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

3 Thomas de Solan | Marc Théry | Damien Picard | Pierre-André Crochet | Patrice David | Jean Secondi

4 **Short title:** Newt phenotypic evolution in sympatry

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14 **Data accessibility statement**

15 The data that support the findings of this study are openly available in Dryad at

16 [doi:10.5061/dryad.b8gtht7dw](https://doi.org/10.5061/dryad.b8gtht7dw)

17

18 **Keywords:** Reproductive character displacement, natural selection, sexual selection, crypsis,

19 *Lissotriton*

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27 **Abstract:**

28 Coexistence with related species poses evolutionary challenges to which populations may react
29 in diverse ways. When exposed to similar environments, sympatric populations of two species
30 may adopt similar phenotypic trait values. However, selection may also favor trait divergence as
31 a way to reduce competition for resources or mates. The characteristics of external body parts,
32 such as coloration and external morphology, are involved to varying degrees in intraspecific
33 signaling as well as in the adaptation to the environment, and consequently may be diversely
34 affected by interspecific interactions in sympatry. Here, we studied the effect of sympatry on
35 various color and morphological traits in males and females of two related newt species
36 *Lissotriton helveticus* and *L. vulgaris*. Importantly, we did not only estimate how raw trait
37 differences between species respond to sympatry, but also the marginal responses after
38 controlling for environmental variation. We found that dorsal and caudal coloration converged
39 in sympatry, likely reflecting their role in adaptation to local environments, especially
40 concealment from predators. In contrast, aspects of male and female ventral coloration, which
41 harbours sexual signals in both species, diverged in sympatry. This divergence may reduce
42 opportunities for interspecific sexual interactions and the associated loss of energy, suggesting
43 reproductive character displacement (RCD). Our study emphasizes the contrasting patterns of
44 traits involved in different functions and calls for the need to consider this diversity in
45 evolutionary studies.

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49 INTRODUCTION

50 Geographic patterns of convergence and divergence among species have attracted a lot of
51 interest in evolutionary ecology, from community assembly (Grime, 2006) to biogeography
52 (Bergmann, 1847) and evolution (Brown and Wilson 1956; Grant 1972). Geographical
53 convergence –the phenotypic convergence of two species where their populations co-occur in
54 the same geographic area- can reflect several evolutionary processes: hybridization (Grant *et al.*,
55 2004; Stern, 2013), selection related to interspecific interactions (e.g. interspecific
56 communication between territorial species: Cody 1969; Secondi *et al.* 2003), adaptation to
57 similar local environmental conditions (Elmer & Meyer, 2011; Losos, 2011), or common plastic
58 responses to these conditions (phenotypic plasticity: e.g. Dennis *et al.* 2011). Likewise,
59 geographical divergence results from several mechanisms. Firstly, resource competition can
60 promote divergence in ecological traits and generate ecological character displacement
61 (Schluter, 2000; Pfennig & Pfennig, 2009). Secondly, reproductive character displacement (RCD)
62 can arise from selection to reduce interspecific reproductive interactions due to imperfect
63 species recognition during mate choice (Goldberg & Lande, 2006). Finally, agonistic character
64 displacement (ACD) can evolve to reduce interspecific interference resulting from agonistic
65 interactions between sympatric species (Grether *et al.*, 2009).

66 At the population level, phenotypic convergence and divergence depend on the interplay
67 between various selective forces, and different body parts may show different responses
68 depending on the sets of selective forces they are exposed to (Shultz & Burns, 2017). Patterns of
69 convergence and divergence may thus help deciphering the role of local adaptation, ecological
70 competition and interspecific reproductive interactions in shaping the different dimensions of
71 the phenotype (e.g. Langerhans *et al.*, 2006; Jang *et al.*, 2009). For example, widespread
72 observations of ecological character displacement, the divergence in sympatry for traits involved

73 in resource acquisition such as size or morphology of the feeding apparatus, provides powerful
74 support for the role of competition in driving phenotypic evolution (reviewed in Grant, 2013;
75 Stuart & Losos, 2013).

76 Body coloration is a prime example of a trait subjected to various selective pressures, as
77 it is involved in sexual signaling, avoiding (crypsis) or deterring (aposematism) predation, or
78 regulating body temperature (Hill & McGraw, 2006; Rudh & Qvarnström, 2013). Colors of
79 different body parts have different main functions. A range of situations exists from body parts
80 usually concealed and exposed only during displays, such as the vocal sac in European tree frogs,
81 whose coloration influence mate attraction (Gomez *et al.*, 2010), to body parts visible most of
82 the time, assumed to be largely affected by environment- or predator-driven natural selection
83 (Stuart-Fox & Ord, 2004). For this reason, investigating convergence and divergence patterns in
84 a set of color traits including both sexual signals and nonsexual traits provides insight into the
85 diversity of selective pressures that shape whole body coloration in animals. Previous studies
86 have suggested that the functions of color patches in different body parts can predict their
87 variation in sympatry: non-sexual colors may more often converge, while sexual signals may more
88 often diverge in sympatry (Stuart-Fox & Ord, 2004; Gomez & Théry, 2007).

89 Patterns of trait convergence or divergence in the zone of sympatry between two related
90 species are usually examined by comparing interspecific differences in phenotypic means in
91 sympatric versus allopatric populations of both species (Kawano, 2002). However, these
92 differences may not reflect a specific evolutionary response to sympatry per se (i.e. to the
93 presence of the other focal species) but simply emerge as a phenotypic response of each species
94 to the environmental gradients experienced within its own range. Indeed, it is common for
95 selective optima of phenotypic traits to vary across a species' range according to large-scale or
96 small-scale environmental gradients (Delhey *et al.*, 2019; Price *et al.*, 2019). As a result,

97 populations of each species may experience more similar environmental conditions in sympatry
98 than in allopatry and thus be phenotypically more similar, independently of the presence of the
99 other species. It is thus essential to account for environmental variations acting on the
100 phenotype, at the scale of the species' distributions, to investigate divergence or convergence in
101 nature (Goldberg and Lande 2006).

102 Our aim here is to investigate patterns of divergence and convergence among sexual and
103 non-sexual traits in a pair of related newt species. We used populations sampled in the wild to
104 compare geographical variation in size, ornaments and coloration of *Lissotriton helveticus* and *L.*
105 *vulgaris* within and outside their zone of sympatry. Specifically, we tested whether each trait
106 diverged or converged in sympatry, and whether this pattern could be simply explained by
107 phenotypic response to environmental gradients, or was also influenced by the presence of the
108 other species. We also investigated whether traits involved in sexual signaling / communication
109 were more likely to exhibit specific response to sympatry than traits assumed to be mainly under
110 natural selection. Although by nature a correlative study, its sampling design, extensive
111 geographical coverage and sophisticated analytical methods makes it a powerful approach to
112 separate between alternative hypotheses of geographical variation in color phenotypes, even if
113 the proximal mechanisms and selective agents remain to be tested experimentally.

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115

116 **MATERIALS AND METHODS**

117 **Biological models**

118 The Smooth Newt *Lissotriton vulgaris* has a large distribution range across Europe (Figure
119 1, we follow Speybroeck et al. 2020 for the taxonomy of the lineages that make up the Smooth
120 Newt complex). From central France to northern Germany, one of its lineages shares a wide area

121 of sympatry with the Palmate Newt *L. helveticus*, while the Palmate Newt has an allopatric range
122 that reaches as far as southern France and north-western Iberia. In European newt species,
123 interspecific reproductive interactions are common (Babik *et al.*, 2003; Pabijan *et al.*, 2017) and
124 in the sympatric zone, *L. helveticus* and *L. vulgaris* are frequently syntopic, and have similar
125 reproductive phenology and ecological requirements during the breeding period (Griffiths, 1986;
126 Chadwick *et al.*, 2006).

127 We described the phenotypes of both species using several characters that we classified
128 *a priori* as sexual or non-sexual, based on the biology of these organisms and the literature. The
129 following characters were considered as sexual characters: (i) morphological ornaments (caudal
130 filament, body and tail crests, hind-foot webbing, listed in Table 1) as they are only found in
131 males, are displayed during courtship (Halliday, 1977), and affect female choice in both species
132 (Gabor & Halliday, 1997; Cornuau *et al.*, 2014). (ii) The conspicuous yellow-orange coloration with
133 black spots, found on the belly of both sexes, because it has been reported to be involved in
134 female choice (Secondi *et al.* 2012; Secondi and Théry 2014; but see Cornuau *et al.* 2012) and to
135 influence male mate choice in a related species (Lüdtke & Foerster, 2018). Ventral coloration is
136 probably not an aposematic signal in our species (*contra* other Salamandridae, see Brodie 1977)
137 since there is no published evidence of toxicity and adults are consumed by a large number of
138 predators (Rosa *et al.*, 2012; Parry *et al.*, 2015; Miró *et al.*, 2020). (iii) Finally, we also classified
139 caudal coloration as a trait under sexual selection because, although its role in mate choice has
140 not been studied specifically, it is sexually dimorphic and the tail plays an overall important part
141 during courtship (Halliday, 1977). On the contrary, dorsal coloration was assumed to be under
142 natural, but not sexual, selection, because both sexes of both species share a dull brown
143 coloration broadly matching the pond substratum, making the newt difficult to detect. It should
144 also be noted that ornaments (crests, webbing and tail filament) are only expressed during the

145 breeding season while dorsal and ventral coloration seem more stable over the year. As each of
146 these traits is likely to be the target of some form of selection, we did not expect any of them to
147 have a purely neutral evolution, though indirect selection via genetic correlations among traits
148 may happen.

149

150 **Sampling**

151 We sampled *L. helveticus* and *L. vulgaris* (belonging to the northern lineage of *L. v. vulgaris*, see
152 Pabijan et al. 2017) in the allopatric and sympatric part of their distribution ranges (Figure 1).
153 Adult newts were caught by dipnetting in ponds during the breeding period from March 2012 to
154 May 2014. All newts were anaesthetized prior to manipulation by immersion in Tricaine methane
155 sulphonate (MS222, 0.2 g/l) for 5 minutes. After the measurements, newts were released at their
156 site of capture. All individuals that we monitored fully recovered from anesthesia. We sampled
157 55 sites in total, 32 in sympatry, 15 in the allopatric distribution of *L. helveticus* and 8 in the
158 allopatric distribution of *L. vulgaris* (location and number of individuals sampled in both sexes for
159 each sampling site in Table S1). In the allopatric areas we caught 59 male and 70 female *L.*
160 *vulgaris*, and 117 male and 100 female *L. helveticus*. In the sympatric zone, we captured 137 male
161 and 127 female *L. vulgaris*, and 311 male and 237 female *L. helveticus*. We excluded males
162 without fully developed sexual ornaments (crests, palmation). Final sample size was 54 male and
163 70 female *L. vulgaris* and 101 male and 100 female *L. helveticus* in allopatry and 124 male and
164 127 female *L. vulgaris* and 309 male and 237 female *L. helveticus* in sympatry.

165

166 **Morphology, pattern and spectrometry measurements**

167 Using a digital caliper, we measured snout-to-vent length and tail height in both sexes, plus the
168 caudal filament length in males. Each measurement was taken twice and the mean was used in

169 further analyses. For males, we also described the sexual ornaments by coding the extent of the
170 hind-foot web, the shape of the dorsal and caudal crest, the number of dark spots on the belly,
171 and the number and size of the dark spots on the throat and the flank (scoring of the traits
172 detailed in Table 1, pictures of males of both species in Figure S1).

173 The morphological variables were all correlated to snout-to-vent length, as it is a
174 measurement of body size in amphibians. We used two methods to remove body-size effects on
175 the other morphological traits. For females, we only had one morphological trait (tail height) in
176 addition to snout-to-vent length, and we used the residuals of the linear regression of tail height
177 on snout-to-vent length to make tail height independent of body size. For males, we had a set of
178 11 traits, so we used a Principal Component Analysis (PCA) to reduce the number of variables to
179 a few independent synthetic traits, while accounting for size effects in the first principal
180 component (see below).

181 For coloration, we measured the reflectance spectra between 300 and 700nm of dorsal,
182 ventral and caudal body parts in all individuals. Measurements were taken on wet animals, and
183 two measurements were taken for each body part. We averaged and smoothed the reflectance
184 spectra for each individual with the R package pavo (Maia *et al.*, 2013). Flank coloration was not
185 measured, as their upper and lower coloration appear to be the same as dorsal and outer (lateral)
186 ventral coloration respectively, at least to the human eye. However, we quantified the number
187 and the size of dark spots on the flank (see above). For ventral coloration, we measured the
188 vividly-colored central band of the belly, which is involved in reproductive interactions in newts
189 (Secondi & Théry, 2014; Lüdtke & Foerster, 2018). The exact location of the measurements on
190 the body is described in Figure S1 (for the ventral coloration of *L. vulgaris* we made sure to
191 measure the vivid coloration between the black spots).

192 We used a Jaz spectrometer (Ocean Optics), a deuterium-halogen light source (Ocean
193 Optics DH-2000), and a coaxial optic fiber (Avantes FCR-7UV200-2-45-ME). The probe was held
194 with an angle of 45°. Reflectance spectra of each body part (Figure 2) are multidimensional traits,
195 and from each of them we were able to extract several variables: the hue in the visible range
196 (401-700nm), the saturation and brightness in the UV range (300-400 nm) and in the visible range
197 separately. We did not describe the full 300-700nm range with only one variable because the
198 spectra were bimodal (Figure 2), and because there is evidence that females use UV to assess
199 males (Secondi & Théry, 2014). We did not estimate hue for dorsal coloration because we
200 observed no peaks in the spectra, and overall a very low reflectance (<6%, see Figure 2). The most
201 appropriate color variables for each part of the spectrum were selected among those described
202 by Montgomerie (2006), and were given the same name (algorithm for each variable described
203 in Supplementary methods). We measured brightness with B2, in UV (B2_UV) and visible range
204 (B2_visible); saturation in the UV range using S1 (S1_UV) and in the visible range using S6
205 (S6_visible). For the ventral coloration, we chose another saturation index (S9), more appropriate
206 to describe carotenoid-based coloration, which probably applies to newt ventral coloration
207 (Kopecký & Šichtař, 2014). We measured hue in the visible range with the H3 variable
208 (H3_visible). In summary, we extracted five (ventral and caudal colorations) or four variables
209 (dorsal coloration) describing the reflectance spectrum in the visible and UV ranges. We do not
210 know of any study assessing whether capture and handling affects coloration in newts but we did
211 not notice any change in coloration during the process. In addition, the photos of animals taken
212 just after capture and after handling and measurements did not show obvious differences. We
213 are thus confident that we recorded live coloration accurately and further note that all
214 populations were treated in the same way, ensuring that none of the reported effects result are
215 artefacts due to capture and handling.

216 We log-transformed several coloration traits before analysis to ensure normality
217 (specified in Table S2). We also standardized all the traits before analysis (mean-centered and
218 divided by the standard deviation) to facilitate comparison between model estimates. Finally, we
219 measured the correlation between coloration traits in both sexes, as coloration traits are known
220 to often coevolve (Figure S2). The correlations between traits describing different body parts
221 were generally low, but they were higher between traits describing the same body parts.

222

223

224 **Environmental factors**

225 We considered both large-scale and local environmental factors that can affect the expression of
226 traits in newts. We extracted 19 bioclimatic variables from WorldClim v2 (Fick & Hijmans, 2017),
227 with 1km resolution, and used the first two axes of a PCA to summarize climatic variation among
228 sites. The first axis accounted for 44% of the variance, and separated warm sites with high winter
229 rainfall (negative side) from cold sites with high summer rainfall (positive side; Table S3). The
230 second axis accounted for 24% of the variance, and described a gradient of temperature
231 seasonality (high seasonality for negative values of PC2). We also included altitude (1-km
232 resolution, Danielson and Gesch 2011) in our models. At a local scale, we quantified variation of
233 water transparency by measuring the water transmission spectrum for each pond, and averaging
234 transmission over the whole spectrum (method described in Secondi et al. 2017). Mean values
235 of environmental variables in the sympatric and allopatric zones are presented in Table S4.

236

237 **Analysis 1: investigating patterns of convergence/divergence**

238 The aim of this first analysis was simply to detect patterns of divergence and convergence in
239 sympatry, without trying to decipher the influence of environmental gradients and sympatry

240 itself. We used Linear Mixed-effect Models, implemented in 'lme4' R package (Bates *et al.*, 2015).
241 The model (1) included species identity, context (sympatry *versus* allopatry) and their interaction
242 as fixed factors, and sampling site as a random factor. Species identity tested for difference in
243 trait value between species, while context tested for difference in trait value between allopatric
244 and sympatric zone, and their interaction tested if the two species showed a similar response to
245 sympatry or not. A significant interaction term indicates either convergence or divergence (the
246 two species react differently to sympatry hence are more similar, or less similar, in sympatry than
247 in allopatry).

248 (1) Trait ~ species * context + sampling site

249 We ran one model for each trait and sex. Sexes were analyzed separately because we did not
250 expect a similar response to context in both sexes and species, and a three-way interaction would
251 have been uninterpretable. We did not include the year of sampling in the models, as this variable
252 was confounded with geographic pattern (most regions were only sampled once, see details in
253 Figure 1). However, we did not expect sampling date to affect the result of the model, because
254 between-year phenotypic variations should be limited compared to phenotypic differences
255 observed at continental scale. We investigated homoscedasticity and normality of the traits using
256 diagnostic plots of models' residuals, but we did not find patterns indicating violation of the linear
257 model hypotheses.

258 Convergence and divergence in sympatry can be inferred from the species * context
259 interaction in linear models, but this requires a new calculus for each model. To provide a global
260 vision of the convergence/divergence patterns observed on all the characters measured, we
261 computed for each trait and sex an index describing the pattern observed in sympatry. We
262 calculated this index following the formula:

263 Index = |Marginal mean Lh sympatry - Marginal mean Lv sympatry |

264 - |Marginal mean Lh allopatry - Marginal mean Lv allopatry |

265 With Lh = *Lissotriton helveticus* and Lv= *Lissotriton vulgaris*. The marginal mean corresponds to
266 the predicted mean value of a trait, after removing the effect of other variables included in the
267 model. For Analysis 1, the marginal means were equal to the mean values of species traits
268 because we did not include additional variables in the model. If the difference is smaller in
269 sympatry, the index is negative and the species have converged. If the sympatric difference is
270 larger, the index is positive and the species have diverged. This index was not subjected to any
271 statistical modelling (since the significance of the sympatry /allopatry effect was tested by means
272 of the context and species * context effects) but was computed for illustrative purpose only.

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

278

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

280 In this second analysis, we assessed the contribution of the environment on phenotypic variation,
281 and tested whether responses to environmental variations entirely explained the divergence and
282 convergence patterns in sympatry detected in Analysis 1. For each sex and trait separately, we
283 first created a full model, which included the fixed and random factors already present in model
284 (1), plus all environmental variables, and their interaction with species identity. This model (2)
285 fitted species-specific responses to each environmental variable for each trait.

286 The full model (2) thus reads:

287 (1) Trait ~ species * (context + Bioclimatic axis 1 + Bioclimatic axis 2 + altitude + water
288 transmission) + sampling site

289 We applied a model selection procedure in order to reduce the number of
290 predictors/explanatory variables. Using the dredge function of MuMIn package (Barton, 2015)
291 we performed the models corresponding to all possible combination of the fixed environmental
292 effects. We forced the model selection procedure to keep species identity, context, and their
293 interaction in the model. Then we selected the simplest model with a deltaAICc of less than 2
294 compared to the model with the lowest AICc. Finally, we computed the index of
295 convergence/divergence in a similar way as in the first analysis for each trait and sex, but this
296 time the marginal means were calculated after removal of the environmental effects. Thus, the
297 part of the difference in a trait observed in sympatry that was due to environmental variables, as
298 well as their possible interactions with species identity, was removed, leaving only the difference
299 brought about by the species-by-context interaction term. The corrected index is positive
300 (respectively negative) when the accentuation of species differences in sympatry (vs allopatry) is
301 larger (respectively weaker) than predicted by large-scale environmental gradients.

302 We performed the same cross-validation procedure as in Analysis 1. Likewise, we checked
303 the validity of each model using the diagnostic plots of the residuals, which did not show any
304 patterns indicating violation of the linear model hypotheses. We also measured the Variance
305 Inflation Factor (VIF) for each model, to estimate the impact of collinearity between variables
306 (Mansfield & Helms, 1982). Finally, the pattern of convergence/divergence observed in sympatry
307 may be partially explained by the non-independence of the phenotypic measurements between
308 populations due to gene flow, thus generating spatial autocorrelation among populations. To test
309 this hypothesis, we performed Moran's tests, using the spdep R package (Bivand *et al.*, 2015), to
310 evaluate the spatial autocorrelation of the residuals.

311

312

313 **RESULTS**

314 **PCA on male morphological traits**

315 The first three PCs on male ornamental traits accounted for respectively 67%, 9% and 8% of the
316 variance and were retained for further analyses. The first axis separated the two species based
317 on the size of the caudal filament, the shape of the foot webs and the crest, the number and size
318 of spots on the throat, the number of spots on the belly and the size of spots on the flank (Table
319 1, see Figure S3 for a plot of individuals' scores on the first axes). The second axis separated
320 individuals with few vs many dark spots on the flanks and the third axis separated large
321 individuals with many dark spots on the flanks from small individuals with few spots.

322

323 **Patterns of divergence and convergence in sympatry**

324 The results of model (1) indicate that the two *Lissotriton* species differ by many traits (9
325 male and 6 female traits), most of which presented significant species-by-context interactions (7
326 for males and 8 for females, model results presented Figure 3A and Table S2). Our index of
327 divergence/convergence indicated the existence of several patterns (Figure 3A). Male
328 ornamental traits (summarized as PC1-3) and female morphological traits (SVL, tail height) did
329 not show any signs of convergence or divergence. Dorsal and caudal coloration traits tended to
330 converge in sympatry, while a pattern of divergence was only observed for the variables
331 describing the coloration of the ventral band (except brightness in the visible range). Overall,
332 males and females showed similar patterns of convergence and divergence.

333

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

335 Environmental effects may result in convergence (i) when both species react similarly to an
336 environmental variable that takes different values in sympatry and in allopatry or (ii) when

337 species react differently to the same variable in such a way that their difference is decreased in
338 sympatry. For these reasons, the patterns visible in Figure 3A may not reflect an actual response
339 of species' phenotypes to sympatry, but rather to the environment.

340 Indeed, model (2) revealed many significant effects of environmental variables on male
341 and female traits (results of model (2) in Table S5). After cross-validation, we found that
342 coloration and morphology co-varied mainly with large scale gradients, bioclimatic variables and
343 altitude (respectively 8 and 7 traits correlated in males and females, Table S5), while local
344 environmental conditions, water transmission, had a generally smaller influence (only one trait
345 in males). Focusing on the effects that were retained for both species, we found that for variation
346 in male morphology, PC1 (which differentiates the two species) was mainly influenced by the
347 second bioclimatic axis (Table S5) and water transmission, while PC2 (which differentiates
348 individuals with low and high number of spots on the flanks) was influenced the two bioclimatic
349 axes. For coloration, we found that dorsal brightness in the UV-spectrum in both sexes and dorsal
350 saturation in the UV spectrum in females both decreased in regions with less thermal contrasts
351 between seasons (axis 2). Dorsal brightness in the visible and UV spectrum and dorsal saturation
352 also increased with altitude in females. However, since dorsal brightness and dorsal saturation
353 are strongly correlated (Table S2), the effect of altitude found in females could be driven by one
354 of these two traits only. For the ventral parts, saturation in the visible spectrum increased in
355 males and females as climate became less seasonal (axis 2).

356 The two species also showed differential responses to some environmental variables
357 (interaction species:environmental variables Table S5). In particular, *L. vulgaris* had stronger
358 responses to altitude than *L. helveticus*. Caudal saturation (S6_visible) and caudal brightness
359 (B2_visible) in the visible spectrum decreased with altitude in both sexes for *L. vulgaris*, while
360 these traits were not affected by altitude in *L. helveticus*. Similarly, male ventral saturation (S9)

361 decreased with altitude in *L. vulgaris* but not *L. helveticus*. However, the pattern was opposite
362 for male ventral hue (H3_visible): it increased with altitude in *L. helveticus* and not *L. vulgaris*.

363 Patterns of convergence and divergence obtained in the first analysis were re-examined
364 based on the model including environmental variables (see Table S5 for model (2) results and
365 Figure 3B for the pattern of convergence/divergence after controlling the effects of
366 environmental variables). To facilitate the interpretation of Figure 3, we will detail one example
367 in which the inclusion of environmental responses makes a visible difference, the *S6_visible*
368 variable describing caudal coloration in females, in which a strong convergence signal is
369 uncovered only when environmental variables are taken into account. For this variable the
370 average trait difference (*L. vulgaris* – *L. helveticus*) in sympatry is small (0.5 on standardized scale)
371 and comparable in absolute value to the difference in allopatry (-0.74); this is why our
372 divergence/convergence index is close to zero, without taking environment into account (0.5-
373 0.74=-0.24 in Figure 3A, Table S2). However, in the model with environmental predictors (Table
374 S5), this variable is significantly sensitive to altitude and to climatic gradients (PC2), and these
375 responses are species-specific (significant interactions). Based on these responses, and on the
376 mean environmental values of the sympatric and allopatric zones (Table S4), environmental
377 gradients would predict a phenotypic difference between the two species of 2.91 in allopatry,
378 and of 2.91 in sympatry, the latter much larger than the observed value. The model fits the data
379 by adding a very large species-by-context interaction (-3.51) that reduces this difference
380 specifically in the sympatric zone, resulting in a very negative divergence index (0.6-2.91=-2.31
381 in Figure 3B). This means that the phenotypic differences between the two species in sympatry
382 is much lower than what would be expected based on their responses to environmental
383 gradients.

384 Looking at all the variables, however, the inclusion of environmental factors did not
385 overall strongly modify the observations on convergence and divergence obtained in the first
386 analysis (Figure 3B). The effect of environmental factors on coloration, although significant,
387 explained a relatively low percentage of the whole variance (examples of relationship between
388 coloration and environment in Figure S4). For three out of the twelve traits that significantly
389 converged in the first analysis, the species-context interaction was no longer significant, though
390 still indicating convergence (in females : caudal B2_visible and S1_UV, in males ventral H3_visible,
391 Figure 3B), suggesting that the initial pattern was largely explained by responses to the
392 environment. For one other trait (caudal S6_visible in females, example detailed above), the
393 pattern of convergence was on the contrary reinforced when the environmental effects were
394 taken into account (Table S5).

395 Patterns of divergence suggested by the first analysis were maintained for two ventral
396 colorimetric variables in the second analysis, S9_visible in males and S1_UV in females. The
397 divergence of the last trait in males (ventral H3_visible), was qualitatively maintained but not
398 statistically supported when accounting for environmental variables as the species-context
399 interaction became non-significant. In Figure 4 we illustrated the variation in the two traits found
400 to be divergent in all models: we displayed the raw data in sympatry and allopatry along with
401 predictions for models including environmental variations only. The comparison between these
402 two graphs indicates if divergence was driven by one or both species. For male ventral coloration
403 (S9), the interspecific difference in raw values increased from 0.23 in allopatry to 1.41 in sympatry
404 (Table S2). This enhanced divergence between raw values and values predicted by environmental
405 variation came from the reduced saturation of *L. helveticus* in sympatry. For female ventral
406 coloration (S1_UV), the interspecific difference in raw values increased from 0.09 in allopatry to
407 0.85 in sympatry (Table S2). This time the divergence arose from the higher saturation of *L.*

408 *helveticus* in sympatry. Thus, for both traits, divergence was the result of a change in *L. helveticus*,
409 while *L. vulgaris* traits exhibited only small variation between sympatry and allopatry (Figure 4).

410 Moran's tests for spatial correlation of the residuals were not significant for any model
411 ($p > 0.05$, Table S6). The VIF was low for most simple effects but high in case of interactions (Table
412 S7). Because collinearity inflates the standard errors, but does not bias the coefficients, the
413 corresponding tests are expected to be less often significant (Morrissey & Ruxton, 2018). A high
414 VIF may also indicate overdispersion, but diagnostic plots of residuals did not show any evidence
415 of it. The results presented above are thus robust, although we may have missed some significant
416 interactions due to a lack of power.

417

418 **DISCUSSION**

419 Our analyses revealed that sympatry affected the phenotype of *L. vulgaris* and *L. helveticus*
420 differently depending on the body part investigated: most of the dorsal and caudal coloration
421 traits converged in sympatry in both sexes, while divergence was observed only on two variables
422 describing ventral coloration, one in males one in females, and no difference between sympatry
423 and allopatry was detected for morphological traits. Divergence was thus restricted to a sexual
424 character (ventral coloration), whereas convergence was observed on both sexual and non-
425 sexual characters (respectively caudal and dorsal colorations). When accounting for
426 environmental variations, the patterns of convergence/divergence were maintained, though
427 environmental variables had significant effects on many traits. Finally, the few ventral
428 colorimetric variables showing divergence in sympatry were not explained by environmental
429 gradients and thus constitute likely cases of character displacement.

430

431 **Environmental effects on newt phenotype mostly lead to convergence**

432 Our analyses revealed that the phenotype of the two *Lissotriton* species was influenced by large-
433 scale gradients more than local water color. This result contrasts with growing evidence for
434 strong effects of local conditions on phenotype in newts (Secondi *et al.*, 2007) and other taxa (de
435 Abreu *et al.*, 2018; Price *et al.*, 2019). It is possible that water color fluctuates too much in time
436 (e.g. reduced transparency after rainfall) or space (i.e. large differences between neighboring
437 sites) to trigger stable plastic and/or evolutionary responses (Sasaki & Ellner, 1995).

438 Environmental selection is known to influence the evolution of both sexual and non-
439 sexual traits (Andersson, 1982; Endler, 1984), leading to phenotypic convergence between sister
440 species (Wilkins, 2001) or distantly related taxa (Mahler *et al.*, 2013) exposed to the same
441 conditions. In many vertebrates, dorsal coloration has a critical role for thermal physiology
442 (Clusella-Trullas *et al.*, 2007; Alho *et al.*, 2010) and crypsis (Rosenblum, 2006; Toledo & Haddad,
443 2009; Rudh & Qvarnström, 2013), which could explain the influence of climate and altitude on
444 newts' dorsal coloration. However, we did not observe a decrease in dorsal brightness in cooler
445 environments (Bogert's rule; Bogert 1949), which would be expected under the thermal
446 adaptation hypothesis. Crypsis is another plausible function of dorsal coloration. Unfortunately,
447 quantifying large-scale variation in predation pressure is a daunting task at such a continental
448 scale, so we were unable to directly evaluate the potential for adaptive geographic variation in
449 coloration in relation to predator avoidance. For the sexual traits, climatic variables could also
450 correlate with conditions of transmission of sexual signals in the breeding habitat, and variable
451 access to trophic resources leading to differential allocation of resources to sexual traits (Snell-
452 Rood, 2012; Macías-Ordóñez, 2013). We wish to stress that climate *per se* is not necessarily the
453 causal agent driving variation in coloration but climatic gradients might also capture large-scale
454 variation in vegetation, predator and prey communities, parasites, pathogens and other biotic or
455 abiotic factors that can affect the selective optima of color traits.

456 Despite their strong effects on body coloration, environmental variables did not explain
457 most of the convergence of dorsal and caudal colors. It is unlikely that the observed convergence
458 arose from introgression because there is no detectable interspecific gene flow between these
459 two species (Arntzen *et al.*, 1998). Convergence due to agonistic interaction is also unlikely. This
460 type of convergence is known to arise in territorial species, because shared signals may help
461 individuals of both species to defend their territories against both conspecifics and heterospecific
462 competitors (Cody, 1969; Grether *et al.*, 2009). However, although reproductive interferences
463 during courtship may occur among male newts (Halliday & Tejedo, 1995; Hardy & Briffa, 2013),
464 *Lissotriton* newts are not territorial (Giacoma & Balletto, 1988). A more likely explanation for
465 residual convergence in dorsal and caudal colours is an environmental selection, not captured by
466 our -mostly climatic- environmental models, that differs in the sympatric zone, and to which
467 species respond by adopting similar dorsal and caudal colorations. It may be predators (as
468 mentioned above), that have been shown to explain patterns of phenotypic convergence in other
469 species (Dennis *et al.*, 2011), but also vegetation structure and composition, prey types, among
470 others.

471 In conclusion, while we cannot definitively identify all environmental drivers of
472 phenotypic variation in *Lissotriton*, our results support that similar responses of the two species
473 to shared environmental factors is the most likely cause of convergence for some traits in
474 sympatry. Common garden experiments would be required to establish if these common
475 responses to environment reveal convergent local adaptation or shared plastic responses.

476

477 **Evidence for character displacement in males and females sexually-selected ventral colors**

478 We found a pattern of divergence in two traits related to ventral coloration in males and females
479 (S9_visible and S1_UV respectively). This pattern was maintained after accounting for

480 environmental effects, and thus constitute a probable case of character displacement (Goldberg
481 & Lande, 2006; Adams & Collyer, 2007; Grether *et al.*, 2009). Divergence, like convergence, could
482 in theory also be explained by plastic responses to unaccounted environmental factors if the two
483 species present species-specific -and opposite- plastic responses to these factors. However, this
484 explanation is unlikely in our situation. Firstly, contrary to the case of convergent responses, it is
485 difficult to imagine how an unmeasured environmental factor could diverge in sympatry when
486 the two species often occur in the same ponds. If the divergence was due to plasticity, it should
487 involve plastic responses to the same factor in opposite directions in two closely related species,
488 a situation for which we know of no empirical example. Secondly, the fact that the traits
489 exhibiting divergence are known sexual signals and diverge in sympatry suggests that this
490 divergence is a direct response to the presence of the other species and that it reduces
491 interspecific interactions. Such divergence in sexual signals in response to the presence of a
492 closely related species typically involve a genetic basis (Servedio & Noor, 2003; Ortíz-Barrientos
493 & Noor, 2005; McGraw *et al.*, 2011), and to our knowledge there are no reports of divergence in
494 sexual characters in related species explained by species-specific plastic responses. We thus
495 believe that the residual unexplained divergence in our models is not the results of unaccounted
496 environmental factors but can rather be explained by sympatry per se and is unlikely to be plastic,
497 even if common garden experiments would be required to formally exclude this possibility.
498 Because ventral colors are mostly visible during courtship, character displacement is probably
499 related to communication, either for mate attraction between sexes (it would then be a
500 reproductive character displacement, RCD), or within sexes, as part of signaling towards
501 competitors (agonistic character displacement, ACD).

502 The first possible advantage of divergence in sexual signals in sympatry is to reduce the
503 production of unfit hybrids. Hybrids obtained experimentally between *L. vulgaris* and *L.*

504 *helveticus* are viable, despite being sterile, yet F1-hybrids are extremely rare in nature (Griffiths
505 *et al.*, 1987; Arntzen *et al.*, 1998; Schlüpmann *et al.*, 1999). This indicates that interspecific
506 fertilization rarely occurs in natural conditions and that hybridization avoidance is already strong
507 between the two species, both suggesting that hybridization avoidance has only a small potential
508 impact on divergence. Yet, species recognition is not perfect in males and females of the two
509 species : males of both species frequently court heterospecific females (Roberts, 1990), and
510 experimental tests showed a low interspecific recognition for female *L. helveticus* and male *L.*
511 *vulgaris* in captivity (Secondi *et al.*, 2010, 2014). We thus suggest that RCD evolved under
512 selection for enhanced interspecific recognition, and associated gains in time and energy
513 (otherwise wasted in interactions that eventually do not result in effective mating), in sympatry.
514 This situation may also explain why divergence affects both sexes in newts, while it is commonly
515 observed in males only (see Dyer *et al.* 2014). In males, mating attempts are costly because of
516 energy and time invested in complex displays, but also because spermatophores used for
517 transferring sperm to the female are a highly limited resource that should not be wasted (Verrell,
518 1986). During heterospecific courtship, males will pay this cost without the benefits of mating.
519 However, the most divergent males could pay a lower cost, because females may identify them
520 more easily and interrupt courtship earlier. Such a situation would select for a divergence of male
521 coloration in sympatry. Divergence in females could be due to genetic cross-sex correlations, but
522 this explanation is not convincing in our case, as the diverging traits were different between
523 sexes. Rather this pattern could stem from the mutual mate choice observed in newts (Roberts,
524 1990; Lüdtké & Foerster, 2018). This could happen in two non-exclusive situations: (i) if males
525 preferentially court females with more recognizable coloration, while females with intermediate
526 coloration have fewer opportunities and may fail to obtain spermatophores, or (ii) if males do not
527 discriminate well between species and females pay a cost when repeatedly courted by

528 heterospecific males. This cost would select for more recognizable coloration in females,
529 reducing the error rate of males (i.e. initiating heterospecific courtship less often) and the
530 associated cost for the females. These conditions are likely to be fulfilled given what we know of
531 the breeding and courtship habits of these newts. Hence, a mutual benefit of enhanced
532 interspecific recognition in both sexes is a likely explanation for divergence of ventral coloration
533 in sympatry. Direct empirical tests are needed to validate this hypothesis by directly measuring
534 sexual preferences.

535 Another explanation for the divergence of coloration in sympatry could be male
536 competition through interspecific reproductive interference. In *Lissotriton*, males sometimes
537 interfere in the courtship of conspecific males (Halliday & Tejedo, 1995; Hardy & Briffa, 2013),
538 and, in males, the pattern of divergence could thus be a case of agonistic character displacement
539 (ACD) – i. e. to avoid unnecessary interference with heterospecific males. It should be noted that,
540 so far, we have no empirical evidence of interference during courtship between males of
541 different newt species in nature, but nothing suggests it cannot happen. On the other hand, ACD
542 is unlikely to explain the pattern observed in females, as reproductive interference has not been
543 reported in this sex.

544 The divergence observed in ventral colors in sympatry, whether in males or in females,
545 was driven by changes in *L. helveticus* only. Asymmetry can be caused by either unidirectional
546 hybridization or asymmetrical cost of interspecific reproductive or agonistic interactions (Smadja
547 & Ganem, 2005; Lemmon, 2009). None of these scenarios can be excluded for now. One potential
548 cause of asymmetrical cost is asymmetry in numbers: the more abundant taxon should pay the
549 lower cost, as encounters with the other taxon are rarer. However, in our case, this is unlikely, as
550 we sampled a higher number of *L. helveticus* in most sympatric sites (24 out of 33). Further

551 investigations are required to investigate the origin of the asymmetrical responses of the two
552 *Lissotriton* studied.

553 If character displacement affects coloration in these species, one might wonder why we
554 did not detected divergence in all coloration traits involved in signaling. In fact, while RCD and
555 ACD can affect any trait as soon as it increases interspecific recognition, not every trait potentially
556 involved in signaling needs to exhibit divergence in a given species pair. First, although traits
557 involved in interspecific recognition are usually sexual (Ryan & Rand, 1993), not all sexual traits
558 signal species identity. For example some of them may be constrained by a shared female
559 preference, maintained in both species as a way to assess male physical condition (Pérez i De
560 Lanuza *et al.*, 2014). This might explain why some sexual traits other than ventral coloration did
561 not diverge in our system: they may simply not be used for interspecific recognition. Another
562 possibility is that these sexual traits indeed allow species recognition, but are already strongly
563 divergent in allopatry, thus limiting the potential benefits of further divergence. This hypothesis
564 is particularly likely for the caudal filament and extent of webbing, sexual ornaments that are
565 only found in *L. helveticus*, see Figure S1). A similar argument can also apply, to some extent, to
566 different variables describing the same color. We observed that among colorimetric variables
567 describing ventral coloration, some did not diverge or even converged in sympatry. This may be
568 because the change in only one component (e.g. the hue) suffices to alter dramatically the
569 perceived ventral coloration, making changes in other components redundant. Amphibians are
570 known to discriminate subtle variations of coloration, even in dim light conditions (e.g. at night,
571 Gomez *et al.* 2010), and the enhanced difference in saturation and hue in sympatric populations
572 can thus certainly be perceived by newts.

573

574 **Conclusion**

575 Our study illustrates how the colors of different parts of the body, subject to different
576 combinations of selection pressures, can follow a diversity of evolutionary trajectories, some
577 convergent, others divergent, when two species meet each other in a sympatric zone. An
578 important point is that, because by definition sympatry means that the two species are
579 confronted to similar environments, at least with respect to large-scale environmental gradients
580 (e.g. driven by latitude), convergence can be considered the “null” expectation for colors
581 predominantly involved in response to environment-related selection – and we indeed
582 frequently observed convergence in dorsal and caudal colors in our newt species. In such cases,
583 the inclusion of environmental variables that potentially differ among regions in the model, as
584 we did, may help to isolate some of the potential drivers of convergence -though it will be difficult
585 to capture them entirely. On the other hand, the opposite pattern, divergence in sympatry, if it
586 resists the inclusion of environmental variables in the model, suggests that the interaction
587 between the two species has specifically resulted in selection for dissimilarity in the
588 corresponding traits.

589 Our observations on newts are consistent with earlier studies suggesting that, in
590 sympatry, non-sexual colors more often converge and sexual signals more often diverge (Stuart–
591 Fox & Ord, 2004; Gomez & Théry, 2007). For the most constantly exposed color patches,
592 potentially involved in thermal regulation or crypsis, we observed convergence (sometimes
593 explained by large-scale environmental gradients) while divergence was only observed in traits
594 of usually concealed body parts (i.e. ventral colors) involved in mate choice. It is important to
595 note however, that RCD or ACD (the most likely sources of selection for dissimilarity in sexual
596 traits), are not expected to apply equally to all sexual traits, because of potential redundancy
597 among them as means of species recognition, and other sources of (non-sexual) selection that
598 may simultaneously affect them. Newt tails, for example, are both sexually selected and visible

599 to predators. A mixture of different selection pressures is likely to apply to many color patches
600 in species where sexual signals are visible under most circumstances (e.g. bright dorsal plumage
601 in northern cardinals, Wolfenbarger 1999). Quantifying the relative contribution of natural
602 selection (e.g. thermal constraints, predation risk), sexual selection and selection for interspecific
603 recognition to the evolution of such exposed sexual color signals would be an interesting
604 research avenue to improve our understanding of the evolution of body coloration in animals.

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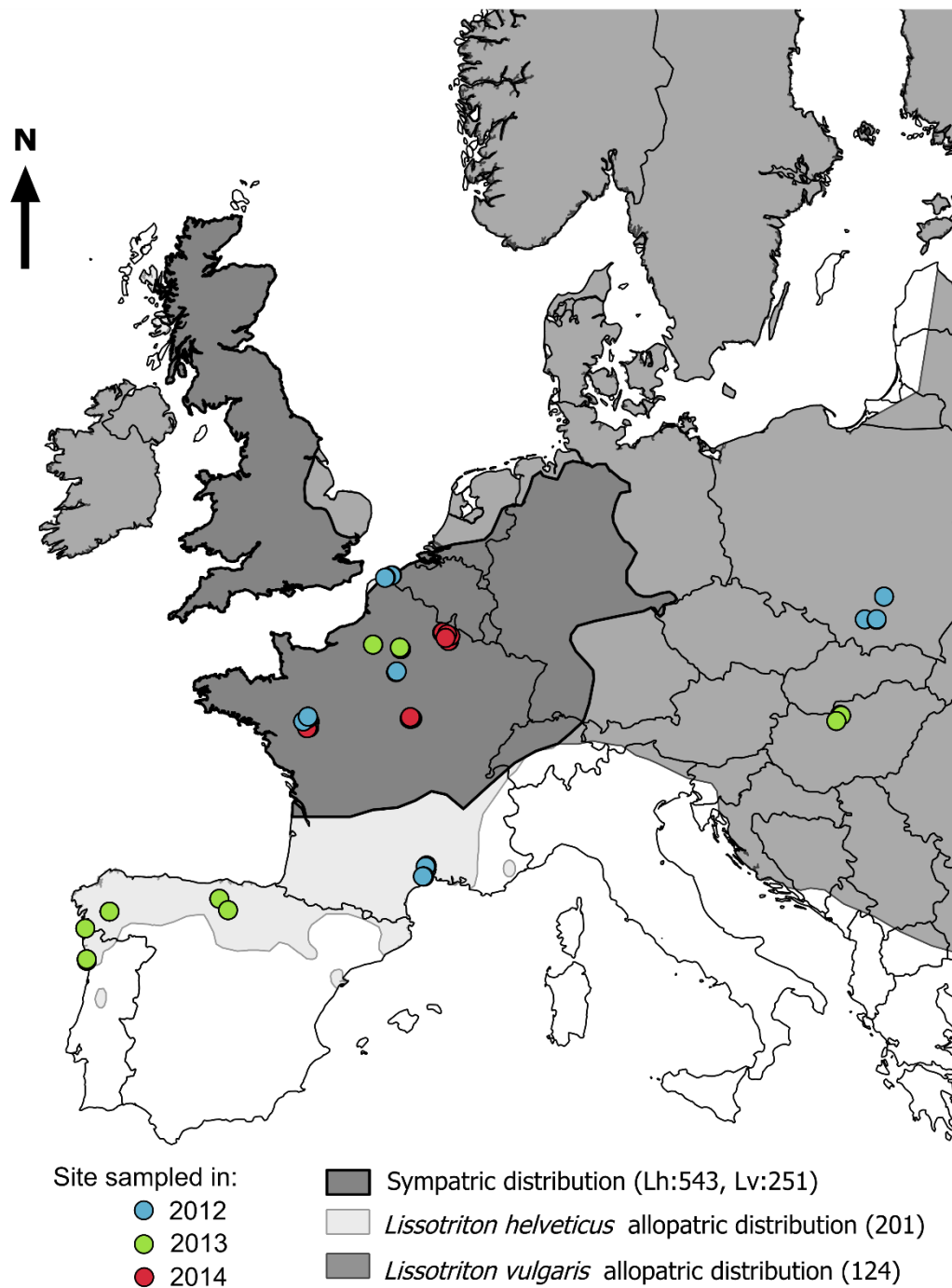
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815 **Table 1.** Description of the morphological and colorations included in the study. Is specified if these
816 traits are expected to be sexual or not (supporting literature in the Material and Methods section of the
817 manuscript). * indicates morphological and patterns traits only measured on males and included in the
818 Principal Component Analysis (PCA). Is also specified the correlations between these traits and the first
819 three PCA axes (values in bold indicate correlations above 0.5). Overall the first axes accounted for 67%
820 of the total variance, the second axis for 9% and the third axis for 8%.

Traits	Variables	Description and trait coding	Sexual	Axis1	Axis2	Axis3
Morphology and Pattern	SVL	Snout to vent length (mm)	No	-0.67	-0.34	0.51
	Tail	Maximum tail height (mm)	yes	-0.72	-0.36	0.44
	Fil *	Caudal filament length (mm)	yes	0.87	-0.23	0.15
	Webshape *	Shape of the web: (0) no web, (1) non-finger-binding web, (2) finger-binding web	yes	0.82	-0.27	0.23
	Crestshape *	Shape of the dorsal crest: (0) no crest, (1) serrated crest	yes	-0.98	0.09	-0.03
	Caudalshape *	Shape of the caudal crest: (0) no crest, (1) smooth crest, (2) serrated crest,	yes	-0.95	0.10	-0.01
	Throat number *	Density of dark spotting on throat: (0) no points, (1) few spots, (2) many spots	yes	-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	yes	-0.90	0.09	-0.03
	Belly *	Number of dark spots on belly	yes	-0.79	0.22	0.09
	Flank number *	Spotting on flanks: (0) no spot, (1) few spots, (2) many spots	yes	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	yes	-0.80	-0.25	-0.15	
Dorsal coloration	S6_visible	Saturation in visible spectrum		-	-	-
	B2_visible	Brightness in visible spectrum	No	-	-	-
	B2_UV	Brightness in UV spectrum		-	-	-
	S1_UV	Saturation in UV spectrum		-	-	-
Ventral coloration	S9	Saturation for carotenoids coloration		-	-	-
	B2_visible			-	-	-
	H3_visible	Hue in visible spectrum	yes	-	-	-
	B2_UV			-	-	-
	S1_UV			-	-	-
Caudal coloration	S6_visible			-	-	-
	B2_visible			-	-	-
	H3_visible		yes	-	-	-
	B2_UV			-	-	-
	S1_UV			-	-	-

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825 **Figure 1.** Map of the distribution ranges of *Lissotriton helveticus* (Lh) and *L. vulgaris* (Lv) with the
 826 location and the date of sampling of the sites included in the study. Sampling size in each sympatric and
 827 allopatric zones is indicated between brackets. Species' distributions were obtained from IUCNRed List
 828 maps (IUCN 2018).

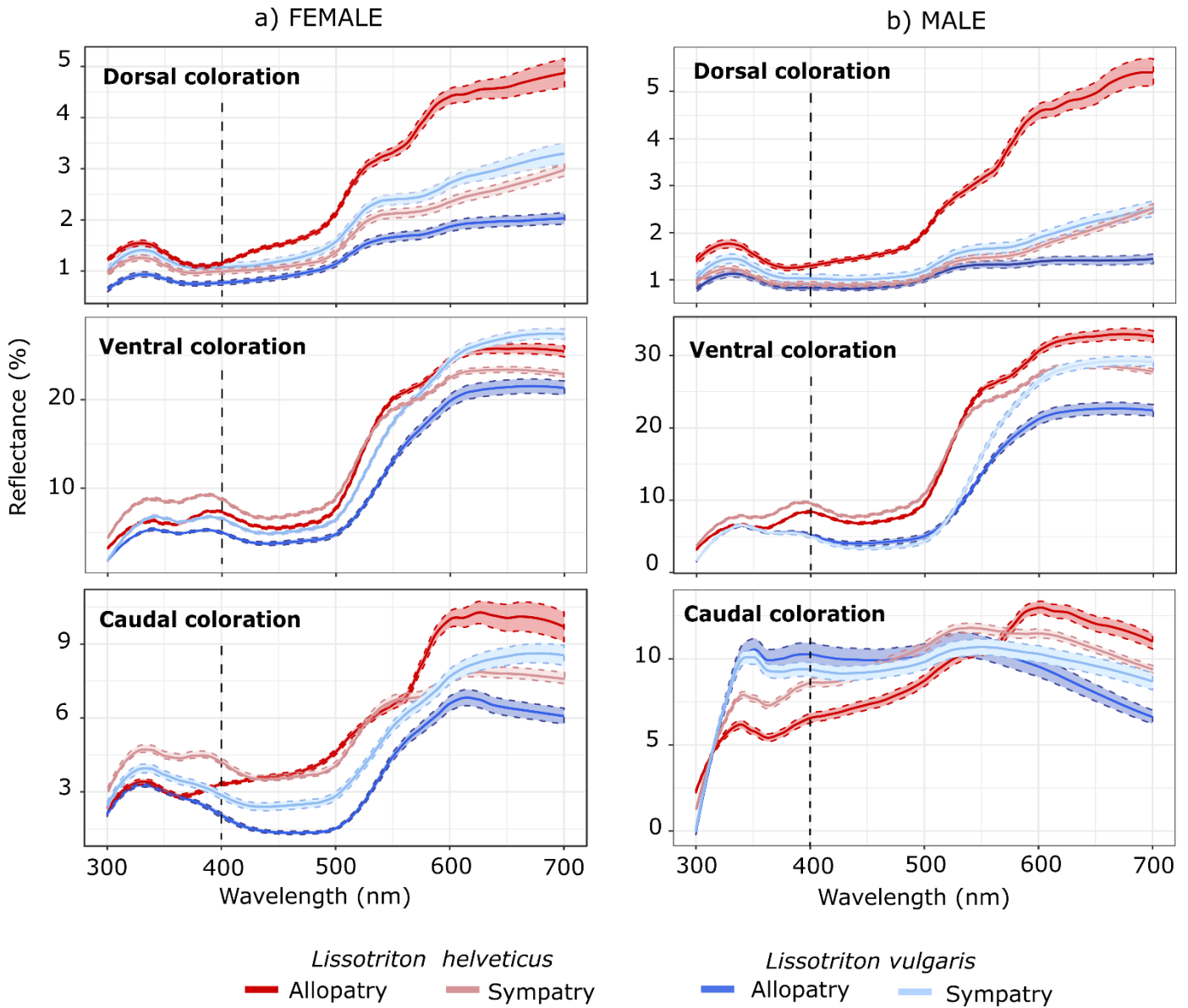
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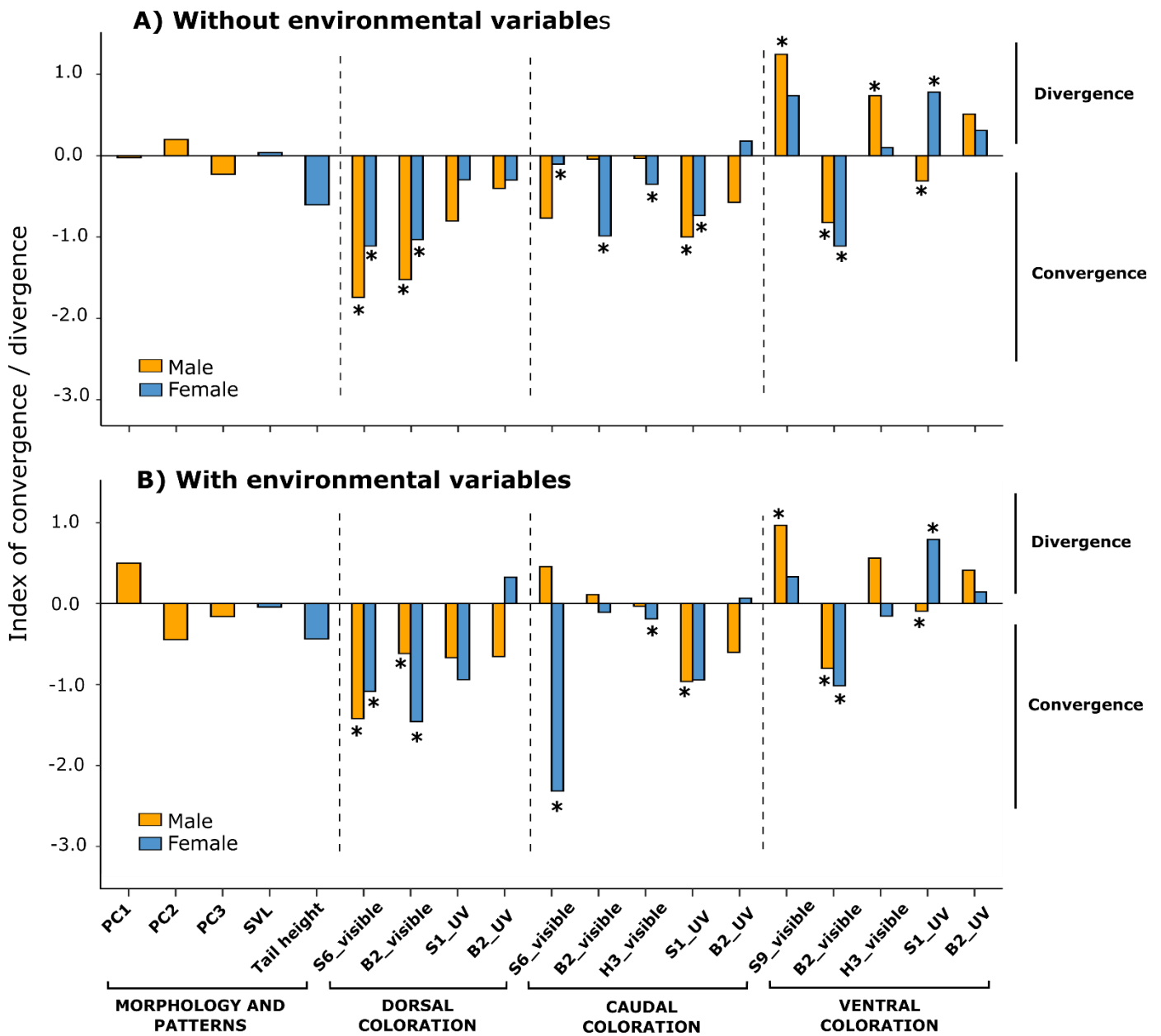
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836 **Figure 2.** Mean spectral reflectance of female (a) and male (b) dorsal, ventral and caudal patches for
837 *Lissotriton vulgaris* and *Lissotriton helveticus* originating from the sympatric and the allopatric zone.
838 Dashed lines and shaded area represent the standard error around each spectrum. The black vertical
839 dashed lines separate the UV range (left) from the visible range (right).

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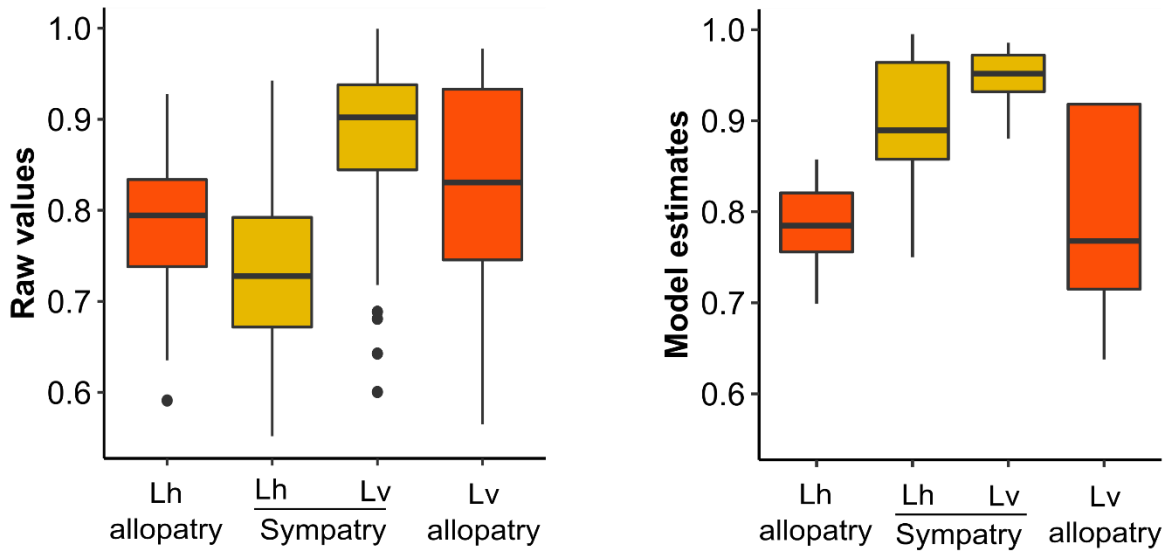
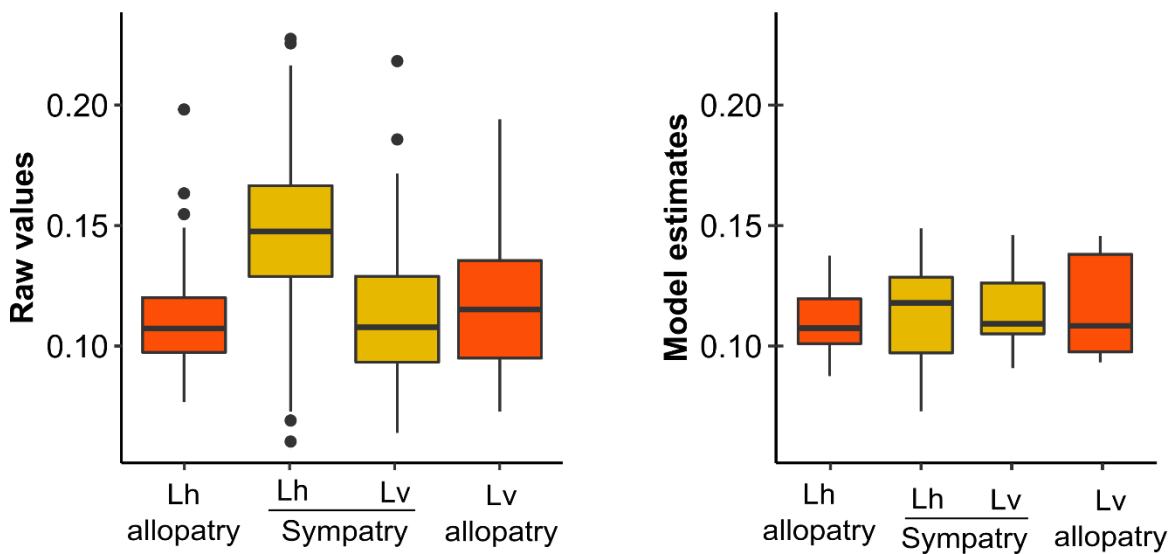


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844 **Figure 3.** Score of the convergence / divergence index for morphological and color variables in males
 845 and females of *Lissotriton helveticus* and *L. vulgaris* obtained from (A) analysis 1, without inclusion of
 846 environmental variables, or (B) analysis 2, with environmental variables included. Index = |marginal
 847 mean LH sympatry - marginal mean LV sympatry| - |marginal mean LH allopatry - marginal mean LV
 848 allopatry| (LH = *L. helveticus*, LV = *L. vulgaris*). For the analysis without environmental variables, the
 849 marginal means were equal to the mean value of species traits. For the analysis with environmental
 850 variables, the marginal means were computed based on residual values after accounting for all linear
 851 environmental effects and their interaction with species, included in the final model. Positive values
 852 represent species divergence in sympatry, and negative values species convergence. To allow for
 853 comparison, all traits were standardized. Stars indicate significant interactions between species and
 854 context (sympatry vs allopatry) i.e. significant pattern of convergence or divergence.

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(a) Male : ventral saturation in the visible spectrum (S9)**(b) Female : ventral saturation on the UV spectrum (S1_UV)**

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858 **Figure 4.** Saturation of ventral body parts in males and females of *Lissostriton helveticus* (Lh) and *L.*
 859 *vulgaris* (Lv) in sympatry and allopatry. See methods for the computation of the variables. The left panel
 860 represents the raw color data for each individual and the right panel the values predicted for each
 861 individual from linear mixed models when accounting for the effects of environmental variables and
 862 species but without the effect of the context (sympatry or allopatry). The difference between the two
 863 graphs shows how sympatry change species coloration (on the left), compared to what is predicted from
 864 species-specific responses to the environmental conditions (on the right).

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