literature, that examples of defensive mimicry almost always involved models with conspicuous aposematic traits. However, there is numerous examples of species that are both camouflaged and defended in the wild. We thus proposed that defensive mimicry can rely not only on signals, which are the traits that evolve to warn the predators about the unprofitability of the model, but also on cues, whose evolution is determined by other selective pressure than predator avoidance. We provided several examples of cue mimicry, some of which being frequently encountered in nature communities (e.g. ant mimicry by spiders). We further explained why cue and signal mimicry are expected to show different evolutionary dynamics. Finally, we focused on the link between cue mimicry and imperfect mimicry, and on the potential role of cues in the maintenance of signal diversity in Müllerian mimicry.

In **chapter three**, we investigated the relative influence of natural and sexual selection on the coloration diversity observed in vertebrates (*manuscript D*). Sexual selection is often thought to increase the diversity and evolution rates, because of the astonishing variety of sexual trait that are observed in some iconic groups (e.g. birds of paradise) and because sexual traits are often the first traits to diverge in recently separated taxa. Previous studies testing this question always found higher rates of evolution for sexual traits. Yet, it remains unclear if this pattern is shared across the animal kingdom, because almost all the studies until now were made on birds. In this study, we used coloration data of birds, lizards and newts to investigate this hypothesis at large taxonomic scale. We found that the effect of sexual selection was clade-dependent, with an increase of evolution rates and disparity for males and sexual colorations in birds, but more mixed results in newts, and almost no difference between sexes and sexual and non-sexual colorations in lizards. These results contrast the previous studies that investigated the question, and support the idea that sexual selection is not always a driver of the phenotypic diversity.

In **chapter four**, we focused on the link between coloration evolution and species diversification. Color polymorphism and sexual selection are both thought to increase the rates of speciation. Sexual selection is predicted to promote reproductive isolation, while polymorphism should favor rapid evolution of new lineages in populations where a morph is lost (morphic speciation). However, none of these two hypothesis have a strong support in the literature, as several studies failed to find a link between proxy of sexual selection and speciation, and only two studies investigated the morphic speciation. In manuscript E, We gather data on the presence/absence of sexual colorations and color polymorphism for all the Lacertids (~330 species). We showed that neither sexual colorations nor color polymorphism were associated with an increase of speciation rates. Lacertids diversification seems rather influenced by ecological factors, as the two most diversified genera live in arid areas. The lack of effect of sexual colorations on species diversification supports the idea that other aspect of sexual section than its intensity influence the rates of speciation. For instance, specie diversification may instead be determined by the rates of evolution of sexual traits. On the other hand, our results for color polymorphism are in opposition with previous studies, which calls into question the morphic speciation hypothesis, and highlights the need of other tests of this hypothesis.

## **The technical challenge of measuring coloration**

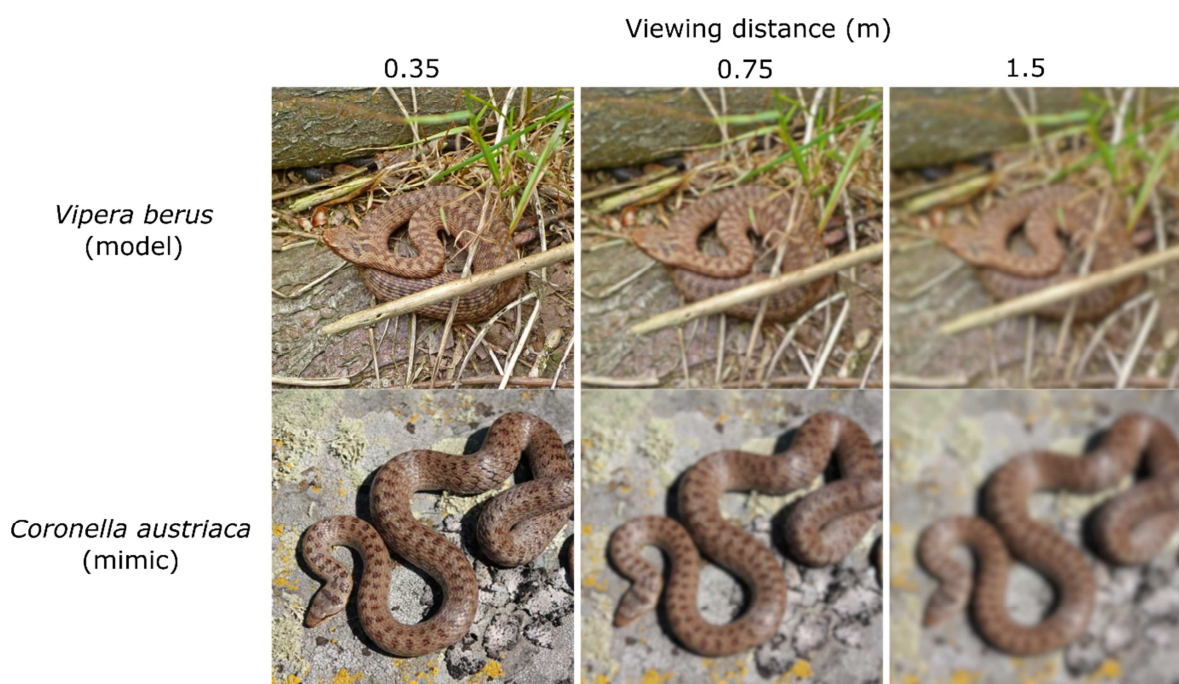
For a long time, coloration was studied and measured with the human vision (e.g. Endler 1984; Heth et al. 1988). However, the generalization of spectrophotometers and the description of photoreceptors sensibility for several species have facilitated the use of vision models, and have revolutionized the field of coloration studies by allowing more pertinent measures of coloration. Today, those models remain the standard methodology (e.g. *manuscript D*; De Lanuza et al. 2014; Shultz and Burns 2017). Yet, they only provide information about a coloration at a specific spot, as they rely on measurement of spectral reflectance, and they thus ignore how the coloration is spatially distributed.

Research on coloration has shown a growing interest for coloration patterns, and especially since patterns were found to contain as much information as spectral reflectance. Indeed, the spatial arrangement of colors is critical in the case of defensive strategy, like background matching and disruptive coloration (Stevens and Merilaita 2009; Egan et al. 2016; Troscianko et al. 2017). In addition, patterns can also play a role in intra and interspecific communication (Bortolotti et al. 2006; Pérez-Rodríguez et al. 2017),



aposematism (Green et al. 2018) and be used as a sexual ornament (Roulin et al. 2003; Sibeaux et al. 2019). All these points support the idea that patterns cannot be ignored when studying coloration.

Not taking into account the spatial arrangement of color patterns leads to the oversight of another vision parameter: the visual acuity. Visual acuity is the ability to resolve spatial details. There are several reasons for which this parameter should be considered. Firstly, the information contained in visual signals often relies on the shape of coloration patterns, which is perceived differently depending on the visual acuity. Secondly, visual acuity varies strongly across the animal kingdom, from less than 1 cycle per degree (c/deg, maximum number of parallel lines that can be seen in one angle degree without seeing a uniform surface) in most invertebrates to more than 100 c/deg for some raptors (Caves et al. 2018). Thirdly, visual acuity of human is one of the highest in the animal kingdom (~60 c/deg, Cronin et al. 2014), and the hypothesis we make about coloration functions may not be plausible when taking into account the actual perception of the concerned organisms.



**Figure 1.** Venomous species (*Vipera berus*) and its potential mimic (*Coronella austriaca*), viewed by a European lynx (*Lynx europaeae*) at three different distances. The visual acuity of the Lynx, 5.5 c/deg (Maffei et al. 1990), is around the average for the mammals predators (e.g. wolves, *Canis lupus*: 14.6 c/deg; European polecat, *Mustela putorius* : 3.6 c/deg; Veilleux and Kirk 2014). I used the R package AcuityView to create these images, which contain only spatial informations above the resolving power of a viewer of given acuity (Caves and Johnsen 2018). Photos of *Vipera berus* by Wikipedia user Wusel007, photos of *Coronella austriaca* by Stephan Tillo.

For instance, taking visual acuity into account could shed new light on the evolution of imperfect mimicry. Among the hypothesis developed to explain imperfect mimicry, only one, the relaxed selection hypothesis, considers the importance of visual acuity (although not explicitly, Kikuchi and Pfennig 2013). Yet, a simple representation of a model and its imperfect mimic, as they can be seen with the visual acuity of their predators (Figure 1), shows that imperfect mimicry could properly be explained by variation of acuity. Accounting for visual acuity is also relevant when several actors that differ in their acuity shape the evolution of coloration (Caves et al. 2018). In such cases, the same coloration can have different functions depending on the acuity of the viewer. For example, reef fish often display yellow and black coloration that are used for communication. One would expect that such conspicuous coloration would hamper the predation capacity of fishes. Yet, this coloration also evolved for predation because the invertebrates, the main prey of fishes, have a very low acuity, which leads the yellow and black patches to blur at distance into one color that matches the background (Marshall 2000).

The fact that neither pattern nor visual acuity are considered in most study on coloration cannot be attributed to a lack of methodological advances. Indeed, the field of pattern analysis has emerged in the 80s (e.g. power spectra and edge detection analysis: Field 1987; Godfrey et al. 1987; Osorio and Srinivasan 1991) and has been continually enriched since then (Endler 2012; Endler et al. 2018). There is now a large range of software and packages to perform such analyses (Stoddard et al. 2014; Van Belleghem et al. 2018; Chan et al. 2019; Maia et al. 2019; van den Berg et al. 2020). In contrast, only two recent methods have been developed to account for visual acuity (Rodríguez-Gironés and Ruiz 2016; Caves and Johnsen 2018). Yet, these methods are easily performed and they could be applied routinely. The time lag between the development and the implementation of new methods may instead reside in the difference of material they require. Pattern analysis are based on standardized photographs taken with special cameras (Troscianko and Stevens 2015), while visual models use spectral reflectance measured with a spectrometer. It is likely that it will take some time for researchers to get new equipment.

The utilization of photographs enables, like spectral reflectance data, to analyze coloration with the point of view of other species. Indeed, standardized photographs can be transformed to match the colors perceived by eyes with nonhuman photoreceptors sensibilities (Verhoeven et al. 2018), and they can capture ultraviolet colorations if they are taken and analyzed properly (Troscianko and Stevens 2015). These features, as well as color and pattern analysis and the ability to account for visual acuity, have been recently implemented in a new framework (Quantitative Colour Pattern Analysis, van den Berg et al.

2020). It would be possible, with this framework, to perform the same analysis than those used currently in studies on coloration, but improved and based on standardized photographs rather than spectral reflectance.

Nonetheless, classic analyses of colors and patterns use the whole information of images. This characteristic is sometime valuable, for instance to compare species and background patterns when studying camouflage (Chiao et al. 2011; Stevens et al. 2013; Kang et al. 2015). However, this feature is a drawback when one wants to compare the coloration and pattern of two species without having to isolate the organisms from their environment (i.e. having a neutral background for the picture). For this task, the methods based on artificial neural networks (ANN) seem to be the most promising. Our analysis in *manuscript C* revealed the powerful capacities of ANN to compare the phenotype of species, despite the fact that the snakes we studied were well camouflaged in their environment and that we did not remove the picture's backgrounds. In comparison, the preliminary analyses that we made with more classic tools of pattern analysis (scale invariant feature, Lowe 1999) were completely unable to identify and compare the object of interest in such complex environment. Depending on the data with which they are fed, ANN could be applied to different questions. If they are trained on unstandardized pictures, like we did in *manuscript C*, they cannot account for visual acuity nor difference of photoreceptors sensibility, but they can handle large datasets without prior image manipulations. On the other hand, ANN may be a proper method to investigate more specific questions if they are fed with standardized photographs. Indeed, ANN are currently trained on RGB images, whose colors were designed for human photoreceptors, and they provide measures of resemblance that match human perception better than any other method (Zhang et al. 2018). I thus expect that ANN, fed with pictures transformed according to the photoreceptors sensibility and visual acuity of an observer, may generate very precise measures of perception. The main limit of ANN is the difficulty to identify the criteria used to quantify the phenotypes, but several methods have been recently developed to respond to this problematic (reviewed in Montavon et al. 2018).

## Future directions

I will provide in this section a few personal ideas on pending questions in the field of coloration evolution.

While we know relatively well the different selective forces that shape the evolution of coloration in diurnal animals, we understand much less the **evolution of coloration in nocturnal animals**. Recent researches showed that, despite very low levels of light, many nocturnal species can perceive both achromatic and chromatic variations of coloration at night (Kelber et al. 2002; Roth and Kelber 2004; Somanathan et al. 2008; Gomez et al. 2010). Just like diurnal species, coloration of nocturnal species was found to play a role in crypsis (Hanlon et al. 2007), aposematism (Mochida 2009; Marek et al. 2011), mating choice (Gomez et al. 2010; Kelley et al. 2019) and even mimicry (Figure 2, Howard and Brodie 1971). Yet, a larger proportion of nocturnal than diurnal species show dull color (Pekár 2014), supporting the idea that coloration of nocturnal species primarily evolves for camouflage. To me, these observations are somewhat contradictory: why are nocturnal animals overall more cryptic? If it is because nocturnal species do not rely (or rely less) on vision for their intra- and interspecific interactions, then why are there so many examples of signaling coloration? Do nocturnal species with signaling colorations share particular suites of ecological or behavioral traits? Or are they living in specific environmental conditions? In addition, signals displayed by nocturnal species seem to be based primarily on achromatic contrasts (e.g. Stankowich et al. 2011), although exceptions exist (Robertson and Greene 2017). Thus, it would be interesting to study why some species rely on chromatic signals, while achromatic signals are predicted to be more conspicuous under low levels of light.



**Figure 2.** Examples of nocturnal species displaying signaling colorations. (a) The eastern spotted skunk (*Spilogale putorius*) have an aposematic coloration, (b) the red salamander (*Pseudotriton ruber*) is a mimic of *Notophthalmus viridescens*, and (c) the red-eyed treefrog (*Agalychnis callidryas*) showed conspicuous sexual signals on its flanks. Photos (a) by Wikipedia user Magnus Manske, photos (a) by Wikipedia user Athene cunicularia and photos (c) by Wikipedia user Careyjamesbalboa.

**Social selection** is the only main selection pressure that I did not study in this thesis, although I discussed its implications for the evolution of coloration in *manuscript D* (the social selection referred here is the social non-sexual selection, as sexual selection is considered as a particular type of social selection, Lyon and Montgomerie 2012). This selective pressure is nonetheless crucial for the evolution of coloration. Indeed, social selection is a plausible cause for the evolution of female ornaments of many species (reviewed in Tobias et al. 2012), and social colorations are often used in cooperative species to indicate dominance (Dale et al. 2015; Bachmann et al. 2017). Yet, it may be difficult to distinguish between sexual and non-sexual social selection, especially in males where competition for territories is often associated with competition for females (e.g. in wall lizards Martin et al. 2015). Due to this difficulty, the role of social selection in coloration evolution is still not fully understood, and may be underestimated in most species. When enough data will be available, it would be interesting to repeat the analysis of *manuscript D* separating social and sexual selection, to see if they have the same consequences on coloration disparity and evolution rates.

The ability of ANN to exploit unstandardized photographs could allow the investigation of another metric: **the phenotypic diversity of a community**. The huge number of pictures available on the web makes this measure conceivable. The data could be gathered automatically, using either automatic google research or online website (e.g. iNaturalist). They could also come from natural history museums, that often photographs of the species present in their collection. Of course, the automation would not prevent the necessity to filter the pictures, as species are often wrongly identified. However, this work would be easily accomplished by an expert as the number of pictures could be relatively limited for most class (~5-10 images per species). Indeed, ANN requires a large number of pictures only for the few classes used for the training, but not for all classes in which the phenotype is measured. The only remaining difficulty is the selection of pictures, since it is necessary that all images represent the same view of the body, so that the measure of phenotypic diversity makes sense (e.g. include photographs of ventral or lateral views would bias the analysis if this view is not available for all species). Once the phenotype of species is measured, many questions could be investigated. I believe it would be interesting to study how light environments, or other constraints, influence the phenotypic space used by a community (e.g. fishes communities at different depth). We could also test the effect of competition and environmental filtering though patterns of phenotypic over or underdispersion of communities. Given that distribution maps are available for most vertebrate terrestrial species (Roll et al. 2017; IUCN 2020), it would be possible to study the

spatial distribution of phenotypic diversity. For instance, we could study the factors (environmental, biotic) that influence the gradients of phenotypic diversity. Such questions have begun to be addressed in a recent paper about moths (Wu et al. 2019), and I believe it is a promising avenue of research.



## References

- Bachmann, J. C., F. Cortesi, M. D. Hall, N. J. Marshall, W. Salzburger, and H. F. Gante. 2017. Real-time social selection maintains honesty of a dynamic visual signal in cooperative fish. *Evol. Lett.* 1:269–278.
- Bortolotti, G. R., J. Blas, J. J. Negro, and J. L. Tella. 2006. A complex plumage pattern as an honest social signal. *Anim. Behav.* 72:423–430.
- Caves, E. M., N. C. Brandley, and S. Johnsen. 2018. Visual Acuity and the Evolution of Signals.
- Caves, E. M., and S. Johnsen. 2018. AcuityView : An r package for portraying the effects of visual acuity on scenes observed by an animal. *Methods Ecol. Evol.* 9:793–797.
- Chan, I. Z. W., M. Stevens, and P. A. Todd. 2019. pat-geom : A software package for the analysis of animal patterns. *Methods Ecol. Evol.* 10:591–600.
- Chiao, C. C., J. K. Wickiser, J. J. Allen, B. Genter, and R. T. Hanlon. 2011. Hyperspectral imaging of cuttlefish camouflage indicates good color match in the eyes of fish predators. *Proc. Natl. Acad. Sci. U. S. A.* 108:9148–9153.
- Cronin, T., S. Johnsen, N. Marshall, and E. Warrant. 2014. *Visual ecology*. Princeton University Press.
- Dale, J., C. J. Dey, K. Delhey, B. Kempenaers, and M. Valcu. 2015. The effects of life history and sexual selection on male and female plumage colouration. *Nature* 527:367–370.
- De Lanuza, G. P. i., P. Carazo, and E. Font. 2014. Colours of quality: Structural (but not pigment) coloration informs about male quality in a polychromatic lizard. *Anim. Behav.* 90:73–81.
- Egan, J., R. J. Sharman, K. C. Scott-Brown, and P. G. Lovell. 2016. Edge enhancement improves disruptive camouflage by emphasising false edges and creating pictorial relief. *Sci. Rep.* 6:1–9.
- Endler, J. A. 2012. A framework for analysing colour pattern geometry: adjacent colours. *Biol. J. Linn. Soc.* 107:233–253.
- Endler, J. A. 1984. Natural and sexual selection on color patterns in poeciliid fishes. Pp. 95–111 in.
- Endler, J. A., G. L. Cole, and A. M. Kranz. 2018. Boundary strength analysis: Combining colour pattern geometry and coloured patch visual properties for use in predicting behaviour and fitness. *Methods Ecol. Evol.* 9:2334–2348.
- Field, D. J. 1987. Relations between the statistics of natural images and the response properties of cortical cells. *J. Opt. Soc. Am. A* 4:2379.
- Godfrey, D., J. N. Lythgoe, and D. A. Rumball. 1987. Zebra stripes and tiger stripes: the spatial frequency distribution of the pattern compared to that of the background is significant in display and crypsis. *Biol. J. Linn. Soc.* 32:427–433.
- Gomez, D., C. Richardson, T. Lengagne, M. Derex, S. Plenet, P. Joly, J. P. Léna, and M. Théry. 2010. Support for a role of colour vision in mate choice in the nocturnal European treefrog (*Hyla arborea*). *Behaviour* 147:1753–1768.
- Green, N. F., H. H. Urquhart, C. P. Van Den Berg, N. J. Marshall, and K. L. Cheney. 2018. Pattern edges improve predator learning of aposematic signals. *Behav. Ecol.* 29:1481–1486.
- Hanlon, R. T., M. J. Naud, J. W. Forsythe, K. Hall, A. C. Watson, and J. McKechnie. 2007. Adaptable night camouflage by cuttlefish. *Am. Nat.* 169:543–551.
- Heth, G., A. Beiles, and E. Nevo. 1988. Adaptive variation of pelage color within and between

- species of the subterranean mole rat (*Spalax ehrenbergi*) in Israel. *Oecologia* 74:617–622.
- Howard, R. R., and E. D. Brodie. 1971. Experimental study of mimicry in salamanders involving *notophthalmus viridescens viridescens* and *pseudotriton ruber schencki*. *Nature* 233:277.
- IUCN. 2020. The IUCN Red List of Threatened Species.
- Kang, C., M. Stevens, J. Y. Moon, S. I. Lee, and P. G. Jablonski. 2015. Camouflage through behavior in moths: The role of background matching and disruptive coloration. *Behav. Ecol.* 26:45–54.
- Kelber, A., A. Balkenius, and E. J. Warrant. 2002. Scotopic colour vision in nocturnal hawkmoths. *Nature* 419:922–925.
- Kelley, J. L., N. J. Tatarinic, G. E. Schröder-Turk, J. A. Endler, and B. D. Wilts. 2019. A dynamic optical signal in a nocturnal moth. *Curr. Biol.* 29:2919–2925.e2.
- Kikuchi, D. W., and D. W. Pfennig. 2013. Imperfect Mimicry and the Limits of Natural Selection. *Q. Rev. Biol.* 88:297–315.
- Lowe, D. G. 1999. Object recognition from local scale-invariant features. Pp. 1150–1157 in *Proceedings of the IEEE International Conference on Computer Vision*.
- Lyon, B. E., and R. Montgomerie. 2012. Sexual selection is a form of social selection. *Philos. Trans. R. Soc. B Biol. Sci.* 367:2266–2273.
- Maffei, L., A. Fiorentini, and S. Bisti. 1990. The visual acuity of the Lynx. *Vision Res.* 30:527–528.
- Maia, R., H. Gruson, J. A. Endler, and T. E. White. 2019. pavo 2: New tools for the spectral and spatial analysis of colour in r. *Methods Ecol. Evol.* 10:1097–1107.
- Marek, P., D. Papaj, J. Yeager, S. Molina, and W. Moore. 2011. Bioluminescent aposematism in millipedes.
- Marshall, J. N. 2000. Communication and camouflage with the same ‘bright’ colours in reef fishes. *Philos. Trans. R. Soc. London. Ser. B Biol. Sci.* 355:1243–1248.
- Martin, M., S. Meylan, S. Perret, and J. F. Le Galliard. 2015. UV coloration influences spatial dominance but not agonistic behaviors in male wall lizards. *Behav. Ecol. Sociobiol.* 69:1483–1491.
- Mochida, K. 2009. A parallel geographical mosaic of morphological and behavioural aposematic traits of the newt, *Cynops pyrrhogaster* (Urodela: Salamandridae). *Biol. J. Linn. Soc.* 97:613–622.
- Montavon, G., W. Samek, and K.-R. Müller. 2018. Methods for interpreting and understanding deep neural networks. *Digit. Signal Process.* 73:1–15.
- Osorio, D., and M. V. Srinivasan. 1991. Camouflage by edge enhancement in animal coloration patterns and its implications for visual mechanisms. *Proc. R. Soc. London. Ser. B Biol. Sci.* 244:81–85.
- Pekár, S. 2014. Comparative analysis of passive defences in spiders (Araneae). *J. Anim. Ecol.* 83:779–790.
- Pérez-Rodríguez, L., R. Jovani, and M. Stevens. 2017. Shape matters: animal colour patterns as signals of individual quality. *Proc. R. Soc. B Biol. Sci.* 284:20162446.
- Robertson, J. M., and H. W. Greene. 2017. Bright colour patterns as social signals in nocturnal frogs. *Biol. J. Linn. Soc.* 121:849–857.
- Rodríguez-Gironés, M. A., and A. Ruiz. 2016. toBeeView: a program for simulating the retinal image of visual scenes on nonhuman eyes. *Ecol. Evol.* 6:7892–7900.
- Roll, U., A. Feldman, M. Novosolov, A. Allison, A. M. Bauer, R. Bernard, M. Böhm, F. Castro-



- Herrera, L. Chirio, B. Collen, G. R. Colli, L. Dabool, I. Das, T. M. Doan, L. L. Grismer, M. Hoogmoed, Y. Itescu, F. Kraus, M. Lebreton, A. Lewin, M. Martins, E. Maza, D. Meirte, Z. T. Nagy, C. D. C. Nogueira, O. S. G. Pauwels, D. Pincheira-Donoso, G. D. Powney, R. Sindaco, O. J. S. Tallwin, O. Torres-Carvajal, J. F. Trape, E. Vidan, P. Uetz, P. Wagner, Y. Wang, C. D. L. Orme, R. Grenyer, and S. Meiri. 2017. The global distribution of tetrapods reveals a need for targeted reptile conservation. *Nat. Ecol. Evol.* 1:1677–1682.
- Roth, L. S. V., and A. Kelber. 2004. Nocturnal colour vision in geckos. *Proc. R. Soc. London. Ser. B Biol. Sci.* 271.
- Roulin, A., A. Ducrest, F. Balloux, C. Dijkstra, and C. Riols. 2003. A female melanin ornament signals offspring fluctuating asymmetry in the barn owl. *Proc. R. Soc. London. Ser. B Biol. Sci.* 270:167–171.
- Shultz, A. J., and K. J. Burns. 2017. The role of sexual and natural selection in shaping patterns of sexual dichromatism in the largest family of songbirds (Aves: Thraupidae). *Evolution (N. Y.)* 71:1061–1074.
- Sibaux, A., G. L. Cole, and J. A. Endler. 2019. The relative importance of local and global visual contrast in mate choice. *Anim. Behav.* 154:143–159.
- Somanathan, H., R. M. Borges, E. J. Warrant, and A. Kelber. 2008. Nocturnal bees learn landmark colours in starlight. *Curr. Biol.* 18:R996–R997.
- Stankowich, T., T. Caro, and M. Cox. 2011. Bold coloration and the evolution of aposematism in terrestrial carnivores. *Evolution (N. Y.)* 65:3090–3099.
- Stevens, M., and S. Merilaita. 2009. Defining disruptive coloration and distinguishing its functions. *Philos. Trans. R. Soc. B Biol. Sci.* 364:481–488.
- Stevens, M., C. P. Rong, and P. A. Todd. 2013. Colour change and camouflage in the horned ghost crab *Ocypode ceratophthalmus*. *Biol. J. Linn. Soc.* 109:257–270.
- Stoddard, M. C., R. M. Kilner, and C. Town. 2014. Pattern recognition algorithm reveals how birds evolve individual egg pattern signatures. *Nat. Commun.* 5:4117.
- Tobias, J. A., R. Montgomerie, and B. E. Lyon. 2012. The evolution of female ornaments and weaponry: Social selection, sexual selection and ecological competition. *Philos. Trans. R. Soc. B Biol. Sci.* 367:2274–2293.
- Troscianko, J., J. Skelhorn, and M. Stevens. 2017. Quantifying camouflage: how to predict detectability from appearance. *BMC Evol. Biol.* 17:7.
- Troscianko, J., and M. Stevens. 2015. Image calibration and analysis toolbox - a free software suite for objectively measuring reflectance, colour and pattern. *Methods Ecol. Evol.* 6:1320–1331.
- Van Belleghem, S. M., R. Papa, H. Ortiz-Zuazaga, F. Hendrickx, C. D. Jiggins, W. Owen McMillan, and B. A. Counterman. 2018. patternize: An R package for quantifying colour pattern variation. *Methods Ecol. Evol.* 9:390–398.
- van den Berg, C. P., J. Troscianko, J. A. Endler, N. J. Marshall, and K. L. Cheney. 2020. Quantitative Colour Pattern Analysis (QCPA): A comprehensive framework for the analysis of colour patterns in nature. *Methods Ecol. Evol.* 11:316–332.
- Veilleux, C. C., and E. C. Kirk. 2014. Visual Acuity in Mammals: Effects of Eye Size and Ecology. *Brain. Behav. Evol.* 83:43–53.
- Verhoeven, C., Z.-X. Ren, and K. Lunau. 2018. False-colour photography: a novel digital approach to visualize the bee view of flowers. *J. Pollinat. Ecol.* 23:102–118.
- Wu, S., C. M. Chang, G. S. Mai, D. R. Rubenstein, C. M. Yang, Y. T. Huang, H. H. Lin, L. C. Shih, S.

W. Chen, S. F. Shen, C. M. Chang, S. W. Chen, Y. T. Huang, C. M. Yang, H. H. Lin, S. Wu, D. R. Rubenstein, and G. S. Mai. 2019. Artificial intelligence reveals environmental constraints on colour diversity in insects. *Nat. Commun.* 10:1–9.

Zhang, R., P. Isola, A. A. Efros, E. Shechtman, and O. Wang. 2018. The Unreasonable Effectiveness of Deep Features as a Perceptual Metric. Pp. 586–595 in *Conference on Computer Vision and Pattern Recognition*.



# **Annex**

## **Opportunistic records reveal Mediterranean reptiles' scale-dependent responses to anthropogenic land use**

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## Abstract

Although classified among the greatest threats to the world's biodiversity, the effects of land use and their scale dependency are left unexplored in many taxonomic groups. Reptiles are among the most data-deficient vertebrates in this respect, although their ecological traits make them highly sensitive to habitat modifications. We tested whether land use gradients shape the distributions of Mediterranean reptiles at regional and local scales, and whether species' ecological traits and phylogeny explain these patterns. Reptiles are generally rare and hard to survey through standardized protocols. We overcame these obstacles by modeling an original data set of 18164 opportunistic occurrence records for 14 reptile species with spatially-explicit point process models incorporating known sources of sampling heterogeneity and spatially autocorrelated error. At a regional scale, reptiles favored open habitats and tended to avoid urban areas. At a local scale, the persistence of open habitats did better than forest resulting from land abandonment in maintaining reptiles within a heavily anthropogenic matrix. Contrary to our expectations, the absence of any clear trait or phylogenetic signals suggests that these responses are mediated by a complex interplay between species' ecology and regional biogeographic history. These results demonstrate that reptile responses to land use are scale-dependent and locally exacerbated when anthropogenic pressure is high. We further show that land abandonment is insufficient to preserve reptiles in the face of anthropogenic pressures unless patches of suitable habitat are effectively maintained. Eventually, our study further illustrates the effectiveness of volunteer-based opportunistic sampling in testing hypotheses on the determinants of rare species' distributions.

**Keywords:** land conversion, multiscale analysis, spatial point process models.



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## **Introduction**

Habitat conversions have modified the taxonomic, functional and phylogenetic compositions of species assemblages (Flynn et al. 2009, Newbold et al. 2015, Kehoe et al. 2015), resulting in scale- and taxa-dependent response patterns to gradients of anthropogenic land use (Dornelas et al. 2014, McGill et al. 2015). Unfortunately, responses to land use of numerous ecosystem service providers, flagship taxa and threatened species are under-reported due to data deficiency (Good et al. 2006, Morais et al. 2013, Howard et al. 2014). In the present study, we aimed to perform a cross-scale, spatially explicit assessment of the responses to human land use of terrestrial Squamata reptiles, one of the most understudied animal orders.

Land use takes a wide range of forms, from a near-complete removal of spontaneous vegetation in urban centres and croplands to less stringent modifications associated with forest exploitation or extensive farming (Foley et al. 2005, Fischer and Lindenmayer 2007, Haberl et al. 2007). Land use modifies habitat quantity and configuration, resource availability within habitats and the spatial distribution of ecological processes associated with ecosystem services such as pollination, seed dispersal and predation (Fischer and Lindenmayer 2007, Fahrig et al. 2011). Depending on the magnitude of land conversions, land use can be associated with changes in species co-occurrence networks (Kay et al. 2018), decreased species diversity or abundances and altered demographic rates (e.g. Foley et al. 2005, Flynn et al. 2009, Newbold et al. 2015, Kehoe et al. 2015). The inverse pattern, post-use land abandonment, results in a gradual recovery of spontaneous vegetation successions (Prévosto et al. 2011). Land abandonment can have a regionally significant imprint on the distribution of habitats and resources (Ellis et al. 2013), but whether it leads to species and diversity recovery or to further losses depends on the initial level of disturbance, studied taxa, community composition and biogeographic history (Falcucci et al. 2007, Sirami et al. 2010). For instance, extensive land abandonment in the north-western Mediterranean region since the 1940's has resulted in diversity increases in some places but diversity losses in others, associated with vegetation successions leading to homogeneous broadleaved forests (Laiolo et al. 2004, Moreira and Russo 2007).

The influence of land use on species distributions is progressively overlaid by climate, topographic barriers and biogeographic legacies while shifting from local to regional scales (Huston 1999, Pearson and Dawson 2003). In spite of this well-established pattern, urbanization, agriculture and other land use forms can decrease assemblage diversity and reduce species distribution ranges over regional extents, typically in birds (Bennett et al.



2004, Meynard et al. 2011). Meanwhile, how land use modifies local species diversity and distributions depends on location, climate, taxa and the types of land use considered: for instance, local changes in species distributions are stronger where land use has removed productive habitats, and for narrow-ranged species (Newbold et al. 2016). As a result, the regional and local outcomes of land use on biodiversity are often uncorrelated and hard to predict, leading to discrepancies in the conclusions of studies performed over different spatial extents or grains (Vellend et al. 2013, Dornelas et al. 2014), which highlights the need for cross-scale comparative studies (McGill et al. 2015).

Reptiles remain one of the most data-deficient groups among vertebrates (Böhm et al. 2013), a consequence of their low detectability and overall rarity (Donnelly et al. 2005, Böhm et al. 2013). Most studies on land use effects on reptiles have consequently been limited to local extents (Trimble and van Aarde 2014), coarse resolution data (Ribeiro et al. 2009) or non-spatial designs (Todd et al. 2017, Keinath et al. 2017). These limitations have prevented a cross-scale assessment of the impacts of land use on reptile distributions, with the notable exception of a region-level study which reported associations between rare Squamata species and near-extirpated habitats in the Carolinas (Todd et al. 2016). In general, and although some reptiles can accommodate relatively high levels of disturbance, many species living in temperate biomes are tied to little-disturbed habitats that retain sufficient levels of prey abundance and microhabitats (Moreira and Russo 2007).

Reptiles have recently undergone declines at regional (Driscoll 2004) and global scales (Gibbons et al. 2000, Keinath et al. 2017), partly explained by ecological trait suites associated with vulnerability to anthropogenic disturbance of resources and habitats (Cardillo et al. 2005, Trimble and van Aarde 2014). As ectothermic predators, most reptile species combine small home ranges, high habitat and dietary specialization, low dispersal and sensitivity to microclimates (Huey 1982). These traits are often associated with high vulnerability to habitat conversion (Tscharntke et al. 2002, Le Viol et al. 2012, Keinath et al. 2017), while broad habitat range, exogenous origin and generalist diet favor tolerance to anthropogenic disturbance (Rayner et al. 2015). In line with these general patterns, a meta-analysis pointed reptiles' habitat specialization to explain their high sensitivity to landscape fragmentation (Keinath et al. 2017). This result was further supported by a regional study in eastern North America, which showed that high human imprint on land use threaten snakes with carnivorous diets, low reproductive rates, fossorial and aquatic habitats (Todd et al. 2017). However, none of these studies distinguished the effects of multiple types of land use, nor did they account for the spatial organization of land use types within a region or a landscape. Hence, although there are empirical reasons to hypothesize that a suite of

ecological traits predicts reptiles' distributional responses to land use; whether these responses are consistent across spatial scales and land use types remains to be tested.

Citizen-based databases of opportunistic herpetological records, which have become widespread in recent years (Wheeler 2004, Silvertown 2009, Callaghan and Gawlik 2015), offer an opportunity to fill this knowledge gap. However, they remain underexploited in spite of the surge of powerful species distribution modelling methods such as point process models (PPMs; Renner et al. 2015, Baddeley et al. 2015). Unlike many other methods for building species distribution models with opportunistic records, PPMs have several advantageous characteristics that facilitate their implementation and interpretation in the face of statistical challenges raised by such data. In particular, they provide a flexible framework for accounting for sources of sampling heterogeneity, clarify key details necessary for model fitting and mapping predictions (including the choice of pseudo-absence locations), and support a large suite of tools for diagnosing spatial dependence among records and model residuals (Renner et al., 2015).

In this study, we showcased the utility of opportunistic records to perform one of the first spatially explicit, cross-scale assessment of reptile responses to land use and land abandonment using PPMs. To this aim, we made use of an extensive volunteer-based database of 18164 individual records which spans all the reptile assemblage of southern France (Geniez and Cheylan 2012). This region is structured by a gradient from urban and agricultural plains in coastal lowlands to inland broadleaved forests resulting from the abandonment of former pastures and cultures during the first half of the 20th century (Sirami et al. 2010, Vimal et al. 2017). We specifically asked the following questions:

- (i) How are reptile species' distributions organized along gradients from abandoned land to densely urbanized and cultivated landscapes?
- (ii) How do these responses change from regional to local scales?
- (iii) Can ecological traits explain these responses?

We hypothesized that reptile occurrence would decrease from the least to the most human-used landscapes, with stronger responses at the regional than at the local scale and a positive effect of land abandonment and open habitats. We expected that interspecific variations in these responses would be explained by body size, dietary and behavioral traits.

## **Material and methods**

### **Study area**

Our study encompassed the French Mediterranean lowlands from the eastern Pyrenees to the Rhône river and north to the Cévennes mountains. This region (hereafter “regional scale” and “regional window”, Fig.1) is characterized by mild winters and warm and dry summers typical of the Mediterranean climate (Table 1). We excluded large open water bodies from the analyses as none of the species considered in our analyses is strictly aquatic. At the regional scale, coastal plains are dominated by marshes, agriculture, groves and several large urban centers. Human presence decreases inland, where dense forests and scrublands result from land abandonment since the 1940’s. In these more hilly landscapes, open habitats are artificially maintained for small livestock pastures and recreational activities around farms and villages (Sluiter and de Jong 2007, Sirami et al. 2010). No historical forests persist apart from residual patches at the extreme south and east of the region, too small to be sampled by our data set.

We delineated a 36x94 km window around Montpellier city (Fig. 1, hereafter “local scale” and “local window”), the largest urban center of the region (600 000 inhabitants). The local window consists of a gradient from a city center to agricultural mosaics with interspersed woods and farms. Variation in altitude is low (Table 1) and climate is typical of the region. In spite of a high level of anthropogenization, most of the habitats found elsewhere in the region are represented in this local window.

### **Reptile data**

#### *Reptile records*

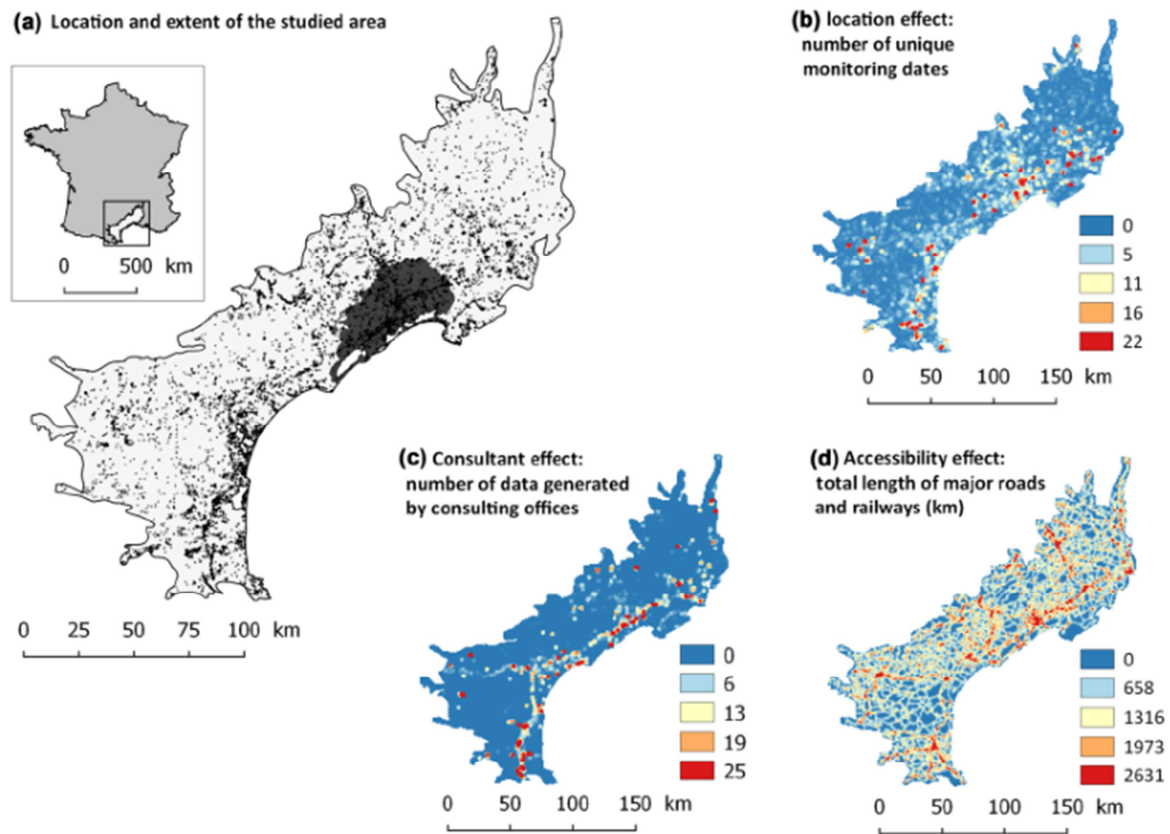
We retrieved reptile occurrence from a citizen science database maintained since the 1970s in southern France, with the aim to increase knowledge of species’ distributions, habitat use and conservation status (The Malpolon Database, Geniez and Cheylan 2012). Approximately 71% of these data consist of opportunistic records gathered by volunteer naturalists without any standardized protocol. The remaining 25% of the records were generated by surveys as part of environmental impact studies, and 4% of the data were obtained from literature records.

We extracted reptile data acquired between 2009 and 2015. This time window corresponds to the years with the highest number of reptile records and maximum spatial coverage; expanding the timeframe would have led to unmanageable variations in sampling effort. Preliminary analyses showed that this timeframe was short enough to neglect temporal variations in species' regional and local distributions, climate and land use (Appendix S1). Thus, our data represent a reasonable snapshot of reptiles' spatial relationships with the studied land use variables. We initially retrieved a total of 20929 occurrence records spanning 23 reptile species over the region, of which 4262 records for 19 species were located in the local window. The geographic location and species identification of each record was validated by local experts prior to inclusion based on observer's expertise, coherence of localization, time period and habitat, and descriptions or photographs if available. We removed records of all exogenous species except *Tarentola mauritanica*, which was established in the region well before the mid-19<sup>th</sup> century (list of excluded species in Appendix S2). We further eliminated three exclusively aquatic species, *Trachemys scripta*, *Emys orbicularis* and *Mauremys leprosa*, which are too rare and too tied to water to expect any interpretable relationship with the studied land use gradients. We retained only species with at least 200 records at the regional scale and 80 records at the local scale to ensure unbiased sampling and sufficient power in statistical analyses. Preliminary exploration revealed that species falling beyond these thresholds had too aggregated or too sparse record distributions with respect to their known pattern of occurrence at regional scale (Appendix S3). These filters resulted in a final data set comprising 18164 occurrences for 14 species at the regional scale and 3168 occurrences for 10 species at the local scale (four species absent in the latter, Appendix S2).

#### *Ecological traits and phylogeny*

We described the phenotypic and behavioral differences among the 14 studied species with nine ecological traits related to resource exploitation, productivity and mobility: body size (distance between the tip of the muzzle to the cloaca in mm), percentages of use of eight prey categories (mammals, birds, reptiles, amphibians, fish, arthropods, mollusks and worms, plants), daily activity (percentage of diurnal and nocturnal activity), yearly activity (average number of days of activity per year), mobility (crawler, walker or climber), main habitat (aquatic, terrestrial, burrowing, arboreal and rupicolous), clutch size (number of eggs per year), age at sexual maturity and reproduction mode (oviparity or viviparity). We compiled these traits from published literature (Andreu et al. 1998, Vacher and Geniez 2010,

Geniez and Cheylan 2012) and expert advice, complemented by body size measurements on collection samples. Niche conservatism forces phylogenetically related species to share similar traits, responses to environmental variations and levels of threat facing human disturbance (McKinney 1997). To account for such potential dependence, we used a dated phylogenetic tree built by a local expert based on topologies and divergence times extracted from the literature (details and references in Appendix S5).



**Figure 1.** (a) Location of the study area (insert), extent of the regional window (light grey) and of the local window (dark grey). Black dots show geolocated reptile records, all species confounded. Panels b-d display the distributions of (b) number of unique sampling dates within 250 m around each reptile record (“location effect”), (c) records from consulting offices (“consultant effect”) and (d) total length of roads and railways in 1 km<sup>2</sup> square cells (“accessibility effect”).

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## Environmental data

### *Regional scale*

We quantified regional land use with Corine Land Cover 2012 (CLC, Bossard et al. 2000). CLC is a European-level standardized description of land-use polygons down to a minimum of 25ha. It represents the best description of land use currently available for regional studies, with an accuracy of 70 to 95% depending on land use classes (Buttner and Maucha 2006, Caetano et al. 2006; the least accurate classes do not appear in our study area). We did not need the level of detail provided by the 48 classes of the original CLC nomenclature to create gradients of land use and land abandonment relevant to reptiles in our region. Furthermore, many CLC classes were absent, lowly represented and / or highly correlated in our study extent. We therefore aggregated polygons into four main land use types: urban, agricultural, open natural or semi-natural habitats and forest (from the most to the least used by humans in the region; correspondence with the original CLC nomenclature in Appendix S6). We quantified the percentage of these four land-use categories in 1 km x 1 km square cells defined independently from the distribution of reptile records (n = 18970 cells, Table 1). Using the same grid, we calculated the distance from cell centroids to the nearest water point (French National Geographic Institute IGN: BD TOPO®, 100 m resolution, <http://www.professionnels.ign.fr/bdtopo>). We needed to ensure that the effects of these land cover variables were controlled for variations in altitude and climate, two typical predictors of species' distributions (Pearson and Dawson 2003). Preliminary investigations of climatic data revealed that in the study area, altitude is highly correlated with temperature variations (Pearson's correlation coefficient  $r = -0.97$ ) and precipitations ( $r = 0.48$ ). Hence, we summarized topoclimatic variations by average altitude per 1 km<sup>2</sup> square cells from a digital elevation model at a 25 m native resolution (data from the IGN BD ALTI®, <http://professionnels.ign.fr/bdalti>).

### *Local scale*

The local window encompasses a dense urban centre, shallow urbanization, small woods, wastelands and numerous farms with small parcels of groves and agriculture. Visual checks and field controls revealed that CLC does not capture these fine-grained gradients adequately ; we therefore replaced it by a land use vector layer created by Montpellier city office and validated with field checks (Table 1 and Appendix S6, <http://data.montpellier3m.fr/>, minimum polygons of 2 ha and 88% of accuracy, Lhernould 2016). We derived the same four land use categories as for the regional scale from the 60

**Table 1.** Quantitative summary of land-use, altitude and sampling variables used in the regional and local point process models. These variables were subsequently scaled for modeling.

<b>Variables</b>		<b>Mean ± SD [range] at regional scale</b>	<b>Mean ± SD [range] at local scale</b>
		(1 km <sup>2</sup> square cells unless otherwise stated)	(0.0625 km <sup>2</sup> square cells unless otherwise stated)
Land use variables	Urbanization (% per cell)	0.06 ± 0.15 [0 ; 1]	0.12 ± 0.25 [0 ; 1]
	Agriculture (% per cell)	0.41 ± 0.35 [0 ; 1]	0.29 ± 0.34 [0 ; 1]
	Open habitats (%per cell)	0.22 ± 0.27 [0 ; 1]	0.25 ± 0.29 [0 ; 1]
	Forest (% per cell)	0.26 ± 0.31 [0 ; 1]	0.16 ± 0.26 [0 ; 1]
	Distance to water (from cell centroid)	125.14 ± 338.56 [0 ; 5448.00]	374.59 ± 378.77 [0 ; 2247.22]
Topographic variable	Average altitude (per cell, m)	208.10 ± 186.80 [0 ; 1229]	69.67 ± 74.01 [0 ; 632]
Sampling variables	Consultant effect (number of records from consulting offices in a 250 m buffer around each record)	0.26 ± 1.40 [0 ; 45.18]	0.26 ± 1.40 [0 ; 25.42]
	Location effect (number of unique sampling dates in a 250 m buffer around each record)	0.47 ± 1.72 [0 ; 175]	0.47 ± 1.72 [0 ; 47.03]
	Accessibility effect (total length of roads and railways per cell, m)	875.39 ± 704.69 [0 ; 5694.94]	68.73 ± 100.49 [0 ; 969.01]

original land use classes of this second layer (Appendix S6), which we overlaid with 250m x 250m square cells constructed independently from the distribution of reptile records (21208 grid cells in total). We retrieved distances to the nearest water point and altitude from the same layer as for the regional scale (correlation with temperature:  $r=-0.97$ , with precipitations:  $r=0.83$ ).

*Sampling effort*

The spatial distribution of opportunistic records is notoriously conditioned by spatial variations in sampling effort (Isaac et al. 2014). We distinguished three sources of sampling heterogeneity in our data (Table 1). First, amateur records are typically isolated in space, while consulting offices dedicated to environmental impact studies generate tens to hundreds of records at non-random locations typically located along motorways, railways or other large infrastructures. We identified records issued by consulting offices and counted

the number of surrounding records, all species combined, within a circular buffer of 250m radius centered on each record (defined as the area surveyed by an herpetologist under usual conditions, from expert advice). This variable identified areas where sampling pressure is increased by consulting offices (“consultant effect”). Second, amateurs tend to over-report records at a few locations (Sastre and Lobo 2009). To control this source of record aggregation, we counted the number of unique sampling dates in a 250 m radius buffer around each individual record, thus separating records acquired during repeated visits to a given location from casual records in under-visited areas (“location effect”). Third, records tend to be aggregated in the most accessible areas. To reflect this sampling bias, we summed the total length of major roads and railways per 1 km<sup>2</sup> square cells (“accessibility effect”, data from the French National Geographic Institute, <http://professionnels.ign.fr/route500>).

#### *Cross-correlations among land use and sampling variables*

Pairwise correlations among all variables included in our analyses ranged from  $r=-0.60$  (percent of forest against percent of agricultural land and altitude against percent of forest at regional scale) to  $r=0.58$  (between altitude and percent of agricultural land at regional scale (correlation matrices in Appendix S7)). We considered that these levels of collinearity did not hinder our analyses, especially as we were interested in the effect of each land use gradient conditional to the others within a multiple regression design, rather than on quantifying their direct effect on reptiles in isolation from each other (Morrisey 2018).

## **Statistical analyses**

### *Reptile distributions*

We used spatial point process models (PPMs, Baddeley et al. 2015) to model reptile distributions as a function of land use, altitude and sampling variables. PPMs relate geolocated point records, such as opportunistic species sightings, and explanatory variables measured at recorded locations and at a set of dummy points chosen along a rectangular grid across the study region (hereby referred to as “quadrature points”). In the simplest PPM of practical use, the number of occurrences over a study area ( $N$ ) and their spatial locations ( $s$ ) are modeled jointly as an inhomogeneous Poisson process with intensity  $\mu(s)$ , the expected number of presence records per unit area at a given spatial location. Inhomogeneous Poisson PPMs are, therefore, a generalization of the widely-used MaxEnt



model (Phillips et al. 2006, Renner and Warton 2013) and pseudo-absence logistic regression (Warton and Shepherd 2010). Defining the model as a PPM clarifies the modeled process and facilitates use of an expansive set of tools for addressing the many challenges inherent in species distribution modelling with opportunistic records. In our case, the PPM framework informed us of an appropriate spatial resolution for the quadrature points, provided a convenient way for accounting for sampling bias, and allowed us to identify spatial dependence and fit a particular class of PPMs that explicitly accounts for this dependence. An extensive demonstration of PPM fitting and of their advantages over closely related methods such as MaxEnt is provided elsewhere (Renner et al. 2015, Velázquez et al. 2016).

For each of our two study scales, we built a multispecies PPM to relate species' occurrence intensity (hereafter "intensity") to a linear combination of the four land-use variables (listed in Table 1) plus altitude and interactions between these variables and species identities to estimate species-specific responses. We scaled all variables to null mean and unit standard deviation prior to modeling to permit the comparison of slopes across variables (Schielzeth 2010). Hence, all species-level effects of explanatory variables can be interpreted in a similar way as effect sizes.

We further included consultant, location and accessibility effects in the PPMs to account for spatial patterns of sampling heterogeneity (Renner et al. 2015). We assumed that consultant and location effects were species-independent, but allowed the accessibility effect to vary among species through an interaction term because some reptile species are mostly detected as dead individuals on roads or tracks.

Although we first fitted inhomogeneous Poisson PPMs to the data, we identified residual spatial aggregation which could lead to biased parameter estimates and underestimated standard errors (Dormann et al. 2007). Consequently, we modelled the number of records of a given species and their locations as a species-varying Geyer process (Geyer 1999). In a Poisson PPM, the intensity measures the limiting expected number of records per unit area for a given species at a given location. In the Geyer process model, this intensity is conditional on the locations of surrounding records. The inhomogeneous Poisson point process and Geyer point process are both examples of a broad class of Gibbs point processes (Cressie 1993), which accommodate spatial dependence through interactions among point locations. The inclusion of this spatial interaction allows the spatial distribution of records to have either lower or higher variance than expected from a Poisson process, analogous to an overdispersion effect. The nature of these point interactions is determined

by a saturation parameter controlling strength and a radius parameter defining the maximum distance of interactions, both maximised by pseudo-likelihood.

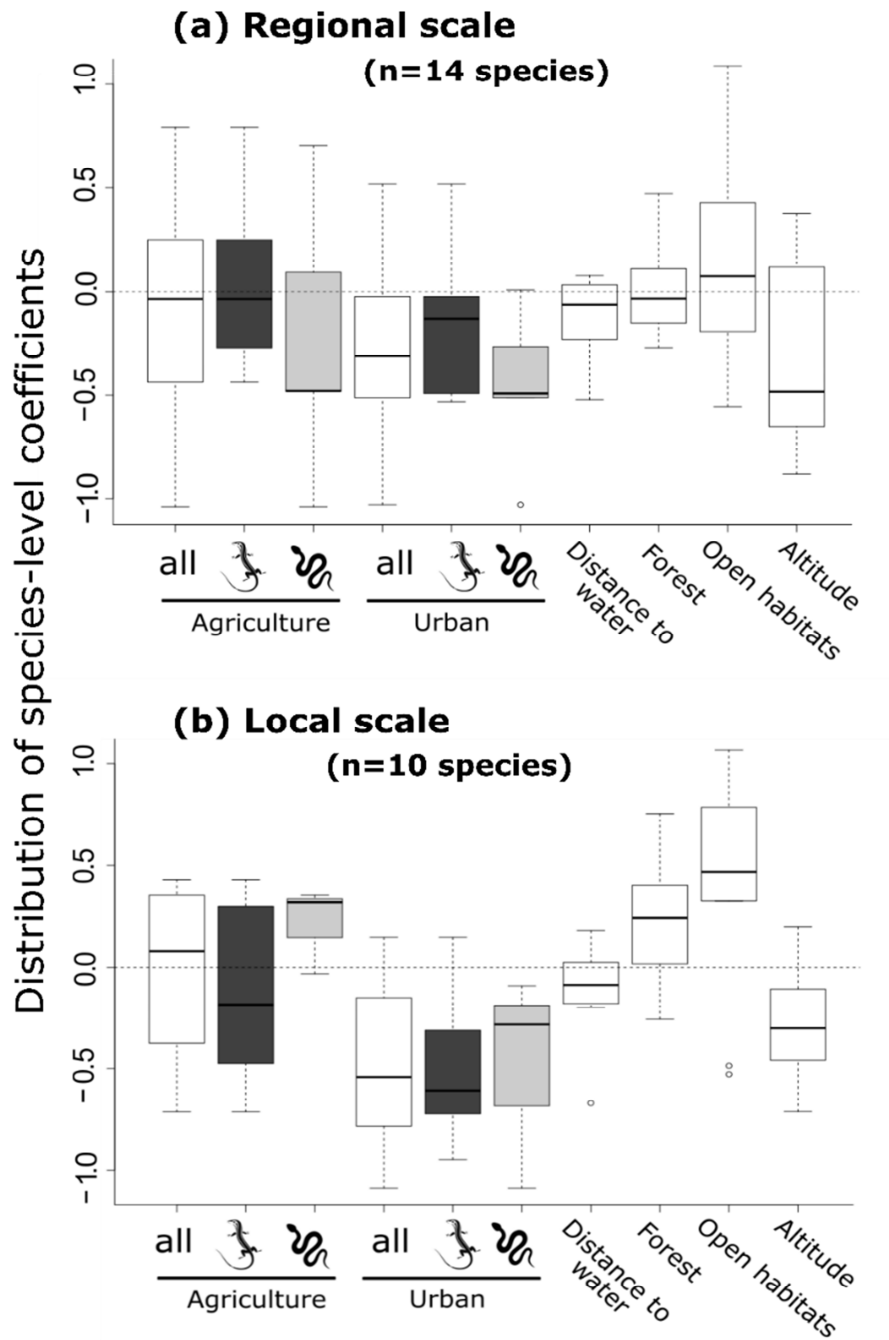
### *Effects of ecological traits*

We summarized the nine ecological traits in a species x species Gower's dissimilarity matrix synthesized in a Principal Coordinates Analysis (PCoA, Legendre and Legendre 2012), in which we weighted categorical traits proportionally to their numbers of modalities and accounted for the dependence among percentages of diet use and daily activity (Pavoine et al. 2009). We measured the contribution of traits to the PCoA axes with Spearman correlation coefficients for quantitative traits, and with an ANOVA and Tukey HSD tests for categorical traits. We quantified phylogenetic signals on ecological traits by measuring Blomberg's K (Blomberg et al. 2003) on each PCoA axis. Blomberg's K measures the strength of a phylogenetic signal on the traits,  $K > 1$  indicating that the traits are more clustered in the phylogeny than expected under the Brownian motion model (Blomberg et al. 2003). We also measured Blomberg's K on the species-level response to land use variables estimated by PPMs.

We estimated the influence of traits on species' responses to land use variables with linear regressions and phylogenetic linear models (PLM, Ho and Ané 2013), fitted separately at the regional and local scales. These models used species-level PPM coefficients as the response variable and species scores on the selected PcoA axes as predictors. We computed non-parametric standard errors for regression slopes with 1000 permutations in order to limit the effects of extreme values and small sample size ( $n = 14$ , regional scale ;  $n = 10$  local scale).

Given the size of our species sample, this phylogenetic analysis should however be interpreted as a guide to assess the sensitivity of our results to non-independence among species related to niche conservatism.

We acquired all environmental variables in raster format under the Lambert 93 projection with QGIS 2.14 (Quantum GIS Development Team 2013), and conducted all statistical analyses under R 3.3.2 (R Core Team 2016) with packages spatstat (Baddeley et al. 2015), ade4 (Dray and Dufour 2007), phylolm (Ho and Ané 2013), picante (Kembel et al. 2014) and ape (Paradis et al. 2017).



**Figure 2.** Distribution of reptile responses to land use gradients in the studied area, as assessed from regression coefficients estimated by a spatial point process model at the regional (a) and local (b) scales. Modelled responses to agriculture and urbanization are shown for all species and for lizards (in dark grey) and snakes (in white grey) separately. All variables were scaled to null mean and unit standard deviation prior to modeling. Boxes denote the interquartile range and the median of reptile' responses values; whiskers expand to the maximum and minimum values.

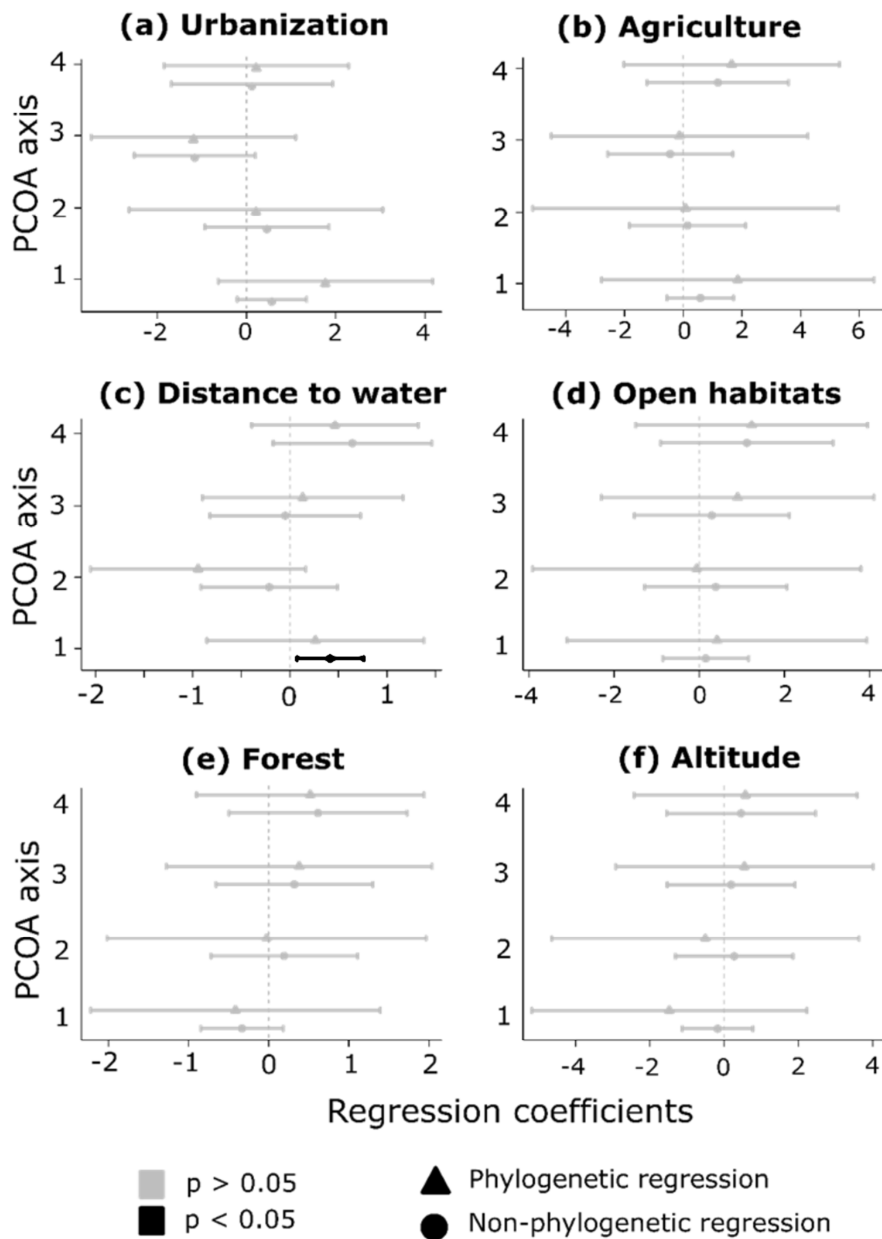
## **Results**

### **Quality of PPMs**

The regional and local models explained respectively 24% and 25% of the total variance in the number and locations of records. The Geyer spatial interaction matrix captured a significant amount of residual variability, suggesting that records were aggregated at both scales (positive value at both scale, regional: estimates =  $0.04 \pm 0.0006$  SE,  $F_{1,1108748} = 10534.5$ ,  $p < 0.0001$  ; local: estimates =  $0.03 \pm 0.001$  SE,  $F_{1,731800} = 695.39$ ,  $p < 0.0001$ , smoothed maps of residuals in Appendix S8).

### **Sampling effects**

All sampling variables contributed significantly to explain spatial variations in species intensities at both regional and local scales (effect sizes in Appendices S9 and S10). Species' intensity increased in areas surveyed by consulting offices (Table 2, "consultant effect"), when the area was surveyed repeatedly (Table 2, "location effect") and in areas served by a large network of roads and railways, with some variation among species (Table 2, "accessibility effect"; interaction species  $\times$  linear elements). This latter result supports the interpretation that increased sampling effort around roads overwhelms their possible impact on reptiles as barriers to dispersal or habitat fragmentation. Spatial variations in predicted species' intensity when all sampling effects were held constant was coherent with experts' knowledge of species' regional distributions, suggesting that sampling effort was properly corrected (Appendix S8). The fitted species intensities can thus be interpreted as proportional to spatial patterns of the species' actual occurrence densities.



**Figure 3.** How land use gradients affect the spatial distribution of reptile traits at the regional scale. Species' responses to six regional land-use or topographic gradients (a-f) were sequentially regressed against one of four PCOA axes constructed from ecological traits (1: snakes vs lizards ; 2: legless lizards vs others ; 3: *Tarentola mauritanica* vs others ; 4: aquatic vs terrestrial species). The figure displays estimates of regression slopes from ordinary least square and phylogenetic regressions, with their 95% confidence intervals. Black bars indicate significant effects at a 0.05 error risk.

## Species responses to land use and altitude

All variables had a significant effect on species intensities (Table 2). The most noticeable result was a decrease in species intensities towards high urbanization cover and high altitude at both scales (Fig. 2). Specifically, urbanization decreased the intensity of seven out of 14 species at the regional scale, all of which were snakes except *Rhinechis scalaris* (Fig. 2a and Appendix S9). Enforcing the role of urbanization as a regional filter of reptile distributions, two of these seven species (*Natrix natrix* and *Zamenis longissimus*) were even absent from the local window, the most urbanized area in the region (Appendix S2). At the local scale, five out of 10 species decreased towards urban areas, with steeper slopes than at the regional scale (Fig. 2b, Appendix S10). *Tarentola mauritanica*, the only exogenous species of our dataset, was the only species to increase towards urban areas at the regional scale, but did not respond to urbanization at the local scale. Counteracting these negative effects, increased proportion of open habitats had low but typically positive effects on distributions at the regional scale (Fig.2a, Appendix S9) and strongly increased species intensities at the local scale, except for the two human commensals *Tarentola mauritanica* and *Podarcis muralis* (Fig. 2b, Appendix S10). Species' responses to agriculture varied among species at both scales (Fig. 2a and 2b, Appendices S9-S10). Of the 14 and 10 species comprised in our regional and local species pools, three decreased towards agricultural areas at regional and local scales (Fig. 2, Appendices S9-S10). Finally, forest cover had no significant effect on species' regional distributions (Fig. 2a, Appendix S9), but it increased significantly the intensity of three lizards (*Lacerta bilineata*, *Podarcis muralis* and *Psammodromus algirus*) and one snake *Malpolon monspessulanus* at the local scale (Fig. 2b, Appendix S10).

**Table 2.** Analysis of variance of the spatial point process models and phylogenetic structure of species-varying parameters as assessed by Blomberg's K. F and the associated p-value correspond to the analysis of variance, Blomberg's K (p) gives the strength of the phylogenetic signal associated with a permutation-based p value.

Variable	Regional scale (n = 18164 records, 14 species)			Local scale (n = 3168 records, 10 species)		
	F	p	Blomberg's K (p)	F	p	Blomberg's K (p)
species	157.6	<0.0001		78.0	<0.0001	
urbanization	9.3	<0.05		25.6	<0.0001	
urbanization × species	7.3	<0.0001	0.29 (0.36)	4.6	<0.0001	0.45 (0.22)
agriculture	6.9	<0.01		145.7	<0.0001	
agriculture × species	12.5	<0.0001	0.15 (0.90)	22.1	<0.0001	0.59 (0.07)
open habitats	136.8	<0.0001		1.9	>0.05	
open habitats × species	53.3	<0.0001	0.21 (0.65)	51.5	<0.0001	0.33 (0.44)
forest	663.5	<0.0001		50.3	<0.0001	
forest × species	30.5	<0.0001	0.22 (51)	21.6	<0.0001	0.25 (0.81)
distance to water	2.13	>0.05		17.5	<0.0001	
distance to water × species	16.8	<0.0001	0.35 (0.13)	3.5	0.0003	0.42 (0.27)
altitude	390.0	<0.0001		362.8	<0.0001	
altitude × species	10.0	<0.0001	0.15 (0.89)	2.1	<0.05	0.90 (0.004)
accessibility effect	138.9	<0.0001		52.9	<0.0001	
accessibility effect x species	4.6	<0.0001	0.31 (0.24)	18.8	<0.0001	1.21 (0.003)
consultant effect	839.5	<0.0001		910.8	<0.0001	
location effect	1046.8	<0.0001		965.9	<0.0001	
Geyer spatial interaction matrix	3118.8	<0.0001		488.7	<0.0001	

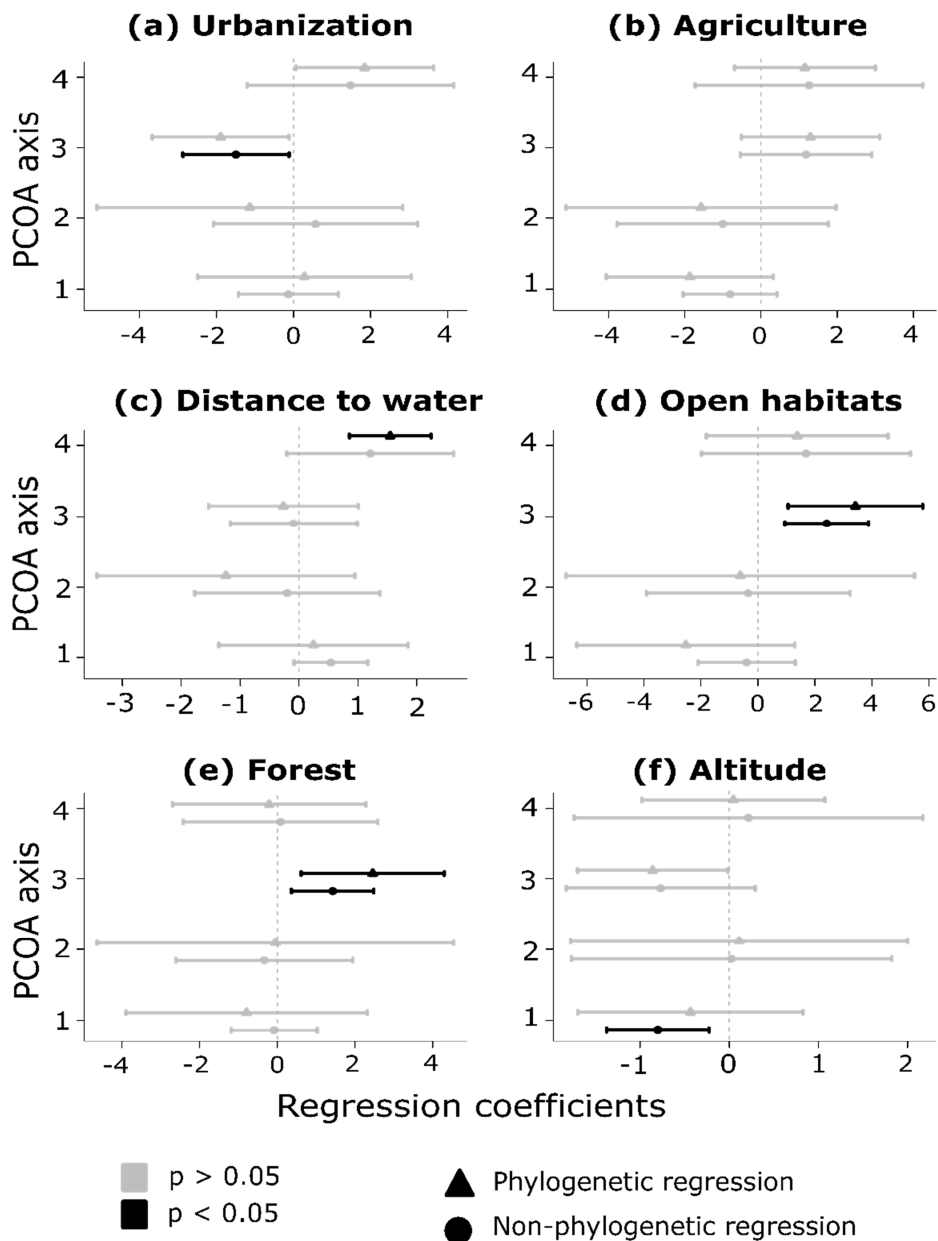
## Trait ordination

The first four PCoA axes accounted for respectively 45, 16, 14 and 10% of the total variance (8% for the fifth axis, not retained). The first axis separated large-sized, late-maturing crawling species from small insectivores (snakes and lizards; correlations of traits with axes in Appendix S4). The second axis separated legless oviparous lizards (*Chalcides striatus* and *Anguis fragilis*) from all other species. Axis 3 isolated the introduced *Tarentola mauritanica* from other species based on its climber behavior, long activity period and nocturnal habits. Axis 4 separated aquatic and terrestrial species. The two first PCoA axes were clustered in the phylogeny (Blomberg's  $K = 2.98$  and  $1.99$  for axes 1 and 2 respectively,  $p < 0.01$ ) while the third axis exhibited a signal of convergent evolution ( $K = 0.80$ ,  $p < 0.01$ ) and the fourth axis had no significant signal ( $K = 0.42$ ,  $p > 0.05$ ).

## Functional signal on species' responses to land use and altitude

Predicted reptile responses to land use were less structured in the phylogeny than expected under a Brownian motion model (Table 2). Contrary to our expectations, ecological traits did not clearly structure these responses either (Fig. 3 and 4). At the local scale only, negative scores along the third PCoA axis were associated with urbanization, while positive scores were related to open habitats and forests (Fig. 4e,  $p < 0.05$  for all variables). These effects were mainly driven by the exogenous *Tarentola mauritanica*, which is mostly present in urban areas. The intensity of snakes increased near water (Fig. 3c and 4c, PCoA axes 1 and 4,  $p < 0.05$ ), mainly because of two partly aquatic species, *Natrix maura* and *Natrix natrix* (Appendices S9 and S10). Phylogenetic linear models yielded similar results, confirming the small effect of niche conservatism on the response of reptiles to land use.





**Figure 4.** How land use gradients affect the spatial distribution of reptile traits at the local scale. Species' responses to six local land-use or topographic gradients (a-f) were sequentially regressed against one of four PCOA axes constructed from ecological traits (1: snakes vs lizards ; 2: legless lizards vs others ; 3: *Tarentola mauritanica* vs others ; 4: aquatic vs terrestrial species). The figure displays estimates of regression slopes from ordinary least square and phylogenetic regressions, with their 95% confidence intervals. Black bars indicate significant effects at a 0.05 error risk.

## **Discussion**

Mediterranean reptiles exhibit negative distributional responses to increasing human pressure at regional and local scales. We further showed that land abandonment, here reflected by forest, and the presence of open habitats such as grasslands and pastures have little effect at the regional scale, but favour species persistence in the most anthropogenized landscapes. Contrary to our initial expectations, no clear signal of trait filtering emerged in relation with these responses, although snakes tended to be more negatively affected by urbanization. Our results reveal scale-dependent effects of land use on reptile distributions mediated by complex processes that cannot be unambiguously attributed to adaptive functional strategies.

On methodological grounds, our study demonstrates that PPMs facilitate the modeling of species that are hard to detect with protocolled studies, taking advantage of the massive amount of geolocated opportunistic records that has emerged in recent years (Dickinson et al. 2012). One major difficulty in the use of this kind of data lies in designing models that separate patterns related to ecological processes from those related to sampling effort, which we achieved through the identification of a set of covariates that reflects the distribution of observers in our study area. Results are convincing with respect to the efficiency of this method, as species' distributions predicted by PPMs do not echo any geographical pattern in sampling effort and are consistent with biological expectations, such as their reluctance to enter in urban or forested areas. Nevertheless, we do not own independent protocolled survey data which would allow estimating detection probabilities as in Dorazio (2014), preventing any formal test of causal relationships between reptiles and land use, a limitation shared by all correlative distribution models. We further admit that our models certainly overlooked some presence areas for the least detected species because PPMs, as most other statistical models, remain limited when sample size becomes too small. In this respect, our decision to remove rare species had probably less undesirable consequences on our results than inappropriate estimations due to biased sampling. However, the particular modifications we incorporated such as applying a correction for sampling bias through surrogate covariates (Warton et al. 2013) and accounting for spatial dependence through a Geyer process (Renner and Warton 2013) improve predictive performance over more standard practices to distribution modelling with opportunistic data.

Our results show that urbanization and, to some extent, agriculture, decrease reptile presence at both study scales. This result mirrors studies on birds (Devictor et al. 2008), insects (Fattorini 2011) and amphibians (Scheffers and Paszkowski 2012). One biologically plausible explanation is that farming and dense urbanization decrease habitat heterogeneity and resource availability through the use of pesticides and mechanization (Benton et al. 2003, Bright et al. 2008, Hamer and McDonnell 2010), but direct mortality through predation by domestic animals (Loyd et al. 2013) and road killing could also contribute to these patterns (Shepard et al. 2008), especially at the local scale. Interestingly, only four species were, as expected, favoured by land abandonment at a local scale, and none responded at the regional scale. Post-abandonment successional dynamics in our study region have led to homogeneous dense forest cover (Sluiter and de Jong 2007), a habitat unsuitable to all reptiles from our regional species pool except *Zamenis longissimus* and *Anguis fragilis*. Hence, our results reflect species' aversion to forest cover rather than a negative effect of land abandonment per se as found in other studies (Laiolo et al. 2004, Moreira and Russo 2007).

Ecological patterns vary across scales as a consequence of different processes affecting regional and local assemblages (Melles et al. 2003, Brind'Amour Anik et al. 2005, Hopkins and Burr 2009). Although the choice of a coarser spatial grain may have attenuated the effects of land use on reptiles at the regional scale, the relative imprints of the gradients considered were largely consistent at both scales. This result implies that, although land use has lower effects relative to topoclimatic variations at broad spatial scales (consistent with Pearson and Dawson 2003), it remains a significant factor shaping species distributions over broad spatial extents (Di Marco and Santini 2015, Kehoe Laura et al. 2016). It also suggests that the processes by which land use affect reptile distributions are less direct at the regional scale than at the local scale, as found in multiscale studies of bird-habitat relationships (Cushman and McGarigal 2004). This result is expected if reptiles, like birds, respond to land use hierarchically by selecting first landscape features such as patch configuration and secondarily local habitat characteristics (Cushman and McGarigal 2004). Reptiles' low dispersal abilities suggest alternatively that their way of selecting habitat is purely local: if so, the negative effect of urbanization on most species at the regional scale probably reflects reduced flow of individuals and low population persistence, while local effects of land use could be more directly related to habitat suitability and prey availability within habitat patches. These hypotheses would need to be tested with mechanistic metapopulation models, and calibrated with PPMs like ours within hierarchical frameworks (e.g. Wikle 2003).

The strong positive effects of open habitats and abandoned land were restricted to the local scale. These habitats are rare within our heavily urbanized local window, concentrating reptiles in residual patches of suitable habitat at the margin of the main urban center. In addition, reptiles are less likely to be found outside their main habitats when the landscape matrix is impermeable to dispersal or temporary colonization, as is the case in anthropogenic centers. This pattern is in line with other studies that reported positive effects of remnant habitat patches on species' persistence within hostile matrices (Fahrig et al. 2011), although these patches might also become ecological traps for some species in the absence of dispersal corridors (Ewers and Didham 2006). However, under this latter hypothesis, it is unlikely reptiles would sustain viable populations over a seven-year period at sufficient densities to be detected by our opportunistic records. Another noticeable result of our local scale analysis is that open habitats had substantially stronger positive effects on reptiles than forest. This pattern supports the idea that maintaining small patches of suitable exploited habitats within anthropogenized matrices has a more positive effect on species persistence than land abandonment (Wretenberg et al. 2010, Loos et al. 2014).

Contrary to our expectations, ecological traits and phylogeny do not imprint a strong structure on species' responses to land use. Snakes tended to correlate more negatively than lizards with urbanization at the regional scale, consistent with previous observations that carnivore, fossorial and ground-dwelling reptiles are negatively affected by the human footprint (Trimble and van Aarde 2014, Todd et al. 2017). However, we did not find a clear signal of traits on species' responses to land use. The regional species pool has not changed substantially in the last 2 million years (Blain and Villa 2006, Bailon and Rage 2012), ruling out the possibility that functionally distinct species could have been removed by past biotic homogenization processes. Thus, the lack of a functional signal in species' responses to land use can more likely be explained by the absence or recent removal of strong environmental constraints on trait distributions; historical records on land use and climate would however be needed for a formal test of this hypothesis. Alternatively, the size of species pools (14 / 10 species) may be insufficient to detect shallow functional and phylogenetic signals, a limitation inherent to the overall paucity of the regional species assemblage. In spite of these limitations, our results are congruent with previous studies (Jung and Threlfall 2016, Todd et al. 2017) and confirm that ecological traits and phylogenetic conservatism do not provide a causal explanation to reptiles' sensitivity to land use.

### Conclusion

We wish to highlight three main conclusions from our study. First, reptile distributions clearly suffer from anthropogenic land use but do not benefit from land abandonment over regional scales. However, maintaining patches of non-used or abandoned habitat in an anthropogenic matrix promotes local species occurrence, suggesting that habitat heterogeneity is key to species' persistence in the face of increasing urbanization. Second, functional strategies or evolutionary history do not account for reptiles' distributional responses to land use, inconsistent with a biotic homogenization scenario (Devictor et al. 2008, Cadotte and Tucker 2017). Overall, our study reveals that opportunistic records combined with cross-scale comparative analyses can provide in-depth insights into the determinants of species distributions for data deficient taxa.

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## References

- Andreu, A. et al. 1998. Fauna ibérica. Vol. 10. Reptiles. - Consejo Superior de Investigaciones Científicas.
- Baddeley, A. et al. 2015. Spatial Point Patterns: Methodology and Applications with R. - Chapman and Hall/CRC.
- Bailon, S. and Rage, J.-C. 2012. Données fossiles et mise en place de l'herpétofaune actuelle de la France. - In: Atlas des Amphibiens et des Reptiles de France. Biotope, pp. 272.
- Bennett, A. F. et al. 2004. Do regional gradients in land-use influence richness, composition and turnover of bird assemblages in small woods? - Biol. Conserv. 119: 191–206.
- Benton, T. G. et al. 2003. Farmland biodiversity: is habitat heterogeneity the key? - Trends Ecol. Evol. 18: 182–188.
- Blain, H.-A. and Villa, P. 2006. Amphibians and squamate reptiles from the early Upper Pleistocene of Bois Roche Cave (Charente, southwestern France).
- Blomberg, S. P. et al. 2003. Testing for Phylogenetic Signal in Comparative Data: Behavioral Traits Are More Labile. - Evolution 57: 717–745.
- Böhm, M. et al. 2013. The conservation status of the world's reptiles. - Biol. Conserv. 157: 372–385.
- Bossard, M. et al. 2000. CORINE Land Cover Technical Guide—Addendum 2000. Technical Report 40 available at <http://www.eea.eu.int>.
- Bright, J. A. et al. 2008. A review of indirect effects of pesticides on birds and mitigating land-management practices.: 66.
- Brind'Amour Anik et al. 2005. Multiscale spatial distribution of a littoral fish community in relation to environmental variables. - Limnol. Oceanogr. 50: 465–479.
- Buttner, G. and Maucha, G. 2006. The thematic accuracy of Corine land cover 2000 - Assessment using LUCAS.
- Cadotte, M. W. and Tucker, C. M. 2017. Should Environmental Filtering be Abandoned? - Trends Ecol. Evol. 32: 429–437.
- Caetano, M. et al. 2006. Accuracy assessment of the Portuguese CORINE Land Cover map. - Glob. Dev. Environ. Earth Obs. Space: 459–467.
- Callaghan, C. T. and Gawlik, D. E. 2015. Efficacy of eBird data as an aid in conservation planning and monitoring. - J. Field Ornithol. 86: 298–304.
- Cardillo, M. et al. 2005. Multiple causes of high extinction risk in large mammal species. - Science 309: 1239–1241.
- Cressie, N. A. C. 1993. Statistics for spatial data. - J. Wiley.
- Cushman, S. A. and McGarigal, K. 2004. Hierarchical Analysis of Forest Bird Species–Environment Relationships in the Oregon Coast Range. - Ecol. Appl. 14: 1090–1105.
- Devictor, V. et al. 2008. Functional biotic homogenization of bird communities in disturbed landscapes. - Glob. Ecol. Biogeogr. 17: 252–261.
- Di Marco and Santini 2015. Human pressures predict species' geographic range size better than biological traits. - Glob. Change Biol. 21: 2169–2178.
- Dickinson, J. L. et al. 2012. The current state of citizen science as a tool for ecological research and public engagement. - Front. Ecol. Environ. 10: 291–297.

- Donnelly, M. A. et al. 2005. Sampling amphibians and reptiles in the Iwokrama Forest ecosystem. - *Proc. Acad. Nat. Sci. Phila.* 154: 55–69.
- Dorazio, R. M. 2014. Accounting for imperfect detection and survey bias in statistical analysis of presence-only data. - *Glob. Ecol. Biogeogr.* 23: 1472–1484.
- Dormann, C. F. et al. 2007. Methods to account for spatial autocorrelation in the analysis of species distributional data: a review. - *Ecography* 30: 609–628.
- Dornelas, M. et al. 2014. Assemblage Time Series Reveal Biodiversity Change but Not Systematic Loss. - *Science* 344: 296–299.
- Dray, S. and Dufour, A. B. 2007. The ade4 package: Implementing the duality diagram for ecologists. - *J. Stat. Softw.* 22: 1–20.
- Driscoll, D. A. 2004. Extinction and outbreaks accompany fragmentation of a reptile community. - *Ecol. Appl.* 14: 220–240.
- Ellis, E. C. et al. 2013. Used planet: a global history. - *Proc. Natl. Acad. Sci.* 110: 7978–7985.
- Ewers, R. M. and Didham, R. K. 2006. Confounding factors in the detection of species responses to habitat fragmentation. - *Biol. Rev.* 81: 117–142.
- Fahrig, L. et al. 2011. Functional landscape heterogeneity and animal biodiversity in agricultural landscapes. - *Ecol. Lett.* 14: 101–112.
- Faluccci, A. et al. 2007. Changes in land-use/land-cover patterns in Italy and their implications for biodiversity conservation. - *Landsc. Ecol.* 22: 617–631.
- Fattorini, S. 2011. Insect extinction by urbanization: A long term study in Rome. - *Biol. Conserv.* 144: 370–375.
- Fischer, J. and Lindenmayer, D. B. 2007. Landscape modification and habitat fragmentation: a synthesis. - *Glob. Ecol. Biogeogr.* 16: 265–280.
- Flynn, D. F. B. et al. 2009. Loss of functional diversity under land use intensification across multiple taxa. - *Ecol. Lett.* 12: 22–33.
- Foley, J. A. et al. 2005. Global consequences of land use. - *Science* 309: 570–574.
- Geniez, P. and Cheylan, M. 2012. Les amphibiens et les reptiles du Languedoc-Roussillon et régions limitrophes - Atlas biogéographique. - Coédition Biotope.
- Geyer, C. J. 1999. Likelihood Inference for Spatial Point Processes. - *Stoch. Geom. Likelihood Comput.*: 79–140.
- Gibbons, J. W. et al. 2000. The Global Decline of Reptiles, Déjà Vu Amphibians. - *BioScience* 50: 653–666.
- Good, T. C. et al. 2006. Addressing Data Deficiency in Classifying Extinction Risk: a Case Study of a Radiation of Bignoniaceae from Madagascar. - *Conserv. Biol.* 20: 1099–1110.
- Haberl, H. et al. 2007. Quantifying and mapping the human appropriation of net primary production in earth's terrestrial ecosystems. - *Proc. Natl. Acad. Sci.* 104: 12942–12947.
- Hamer, A. J. and McDonnell, M. J. 2010. The response of herpetofauna to urbanization: Inferring patterns of persistence from wildlife databases. - *Austral Ecol.* 35: 568–580.
- Ho, L. S. T. and Ané, C. 2013. Package “phylolm” - Phylogenetic linear regression.
- Hopkins, R. L. and Burr, B. M. 2009. Modeling freshwater fish distributions using multiscale landscape data: A case study of six narrow range endemics. - *Ecol. Model.* 220: 2024–2034.
- Howard et al. 2014. Amphibians over the edge: silent extinction risk of Data Deficient species. - *Divers. Distrib.* 20: 837–846.

- Huey, R. B. 1982. Temperature, physiology and the ecology of reptiles. - In: *Biology of the Reptilia*, Vol. 12, Physiology (C). Academic Press, pp. 25–91.
- Huston, M. A. 1999. Local processes and regional patterns: appropriate scales for understanding variation in the diversity of plants and animals. - *Oikos* 86: 393–401.
- Isaac, N. J. B. et al. 2014. Statistics for citizen science: extracting signals of change from noisy ecological data. - *Methods Ecol. Evol.* 5: 1052–1060.
- Jung, K. and Threlfall, C. G. 2016. Urbanisation and Its Effects on Bats—A Global Meta-Analysis. - In: *Bats in the Anthropocene: Conservation of Bats in a Changing World*. Springer, Cham, pp. 13–33.
- Kay, G. M. et al. 2018. Species co-occurrence networks show reptile community reorganization under agricultural transformation. - *Ecography* 41: 113–125.
- Kehoe, L. et al. 2015. Global patterns of agricultural land-use intensity and vertebrate diversity. - *Divers. Distrib.* 21: 1308–1318.
- Kehoe Laura et al. 2016. Agriculture rivals biomes in predicting global species richness. - *Ecography* 40: 1118–1128.
- Keinath, D. A. et al. 2017. A global analysis of traits predicting species sensitivity to habitat fragmentation. - *Glob. Ecol. Biogeogr.* 26: 115–127.
- Kembel, S. W. et al. 2014. *picante*: R tools for integrating phylogenies and ecology.
- Laiolo, P. et al. 2004. Consequences of pastoral abandonment for the structure and diversity of the alpine avifauna. - *J. Appl. Ecol.* 41: 294–304.
- Le Viol, I. L. et al. 2012. More and more generalists: two decades of changes in the European avifauna. - *Biol. Lett.* 8: 780–782.
- Legendre, P. and Legendre, L. F. J. 2012. *Numerical Ecology*. - Elsevier.
- Lhernould, A. 2016. Mise à jour et évolution en 2015 de l'occupation du sol de la métropole de Montpellier : Actualisation d'une base de données.
- Loos, J. et al. 2014. Low-Intensity Agricultural Landscapes in Transylvania Support High Butterfly Diversity: Implications for Conservation. - *PLoS ONE* in press.
- Loyd, K. A. T. et al. 2013. Quantifying free-roaming domestic cat predation using animal-borne video cameras. - *Biol. Conserv.* 160: 183–189.
- McGill, B. J. et al. 2015. Fifteen forms of biodiversity trend in the Anthropocene. - *Trends Ecol. Evol.* 30: 104–113.
- McKinney, M. L. 1997. Extinction vulnerability and selectivity: Combining ecological and paleontological views. - *Annu. Rev. Ecol. Syst.* 28: 495–516.
- Melles, S. et al. 2003. Urban Bird Diversity and Landscape Complexity: Species–environment Associations Along a Multiscale Habitat Gradient. - *Conserv. Ecol.* in press.
- Meynard, C. N. et al. 2011. Beyond taxonomic diversity patterns: how do  $\alpha$ ,  $\beta$  and  $\gamma$  components of bird functional and phylogenetic diversity respond to environmental gradients across France? - *Glob. Ecol. Biogeogr.*: 893–903.
- Morais, A. R. et al. 2013. Unraveling the conservation status of Data Deficient species. - *Biol. Conserv.* 166: 98–102.
- Moreira, F. and Russo, D. 2007. Modelling the impact of agricultural abandonment and wildfires on vertebrate diversity in Mediterranean Europe. - *Landsc. Ecol.* 22: 1461–1476.
- Morrissey, M. B. ; R. 2018. Multiple Regression Is Not Multiple Regressions: The Meaning of Multiple Regression and the Non-Problem of Collinearity. - *Philos. Theory Pract. Biol.* in



press.

- Newbold, T. et al. 2015. Global effects of land use on local terrestrial biodiversity. - *Nature* 520: 45–50.
- Newbold, T. et al. 2016. Global patterns of terrestrial assemblage turnover within and among land uses. - *Ecography*: n/a-n/a.
- Paradis, E. et al. 2017. *ape: Analyses of Phylogenetics and Evolution*.
- Pavoine, S. et al. 2009. On the challenge of treating various types of variables: application for improving the measurement of functional diversity. - *Oikos* 118: 391–402.
- Pearson, R. G. and Dawson, T. P. 2003. Predicting the impacts of climate change on the distribution of species: are bioclimate envelope models useful? - *Glob. Ecol. Biogeogr.* 12: 361–371.
- Phillips, S. J. et al. 2006. Maximum entropy modeling of species geographic distributions. - *Ecol. Model.* 190: 231–259.
- Prévosto, B. et al. 2011. Impacts of Land Abandonment on Vegetation: Successional Pathways in European Habitats. - *Folia Geobot.* 46: 303–325.
- Quantum GIS Development Team 2013. Quantum GIS Geographic Information System. - Open Source Geospatial Foundation Project.
- R Core Team 2016. R: A language and environment for statistical computing. - R Foundation for Statistical Computing.
- Rayner, L. et al. 2015. Avifauna and urban encroachment in time and space. - *Divers. Distrib.* 21: 428–440.
- Renner, I. W. and Warton, D. I. 2013. Equivalence of MAXENT and Poisson Point Process Models for Species Distribution Modeling in Ecology. - *Biometrics* 69: 274–281.
- Renner, I. W. et al. 2015. Point process models for presence-only analysis. - *Methods Ecol. Evol.* 6: 366–379.
- Ribeiro, R. et al. 2009. Biodiversity and land uses at a regional scale: is agriculture the biggest threat for reptile assemblages? - *Acta Oecologica* 35: 327–334.
- Sastre, P. and Lobo, J. M. 2009. Taxonomist survey biases and the unveiling of biodiversity patterns. - *Biol. Conserv.* 142: 462–467.
- Scheffers, B. R. and Paszkowski, C. A. 2012. The effects of urbanization on North American amphibian species: Identifying new directions for urban conservation. - *Urban Ecosyst.* 15: 133–147.
- Schielzeth, H. 2010. Simple means to improve the interpretability of regression coefficients. - *Methods Ecol. Evol.* 1: 103–113.
- Shepard, D. B. et al. 2008. Reptile Road Mortality around an Oasis in the Illinois Corn Desert with Emphasis on the Endangered Eastern Massasauga. - *Copeia* 2008: 350–359.
- Silvertown, J. 2009. A new dawn for citizen science. - *Trends Ecol. Evol.* 24: 467–471.
- Sirami, C. et al. 2010. Long-term anthropogenic and ecological dynamics of a Mediterranean landscape: Impacts on multiple taxa. - *Landsc. Urban Plan.* 96: 214–223.
- Sluiter, R. and de Jong, S. M. 2007. Spatial patterns of Mediterranean land abandonment and related land cover transitions. - *Landsc. Ecol.* 22: 559–576.
- Todd, B. D. et al. 2016. Using citizen science data to identify the sensitivity of species to human land use. - *Conserv. Biol.* 30: 1266–1276.

- 
- Todd, B. D. et al. 2017. Species traits explaining sensitivity of snakes to human land use estimated from citizen science data. - *Biol. Conserv.* 206: 31–36.
- Trimble, M. J. and van Aarde, R. J. 2014. Amphibian and reptile communities and functional groups over a land-use gradient in a coastal tropical forest landscape of high richness and endemism. - *Anim. Conserv.* 17: 441–453.
- Tscharntke, T. et al. 2002. Characteristics of insect populations on habitat fragments: A mini review. - *Ecol. Res.* 17: 229–239.
- Vacher, J.-P. and Geniez, M. 2010. *Les Reptiles de France, Belgique, Luxembourg et Suisse.* - Biotope Editions.
- Velázquez, E. et al. 2016. An evaluation of the state of spatial point pattern analysis in ecology. - *Ecography* 39: 1042–1055.
- Vellend, M. et al. 2013. Global meta-analysis reveals no net change in local-scale plant biodiversity over time. - *Proc. Natl. Acad. Sci.* 110: 19456–19459.
- Vimal, R. et al. 2017. Integrating habitat diversity into species conservation in the Mediterranean mosaic landscape. - *Basic Appl. Ecol.* 22: 36–43.
- Warton, D. I. and Shepherd, L. C. 2010. Poisson point process models solve the “pseudo-absence problem” for presence-only data in ecology. - *Ann. Appl. Stat.* 4: 1383–1402.
- Warton, D. I. et al. 2013. Model-Based Control of Observer Bias for the Analysis of Presence-Only Data in Ecology. - *PLOS ONE* 8: e79168.
- Wheeler, Q. D. 2004. What if GBIF? - *Bioscience* 54: 717–717.
- Wikle, C. K. 2003. Hierarchical Bayesian Models for Predicting the Spread of Ecological Processes. - *Ecology* 84: 1382–1394.
- Wretenberg, J. et al. 2010. Changes in local species richness of farmland birds in relation to land-use changes and landscape structure. - *Biol. Conserv.* 143: 375–381.

## **Annexes: Supplementary Tables and Figures**

The supplementary Tables and Figure are not available in the printed version of the thesis due to their size, but can be found in the file Annex reptiles distributions at: <https://1drv.ms/f/s!AhWgyBtt5X3FamXDhTlzauD5uXo>

## **Résumé de la thèse en Français**

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L'incroyable diversité de phénotypes observés dans la nature, c'est-à-dire l'ensemble des traits observables des organismes, est un sujet qui fascine les biologistes depuis des siècles. Une grande partie de cette diversité est le résultat de variations de coloration entre organismes. Comprendre l'origine de ces variations de coloration est donc un sujet fondamental en évolution. L'incroyable diversité de coloration observée dans la nature s'explique par deux facteurs : (i) le grand nombre de « pressions de sélections » (contraintes) affectant la coloration, (ii) le fait que ces pressions agissent dans des directions opposées.

La diversité des pressions de sélection s'exerçant sur la coloration s'explique par les multiples fonctions dans lesquelles est impliqué ce trait. Tout d'abord, la coloration peut servir au camouflage des espèces, afin d'éviter aux proies de se faire voir par leur(s) prédateur(s), et aux prédateurs de se faire voir par leurs proies. Elle permet aussi de répondre à des contraintes environnementales. Ainsi la coloration de la peau chez l'homme est utilisée pour la résistance aux ultraviolets, et la coloration des espèces ectothermes s'assombrit souvent avec l'altitude pour faciliter la régulation de la température corporelle. La coloration entre également en compte dans la communication entre espèces. C'est notamment le cas des colorations vives qui servent de signaux d'alerte en indiquant aux prédateurs que l'organisme est protégé (venin, piquants...). Ces signaux d'alerte peuvent être « honnêtes », comme l'alternance de bandes rouges jaunes et noires chez les serpents corail qui indiquent sa dangerosité. Ils peuvent aussi être « malhonnêtes », lorsqu'une espèce qui ne possède pas de défense mime une espèce protégée (par ex. les faux serpents corail). La coloration intervient enfin dans la communication entre individus d'une même espèce. Elle peut indiquer le rang social, l'état de santé de l'individu ou encore l'identité de l'espèce à laquelle appartient l'organisme. Elle influence ainsi directement les relations sociales et la recherche du partenaire sexuel.

Les différentes pressions de sélection agissant sur la coloration ont souvent des directions opposées. Par exemple, la sélection pour le camouflage va sélectionner des colorations ressemblant au milieu dans lequel vit l'espèce, alors que la sélection pour la communication va sélectionner des colorations différentes de celle du milieu environnant pour que l'organisme soit bien visible.

L'objectif de cette thèse est d'étudier les différentes pressions de sélection qui s'exercent sur les vertébrés. Pour cela nous avons étudié les variations de coloration à l'échelle intraspécifique (au sein de l'espèce) aussi bien qu'interspécifique (entre espèces), au travers de différentes recherches. Dans le chapitre un, nous nous sommes intéressés à

l'effet de l'environnement et de la sélection pour la reconnaissance entre espèces sur les variations de couleurs et de morphologies. Dans le chapitre deux, nous avons étudié l'impact d'une stratégie de défense, le mimétisme Batésien, sur l'évolution de la coloration. Dans le chapitre trois nous avons comparé l'influence de la sélection naturelle et sexuelle sur la disparité et les taux d'évolution de la coloration. Enfin dans le chapitre quatre, nous avons testé si la présence de polymorphisme de coloration était corrélée à une augmentation de la diversification.

### **Chapitre 1 – Manuscrit A : effet de l'environnement et de la sélection pour la reconnaissance interspécifique sur la coloration et la morphologie de deux espèces de tritons**

Nous avons tout d'abord étudié des facteurs expliquant les variations de couleurs et de morphologies à l'échelle intraspécifique chez deux espèces de triton du genre *Lissotriton*. Ces deux espèces possèdent de larges aires de répartition qui couvrent la majorité du territoire européen et qui se chevauchent dans l'ouest de l'Europe. Les deux espèces se reconnaissent mal lorsqu'elles cohabitent ce qui peut conduire à des parades interspécifiques et à de l'hybridation. Cependant les hybrides sont stériles et il n'y a pas de flux de gènes entre les deux espèces. Dans cette situation, une forte sélection pour une meilleure reconnaissance entre espèces est attendue lorsque les deux espèces vivent ensembles, afin de limiter la production d'hybrides.

Nous nous sommes intéressés à la coloration dorsale, ventrale et caudale des tritons. La coloration dorsale est utilisée pour le camouflage, alors que la ventrale et caudale sert pour le choix des partenaires. Nous avons aussi quantifié la taille du corps et plusieurs traits morphologiques impliqués dans la parade nuptiale des mâles (palmure, filament caudal...). Pour chacun de ces caractères, nous avons testé l'influence de plusieurs facteurs : le climat, l'altitude, la coloration de l'eau dans les sites de pontes et la présence des deux espèces en cohabitation.

Nous montrons que les colorations visibles à distance (coloration dorsale et caudale) convergent lorsque les deux espèces vivent ensemble, alors que la coloration ventrale, cachée aux prédateurs, diverge lorsqu'elles cohabitent. Plus précisément, nous trouvons que la morphologie des tritons est peu influencée par les facteurs inclus dans l'étude. Nous trouvons aussi que la coloration des deux espèces varie avec les gradients climatiques et altitudinaux, ainsi qu'avec la coloration locale de l'eau, mais que cette dernière à un effet

moindre. Enfin, nous montrons que la coloration ventrale des deux espèces diffère davantage que ce qui était attendu au vu des conditions environnementales lorsqu'elles cohabitent. Ce patron supporte l'existence d'un déplacement de caractères sexuels, c'est-à-dire une divergence accrue des caractères afin d'améliorer la reconnaissance interspécifique lorsque les deux espèces sont ensemble. Cette divergence est observée à la fois chez les mâles et les femelles, ce qui peut s'expliquer par le fait que le choix de partenaire est mutuel chez les tritons.

## **Chapitre 2 – Manuscrit B : évolution du mimétisme Batésien chez les serpents du Paléarctique de l'Ouest**

Le mimétisme Batésien est une stratégie de défense dans laquelle une espèce sans défense (espèce *mime*) est protégée des prédateurs par sa ressemblance à une espèce ayant des défenses (espèce *modèle*). Bien que le mimétisme Batésien soit une des stratégies de défense les plus étudiées, on connaît encore mal sa fréquence dans les communautés naturelles. Cette incapacité à détecter le mimétisme provient en partie du fait qu'il n'y a pas, à l'heure actuelle, de méthode qui soit adaptée pour mesurer la ressemblance générale entre un grand nombre d'espèces. Les méthodes déjà développées permettent uniquement de comparer la coloration à un point précis des individus (pas sur tout le corps), ou nécessitent des données standardisées, difficiles à obtenir. Dans ce manuscrit, nous avons développé une nouvelle manière de quantifier la ressemblance entre espèces en utilisant des photographies non standardisées et des Réseaux de Neurones Artificiels (RNA). Nous avons ensuite développé une méthode permettant de détecter les espèces potentiellement mimétiques sur la base des mesures de ressemblances des RNA complétées par des mesures de sympatrie (chevauchement des aires de distributions de deux espèces). Dans le cadre de l'étude du mimétisme Batésien, nous avons considéré les espèces non venimeuses comme potentiellement mimétiques lorsqu'elles présentaient un excès de ressemblance avec les espèces modèles (venimeuses) sympatriques. Nous avons appliqué cette méthode à la détection du mimétisme Batésien chez les serpents du Paléarctique de l'Ouest (cette région comprend l'Europe, l'Afrique du Nord et le Moyen Orient).

Nous montrons que les RNA sont des outils fiables pour estimer la ressemblance entre espèces. Une fois entraînés, ils sont capables d'identifier les espèces de serpents venimeux avec une forte précision et indépendamment de l'arrière-plan de l'image. Parmi les serpents du Paléarctique de l'Ouest, nous détectons 7 espèces mimétiques. Ces espèces présentent deux caractéristiques : elles ne ressemblent pas parfaitement à leur espèce



modèle et elles ne présentent pas de coloration vive. Ces deux caractéristiques contrastent avec la grande majorité des exemples de mimétisme Batesien décrit à ce jour, qui sont basés sur une ressemblance souvent stupéfiante avec le modèle et des couleurs vives. Cette différence est probablement due à un biais de recherche pour les cas de mimétisme les plus évidents. En permettant de tester le mimétisme pour toutes les espèces, notre méthode apporte une vision plus complète de cette stratégie de défense, en supportant l'idée que le mimétisme est peut-être plus fréquent mais moins visible qu'imaginé jusqu'alors.

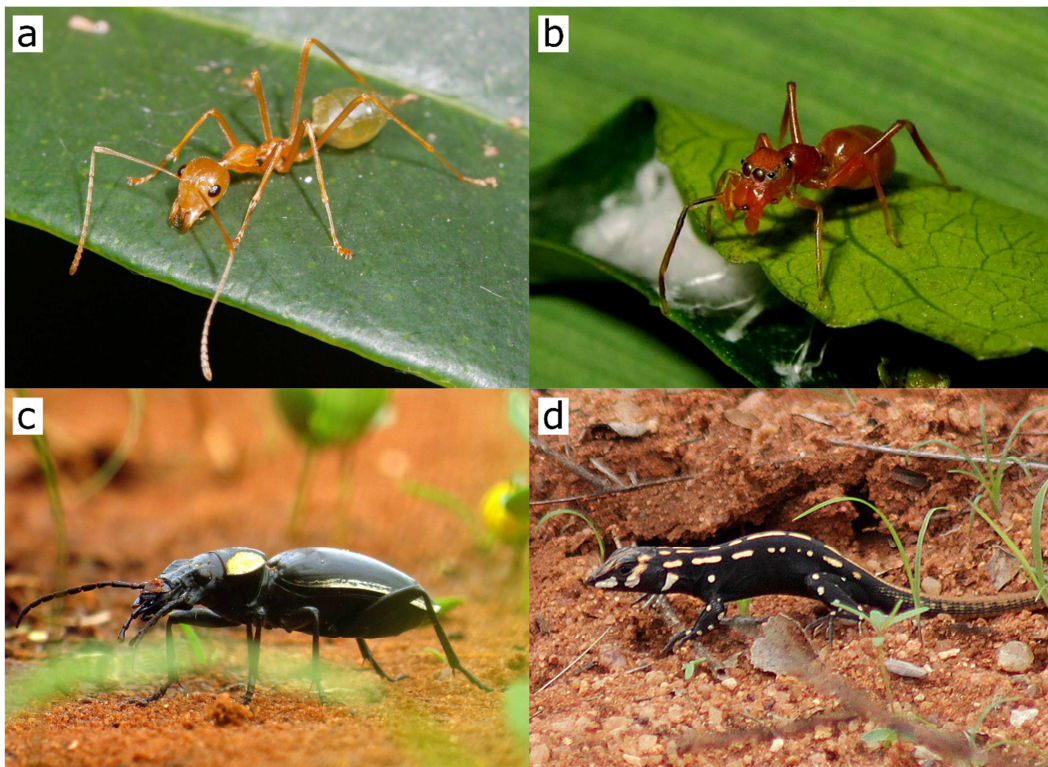
## **Chapitre 2 – Manuscrit C : importance des indices et des signaux dans l'évolution du mimétisme défensif**

De nombreuses espèces protégées (venin, piquants...) présentent des signaux - appelés signaux aposématiques - qui servent à avertir de la présence de défenses ; au fil du temps ces signaux ont évolué du fait de la pression de sélection des prédateurs. Cependant, certaines espèces défendues ne possèdent pas de signaux aposématiques. C'est par exemple le cas des fourmis, qui sont défendues par leur nombre, leurs mandibules et l'acide formique, mais qui ne présentent aucune caractéristiques physiques ayant évolué spécifiquement pour prévenir les autres espèces de leur défense.

Dans le cas du mimétisme, la très grande majorité des exemples décrits à ce jour concernent le mimétisme de signal, c'est-à-dire le cas où le mime copie les signaux aposématiques du modèle. Cependant, puisque certaines espèces défendues ne présentent pas de signaux certains mimes ont développé un mimétisme d'indice. Le mimétisme d'indice correspond au cas où le mime copie des caractéristiques du modèle qui n'ont pas évoluées pour prévenir les autres de la présence de défense (par exemple : la forme du corps, la couleur utilisée pour le camouflage). L'objectif de ce manuscrit est de montrer l'importance des indices dans l'évolution du mimétisme défensif.

Nous décrivons tout d'abord plusieurs exemples de mimétisme d'indices (Figure 1). Ces exemples montrent que ce type de mimétisme n'est pas anecdotique, bien qu'il ait été négligé par la recherche jusqu'à maintenant. Nous discutons ensuite de l'effet des indices sur la dynamique évolutive du mimétisme. Nous montrons que le mimétisme d'indice pourrait servir en complément du mimétisme de signal, en particulier dans le cas du mimétisme imparfait où le modèle et le mime sont très différents, et ce afin de renforcer la ressemblance. Nous avançons aussi l'idée que le mimétisme d'indices, lorsqu'il est utilisé en

même temps que le mimétisme de signal, peut participer à la maintenance de la diversité des signaux aposématifs.



**Figure 1.** Exemple de mimétisme d'indice. La forme du corps de la fourmi *Oecophylla smaragdina* (a) est imitée par l'araignée *Myrmaplata plataleoides* (b). En plus de l'imitation du signal coloré, le lézard juvénile *Heliobolus lugubris* (d) copie la démarche du coléoptère *Anthia thoracica* (c). Crédits photos : (a) Lek Khauv ; (b) Jason Alexander ; (c) Tjeerd de Wit ; (d) Christine Sydes.

### Chapitre 3 – Manuscrit D : effet de la sélection naturelle et sexuelle sur la disparité et les taux d'évolution de la coloration des vertébrés

La sélection naturelle est responsable de l'évolution des traits utilisés pour la survie (par exemple pour le camouflage). La sélection sexuelle agit elle, sur les traits influençant la reproduction des individus, qui peuvent être de deux types : (i) les ornements utilisés par les mâles pour séduire les femelles (par ex. les plumes colorées de la queue du paon) ou plus exceptionnellement par les femelles pour séduire les mâles. (ii) Les armements, qui interviennent dans la compétition au sein d'un même sexe, pour trouver un partenaire (par exemple les bois chez les cerfs).

Il est souvent supposé que la sélection sexuelle produit une plus grande diversité de couleur que la sélection naturelle. Cependant cette hypothèse n'a été que rarement testée et toutes les études, ou presque, concernaient les oiseaux.

Dans notre étude, nous avons comparé la disparité et les taux d'évolution de colorations influencés par la sélection sexuelle et naturelle dans trois groupes de vertébrés : chez les oiseaux, chez les lézards et chez les tritons. Nous trouvons que, comme attendu, la sélection sexuelle augmente la disparité et les taux d'évolution de la coloration chez les oiseaux. Cependant, les résultats sont moins clairs chez les tritons car certaines colorations non-sexuelles évoluent aussi vite que les colorations sexuelles. Pour les lézards, nous observons un patron inverse à celui attendu, avec des traits sexuels ayant une plus faible disparité et de plus petits taux d'évolution que les traits non-sexuels en terme de coloration. Ces observations contredisent donc l'idée que la sélection sexuelle est toujours une force de diversification pour la coloration.

#### **Chapitre 4 – Manuscrit E : influence du polymorphisme et de la sélection sexuelle sur la diversification des Lacertidae**

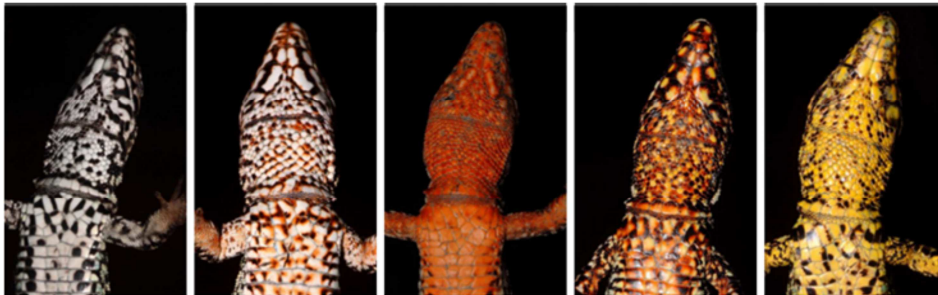
Dans ce dernier chapitre, nous avons regardé comment l'évolution de la coloration pouvait interagir avec les processus de formation de nouvelles espèces. Les Lacertidae sont un groupe de lézards qui présentent une coloration très variable : certaines espèces ont des colorations sexuelles, d'autres présentent du polymorphisme de couleur (Figure 2).

Il a été avancé que la sélection sexuelle pourrait augmenter la formation de nouvelles espèces en favorisant la divergence des caractères sexuels (y compris la coloration). Par ailleurs, certains auteurs ont proposé une théorie appelée « spéciation morphique » ; celle-ci affirme que le polymorphisme pourrait faciliter la création de nouvelles espèces de part la spécialisation compétitive des différents morphes (rouge, jaune, blanc...). Par exemple le morphe blanc va gagner la compétition contre le morphe rouge, mais perdre contre le morphe jaune. Dans ce cas-là, si une population perd un morphe (par exemple lors de la colonisation d'un nouvel habitat), il y aura un déséquilibre de la compétition entre individus, ce qui faciliterait la formation d'une nouvelle espèce à partir de cette population.

Dans cette étude nous avons testé ces deux postulats chez les 350 espèces de Lacertidae. Nous avons utilisé des modèles d'évolution qui mesurent l'influence d'un caractère, ici la coloration, sur la formation et l'extinction des espèces à partir d'un arbre

représentant la parenté entre espèces, et les caractéristiques colorimétriques de chaque espèce vivant actuellement.

Nous montrons tout d'abord que la présence de coloration sexuelle n'influence pas la formation de nouvelles espèces. Ce résultat est en accord avec de nombreuses études précédentes, ce qui indique que les processus qui déterminent la création de nouvelles espèces ne sont pas les mêmes que ceux qui influencent la persistance des espèces dans le temps. Nous montrons aussi que la présence de polymorphisme n'a pas conduit à une augmentation du nombre d'espèces. La théorie de la spéciation morphique n'avait été testée que par une seule étude réalisée à large échelle, et ce résultat remet donc en question la généralité de cette théorie.



**Figure 2.** Exemple de polymorphisme observé chez les Lacertidae. Ici, le cas du mâle lézard des murailles (*Podarcis muralis*) dont la coloration ventrale peut prendre plusieurs teintes. Ces photos proviennent de l'article de Andrade et al. 2019.



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## **Evolution of coloration: a study of the palette of vertebrates**

Coloration often have an essential role in both the survival and the reproduction of individuals. Because of this role, its evolution is determined by multiple, and often opposite, selective pressures. These characteristics make coloration an interesting trait to understand the fine details of the processes that shape the evolution of phenotypes. During my PhD, my aim was to disentangle the different selective forces influencing the evolution of coloration and to understand the effect of coloration evolution on species diversification. Although, there is already a solid literature on these subjects, most of the previous studies were made at the species levels. The originality of my work lies in the utilization of large-scale comparative analyses, applied to different species coming from three major clades of vertebrates, Aves, Amphibia and Squamata. I first investigated the selective pressures determining coloration variations at the intraspecific scale. I showed that, in two species of newt, selection for interspecific recognition influenced the evolution of colorations visible at short range, while selection for crypsis determined the colorations visible at distance. Secondly, I investigated the selective forces shaping coloration at the interspecific scale. I studied how coloration mimicry evolves in communities. I developed a new method, based on artificial neural network, to detect mimetic species. I also proposed a new framework to study defensive mimicry, which emphasizes the role of both cues and signals in the evolution of aposematic coloration. Besides mimicry, I tested the relative influence of natural and sexual selection on the evolution of coloration in several clades of vertebrates. This analysis highlighted that sexual trait do not always have higher rates of evolution nor a higher disparity than non-sexual traits. Finally, I focused on the interplay between the evolution of coloration and species diversification, and I showed that, in Lacertids, neither the evolution of sexual colorations nor color polymorphism affected the rates of speciation.

## **Évolution de la coloration: une étude de la palette des vertébrés**

La coloration joue souvent un rôle essentiel dans la survie et la reproduction des individus. En raison de ce rôle, son évolution est déterminée par des pressions sélectives multiples et souvent opposées, ce qui en fait un trait intéressant pour comprendre le détail des processus qui façonnent l'évolution des phénotypes. Au cours de mon doctorat, mon objectif était d'identifier les différentes forces sélectives qui agissent sur l'évolution de la coloration et de comprendre comment l'évolution de la coloration pouvait influencer les processus de diversification. Ces questions ont déjà été étudiées de nombreuses fois précédemment, mais cela a généralement été fait à l'échelle de l'espèce. L'originalité de mon travail réside dans l'utilisation d'analyses comparatives à grande échelle, appliquée à des espèces provenant de trois principaux clades de vertébrés: Aves, Amphibia et Squamata. J'ai d'abord étudié les pressions de sélection déterminant les variations de colorations à l'échelle intraspécifique. En utilisant les données de coloration de deux espèces de tritons, j'ai montré que la sélection pour la reconnaissance interspécifique influençait l'évolution des colorations visibles de près, tandis que la sélection pour le camouflage déterminait les colorations visibles à distance. J'ai ensuite étudié les forces sélectives qui façonnent la coloration à l'échelle interspécifique. Je me suis intéressé à l'évolution du mimétisme de coloration dans les communautés. J'ai développé une nouvelle méthode, basée sur le réseau neural artificiel, pour détecter les espèces mimétiques. J'ai également proposé un nouveau cadre de recherche pour étudier le mimétisme défensif, prenant en compte le rôle des indices et des signaux dans l'évolution de la coloration aposématique. Outre le mimétisme, j'ai aussi étudié l'influence relative de la sélection naturelle et sexuelle sur l'évolution de la coloration chez trois clades de vertébrés. Cette analyse a mis en évidence que les traits sexuels n'ont pas toujours un taux d'évolution plus élevé ni une disparité plus grande que les traits non sexuels. Enfin, j'ai étudié l'interaction entre l'évolution de la coloration et la diversification des espèces. J'ai montré que, chez les Lacertidae, ni l'évolution des coloration sexuelles ni le polymorphisme de coloration n'avaient d'effets sur les taux de spéciation.